Pathological Evaluation, Classification, and Staging of Gastrointestinal Cancers

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Introduction

Pathology as a diagnostic branch is an important pillar in the multidisciplinary management of most of the diseases including management of cancers. The insight provided by the microscopic features of any disease, *pathological evaluation of tissue,* is pivotal and an essential component in securing the best outcome in the multidisciplinary/multispecialty management of any cancer including gastrointestinal (GI) cancer.

GI cancer diagnosis involves multiple steps with various specialties including clinical examination for evaluating symptoms and signs, which guides the selection of an appropriate combination of imaging modalities, endoscopy, and various approaches for tissue diagnosis. The ultimate step is tissue diagnosis, the gold standard, with help of various biopsy methods. Sampling artifact due to missing of the actual pathology by random approach may be avoided by applying targeted methods guided by high-resolution endoscopy, such as different types of endomicroscopy in an effort to achieve in vivo histology-like real-time details (optical biopsy) [1–5].

Any of these methodologies has to conclude with appropriate expertise in ruling out various morphological mimickers by weeding out potential pitfalls in marching toward the correct diagnosis. Careful scrutiny of a variety of morphological features in the tissue specimens under examination is the most important step. Generally, the differential diagnosis involves a wide spectrum, spanning from reactive process at one end to various benign and malignant tumors at other end. If the morphological features are not sufficient enough to reach conclusive interpretation, a variety of ancillary tests may have to be applied. These ancillary tests include immunophenotyping by immunohistochemistry (IHC) or flow cytometry, fluorescence in situ hybridization/chromogenic in situ hybridization (FISH/CISH), cytogenetics, various molecular tests, electron microscopy, etc. Because it is easily adaptable to the routine anatomic pathology workflow using light microscopy, IHC is the most frequently used tool for evaluating diagnostic and prognostic immunomarkers. In addition, IHC has many other practical benefits, including feasibility to perform the immunostaining on archivable formalin-fixed paraffin-embedded (FFPE) tissue/cell-blocks. IHC slides can be stored like surgical pathology slides for future record. Ongoing refinement and availability of an ever-widening battery of immunomarkers along with increasing availability of multicolor immunostaining options for improved interpretation are continuously strengthening its ancillary status.

Thus, the interpretation of tissue for the diagnosis of any cancer is based on microscopic evaluation of morphological features with or without ancillary tests including immunophenotyping (immunohistochemistry/flow cytometry), cytogenetics, and variety of molecular pathology tests. Another component of interpretation is proper classification, which by itself, is an ongoing process based on increasing understanding with advances in the field of molecular pathology. Due to this, there are many tumor classifications for various cancers. However, depending on regional/local preferences and standard of practice, one or other classification is favored. In general, some classifications, such as the World Health Organization (WHO) classification [6] are favored over others. The tumors are generally classified based on their morphological features matching with its normal counterpart. This has been termed *histogenesis* (tissue of tumor origin). However, the preferred approach would be to consider the resemblance of a particular tumor to a particular type of normal tissue as its *differentiation* into that tissue type rather than as evidence of tissue of origin or *histogenesis*.

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Although not significantly important for all tumors, ***grading*** of tumor is an additional component of tissue diagnosis. Most of the approaches involve comparison of the tumor differentiation with the normal counterpart. Tumors with morphological resemblance closer to the normal spectrum would be "well differentiated" and the one lacking significant differentiation as "poorly differentiated," with "moderately differentiated" falling between the two extremes. This approach may be modified in some specific tumor/organ systems, such as in the application of mitotic figure count (proliferation status) and necrosis for grading neuroendocrine tumors (NET) [7] and gastrointestinal stromal tumors (GIST) [8, 9]. Ancillary tests such as KI-67 index may be applied for improved objectivity in tumor grading based on parameters related to proliferation [9a] are important factors to be considered for making treatment decisions. These features should be included in final pathology report under summary/synoptic report [10].

After tissue diagnosis and its proper classification, ***staging*** of that tumor has *prognostic significance* and is a critical component of any surgical pathology report on the resection specimen for proper clinical management. Currently, TNM (Tumor, Node, Metastasis) staging is the most widely practiced staging system. Based on various experiences, The American Joint Committee on Cancer (AJCC), in cooperation with the TNM Committee of the International Union Against Cancer (UICC), has incorporated these factors and developed a comprehensive TNM staging system, which is revised periodically [11–13, 15]. Each of the three components in TNM is given an incremental number as the tumor shows worsening features in that category. T (Tumor topography) is usually based on the size of tumor or the depth of the tumor invasion in tubular GI organs. Larger tumor size and/or deeper tumor invasion equates with a higher stage. N (extent of regional lymph node involvement) and M (evidence of distant metastasis) indicate the status regarding the spread of the tumor beyond the primary site as additional prognostic indicators. Depending on T, N, and M status, the AJCC has compiled various permutations and combinations into progressive groups from Stage 0 to Stage IV. In addition to TNM, other features such as Tumor deposits, Preoperative blood level of CEA, Tumor regresion score, Circumferential resection margin, Lymphovascular invasion, Perineural invasion, Microsatellite instability, KRAS and NRAS mutation, and BRAF mutation. Currently, this staging is one of the most important prognostic determinants and is important information in guiding the treatment plan [13]. Please see Table 2.1 in which "Colon carcinoma" is chosen as the organ system as an example for TNM staging [13]. The prognosis of higher stage cancer is poorer than lower stage cancers with shorter 5-year survival rates, even after curative resection [14].

Table 2.1 TNM staging based on AJCC eighth edition using *colon* as example (comparable approach with organ-specific details is applied for other tubular GIT) (see Fig. 2.5)

Definition of primary tumor (T)	
T category	T criteria
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma in situ: intramucosal carcinoma (involvement of lamina propria with no extension through muscularis mucosae)
T1	Tumor invades the submucosa (through the muscularis mucosa but not into the muscularis propria)
T2	Tumor invades muscularis propria
T3	Tumor invades through the muscularis propria into pericolorectal tissues
T4	Tumor invades the visceral peritoneum or invades or adheres to adjacent organ or structure
T4a	Tumor invades through the visceral peritoneum (including gross perforation of the bowel through tumor and continuous invasion of tumor through areas of inflammation to the surface of the visceral peritoneum)
T4b	Tumor directly invades or adheres to adjacent organs or structures

Definition of regional lymph node (N)	
N category	N criteria
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	One to three regional lymph nodes are positive (tumor in lymph nodes measuring ≥0.2 mm), or any number of tumor deposits are present and all identifiable lymph nodes are negative
N1a	One regional lymph node is positive
N1b	Two or three regional lymph nodes are positive
N1c	No regional lymph nodes are positive, but there are tumor deposits in the Subserosa Mesentery Nonperitonealized pericolic or perirectal/mesorectal tissues
N2	Four or more regional nodes are positive
N2a	Four to six regional lymph nodes are positive
N2b	Seven or more regional lymph nodes are positive

Definition of distant metastasis (M)	
M category	M criteria
M0	No distant metastasis by imaging, etc.; no evidence of tumor in distant sites or organs (this category is not assigned by pathologists)
M1	Metastasis to one or more distant sites or organs or peritoneal metastasis is identified
M1a	Metastasis to one site or organ is identified without peritoneal metastasis
M1b	Metastasis to two or more sites or organ is identified without peritoneal metastasis
M1c	Metastasis to the peritoneal surface is identified alone or with other site or organ metastasis

Table 2.1 (continued)

AJCC prognostic stage groups			
When T is…	And N is…	And M is…	Then the stage group is…
Tis	N0	M0	0
T1, T2	N0	M0	I
T3	N0	M0	IIA
T4a	N0	M0	IIB
T4b	N0	M0	IIC
T1–T2	N1/N1c	M0	IIIA
T1	N2a	M0	IIIA
T3–T4a	N1/N1c	M0	IIIB
T2–T3	N2a	M0	IIIB
T1–T2	N2b	M0	IIIB
T4a	N2a	M0	IIIC
T3–T4a	N2b	M0	IIIC
T4b	N1–N2	M0	IIIC
Any T	Any N	M1a	IVA
Any T	Any N	M1b	IVB
Any T	Any N	M1c	IVC

Used with permission of the American Joint Committee on Cancer (AJCC), Chicago, Illinois [15]. The original and primary source for this information is the *AJCC Cancer Staging Manual*, *Eighth Edition* (2017) published by Springer International Publishing

Table 2.2 Appendix: Comparative TNM staging according to AJCC applied to carcinoma *versus* neuroendocrine tumor [15]. (Note that for Appendix, in addition to TNM, grade of the tumor is also a consideration for staging of carcinoma, especially subcategorization of stage IV)

(a) Carcinoma	
Definition of primary tumor (T)	
T category	T criteria
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma in situ (intramucosal carcinoma; invasion of the lamina propria or extension into but not through the muscularis mucosae)
Tis(LAMN)	Low-grade appendiceal mucinous neoplasm confined by the muscularis propria. Acellular mucin or mucinous epithelium may invade into the muscularis propria T1 and T2 are not applicable to LAMN. Acellular mucin or mucinous epithelium that extends into the subserosa or serosa should be classified as T3 or T4a, respectively
T1	Tumor invades the submucosa (through the muscularis mucosa but not into the muscularis propria)
T2	Tumor invades muscularis propria
T3	Tumor invades through the muscularis propria into the subserosa or the mesoappendix
T4	Tumor invades the visceral peritoneum, including the acellular mucin or mucinous epithelium involving the serosa of the appendix or mesoappendix, and/or directly invades adjacent organs or structures
T4a	Tumor invades through the visceral peritoneum, including the acellular mucin or mucinous epithelium involving the serosa of the appendix or serosa of the mesoappendix
T4b	Tumor directly invades or adheres to adjacent organs or structures

Table 2.2 (continued)

Definition of regional lymph node (N)	
N category	N criteria
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	One to three regional lymph nodes are positive (tumor in lymph nodes measuring ≥0.2 mm), or any number of tumor deposits are present, and all identifiable lymph nodes are negative
N1a	One regional lymph node is positive
N1b	Two or three regional lymph nodes are positive
N1c	No regional lymph nodes are positive, but there are tumor deposits in the subserosa or mesentery
N2	Four or more regional nodes are positive

Definition of distant metastasis (M)	
M category	M criteria
M0	No distant metastasis
M1	Distant metastasis
M1a	Intraperitoneal acellular mucin, without identifiable tumor cells in the disseminated peritoneal mucinous deposits
M1b	Intraperitoneal metastasis only, including peritoneal mucinous deposits containing tumor cells
M1c	Metastasis to sites other than peritoneum

AJCC prognostic stage groups				
When T is…	And N is…	And M is…	And grade is…	Then the stage group is…
Tis	N0	M0		0
Tis(LAMN)	N0	M0		0
T1	N0	M0		I
T2	N0	M0		I
T3	N0	M0		IIA
T4a	N0	M0		IIB
T4b	N0	M0		IIC
T1	N1	M0		IIIA
T2	N1	M0		IIIA
T3	N1	M0		IIIB
T4	N1	M0		IIIB
Any T	N2	M0		IIIC
Any T	N0	M1a		IVA
Any T	Any N	M1b	G1	IVA
Any T	Any N	M1b	G2, G3, or GX	IVB
Any T	Any N	M1c	Any G	IVC

(b) Neuroendocrine tumor	
Definition of primary tumor (T)	
T category	T criteria
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
T1	Tumor 2 cm or less in greatest dimension
T2	Tumor more than 2 cm but less than or equal to 4 cm
T3	Tumor more than 4 cm or with subserosal invasion or involvement of the mesoappendix
T4	Tumor perforates the peritoneum or directly invades other adjacent organs or structures (excluding direct mural extension to adjacent subserosa of adjacent bowel), e.g., abdominal wall and skeletal muscle

(continued)

Table 2.2 (continued)

Definition of regional lymph node (N)	
N category	N criteria
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Regional lymph node metastasis

Definition of distant metastasis (M)	
M category	M criteria
M0	No distant metastasis
M1	Distant metastasis
M1a	Metastasis confined to liver
M1b	Metastases in at least one extrahepatic site (e.g., lung, ovary, nonregional lymph node, peritoneum, and bone)
M1c	Both hepatic and extrahepatic metastases

AJCC prognostic stage groups			
When T is…	And N is…	And M is…	Then the stage group is…
T1	N0	M0	I
T1	N1	M0	III
T1	N0, N1	M1	IV
T2	N0	M0	II
T2	N1	M0	III
T2	N0, N1	M1	IV
T3	N0	M0	II
T3	N1	M0	III
T3	N0, N1	M1	IV
T4	N0	M0	III
T4	N1	M0	III
T4	N0, N1	M1	IV

Used with permission of the American Joint Committee on Cancer (AJCC), Chicago, Illinois [15]. The original and primary source for this information is the *AJCC Cancer Staging Manual, Eighth Edition* (2017) published by Springer International Publishing

Various organ systems have comparable methods to TNM staging, which may be modified in some cases based on the type of neoplasm. For example, TNM staging of the appendix for adenocarcinoma including goblet cell carcinoid (crypt cell carcinoma) is different than for neuroendocrine tumor (carcinoid) for the same organ (Table 2.2) [15].

The role of ***molecular pathology*** is evolving due to the ongoing introduction of a variety of targeted therapy for various GI cancers. The classical example is the role of *KIT* (CD117) in establishing the diagnosis of gastrointestinal stromal tumor (GIST) with evaluation for various KIT mutations related to the response to tyrosine kinase inhibitors such as Gleevec [16]. Other molecular tests are evolving continuously with an increasing role not only in treating GI cancer patients but also in monitoring/evaluating their relatives. An example includes evaluation for mismatch repair (MMR) genes for microsatellite instability (MSI), which is linked with the hereditary form of colorectal cancer in Lynch syndrome [17].

Most of this information is currently included as part of the final report on most of the definitive resections and some of the biopsies as per the College of American Pathologists (CAP) checklist for a particular tumor/organ [10, 18].

Pathological Evaluation

The standard of practice requires tissue diagnosis prior to initiation of treatment. Many lesions – both *benign* (including benign ulceration [usually due to ischemia or inflammatory processes, such as *Helicobacter pylori* infection in the stomach or cytomegalovirus infection in the colon], inflammatory conditions such as inflammatory bowel disease [Crohn's disease or ulcerative colitis], solitary rectal ulcer syndrome, and diverticular disease with mural stricturing, hamartomas, endometriosis, and adenomas) and *malignant* (including neuroendocrine tumors, lymphomas, mesenchymal tumors [e.g., GIST]), metastatic tumors with tendency for gastrointestinal tract metastases (e.g., melanomas), and malignancies growing into GI tract (GIT) from adjacent organs (e.g., cancers of the ovary, endometrium, urinary bladder, or prostate]) – *may clinically resemble GI carcinomas*. Due to this, it is critical to confirm the tissue diagnosis prior to definitive therapy as a standard of practice for the best outcome.

Tissue diagnosis and pathological evaluation may be achieved by various biopsy methods including fine-needle aspiration (FNA) biopsy (with its variants such as endoscopic ultrasound [EUS]-guided FNA, which is very important for evaluation of lesions of deeper organs such as the pancreas and other sites accessible through the tubular GI system) and other cytopathology methods including brushings, washings/lavages, and cyst aspirations. Surgical pathology approaches include endoscopic forceps biopsies/resection of small lesions such as polyps, core biopsy (including image-guided core biopsy), wedge biopsy (including laparoscopic biopsies), and ultimately resection specimens. Each of these approaches has benefits and limitations discussed briefly as follows.

Cytopathological Evaluation

Cytology has multiple advantages with the ability to evaluate excellent cytomorphological details (Fig. 2.1) over surgical pathology biopsy (Fig. 2.2). The principal mechanism by which the diagnostic material is retrieved by FNA facilitates selective suction of poorly cohesive neoplastic cells (Fig. 2.3) over supporting stroma, as compared to coring out of both stroma and tumor cells by core biopsy along the tract for that core (Fig. 2.3). FNA procedure samples a relatively wider area of the lesion because of the nature of the procedure in which the sampling FNA needle has to be moved back and forth in different directions in the tumor. Most of the sampled material is seen directly on the slides under scrutiny (in contrast to just a tiny fraction of the sampled surgical biopsy tissue as just a 4-micron thick tissue section) [19]. In addition to rapid turnaround time and lower cost, these specimens provide the opportunity to evaluate the cytomorphological features of tumor/lesion cells at a higher level of clarity with excellent nuclear details allowing precise diagnosis even with limited material (Fig. 2.1). In addition to the initial tissue diagnosis (Fig. 2.1), cytopathology contributes to

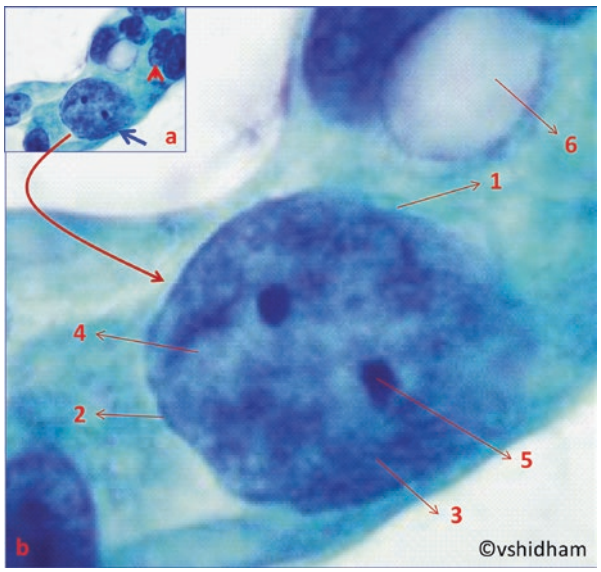

Fig. 2.1 Diagnostically crisp cytomorphological details in cytopathology samples (e.g., pancreatic ductal adenocarcinoma) (Pap stain – direct smear). (**a**) (inset): Cohesive group of neoplastic cells with sudden nucleomegaly. Variation in size of tumor nuclei: The difference in size between smallest (red arrowhead) and largest (blue arrow) nucleus in the group is at least 1:4. (**b**): 1. Large cell with high nuclear:cytoplasmic ratio; 2. irregular nuclear margin; 3. coarsely clumped irregularly distributed hyperchromatic chromatin; 4. parachromatin clearing; 5. nucleoli with irregular outlines; 6. cytoplasmic vacuoles with secretion (all these features collectively are consistent with adenocarcinoma)

the staging of many GI cancers such as TNM staging of colon cancer. Positivity of tumor cells in peritoneal fluid cytology is equivalent to the distant metastasis properly assigning a status of AJCC stage IV to these cases.

However, depending on a particular situation, invasion cannot be evaluated directly in the cytology specimens, although some indirect evidence such as tumor diathesis in the background with relatively higher cellularity may suggest that. Similarly, although some architectural details may be observed, it may not be comparable to that seen in surgical pathology (histopathology) tissue sections. Both these limitations could be overcome by using improved techniques for achieving best cellularity in cell-block sections from an adequately cellular cell-block [20, 21]. Recent advances for improving cellularity of cell-blocks allows maximum retrieval of diagnostic material in cell-block sections [19] [20a]. Cell-block also allows application of ancillary tests including IHC for differential diagnosis, for evaluation of prognostic markers, and for evaluating primary versus metastatic nature of the tumor. With the ever-increasing role of molecular tests, the cell-block is an excellent resource for many of these tests to be performed as indicated synchronously or at a later time on the archived FFPE cell-blocks. During on-site adequacy evaluation, it should be recommended to submit dedicated passes/material for cell-block preparation for future elective tests as clinically indicated. All these advantages of cell-blocks with

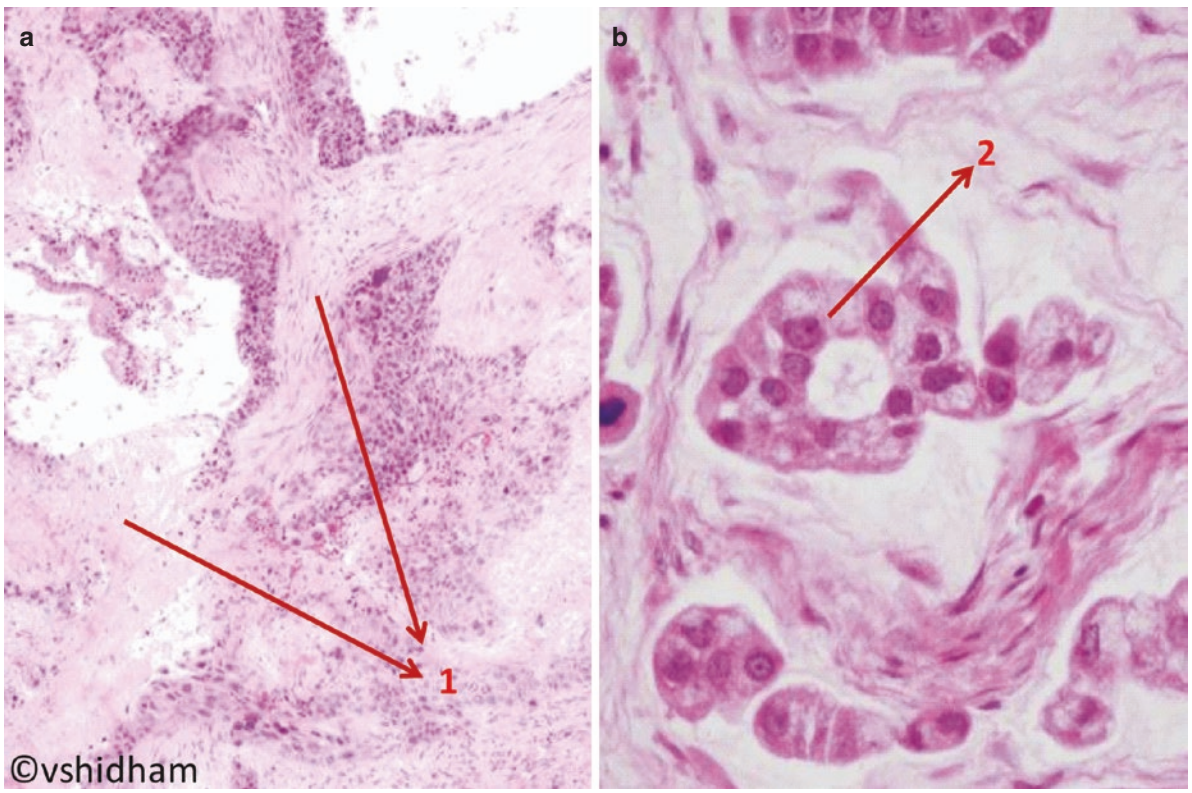

Fig. 2.2 Pancreatic adenocarcinoma (H&E) (**a**). Surgical pathology biopsy samples all tissue in the trajectory of the biopsy needle. Four-micron section of pancreatic ductal adenocarcinoma shows only fraction of the neoplastic epithelial component with predominance of stroma in section from tumors with predominance of desmoplastic stroma (compare with Fig. 2.3) (**b**). The morphology of individual tumor cells is relatively suboptimal as compared to cytology specimen (compare with Fig. 2.1). Similarly, the evaluation of sudden nucleomegaly is also relatively less dependable in surgical pathology sections

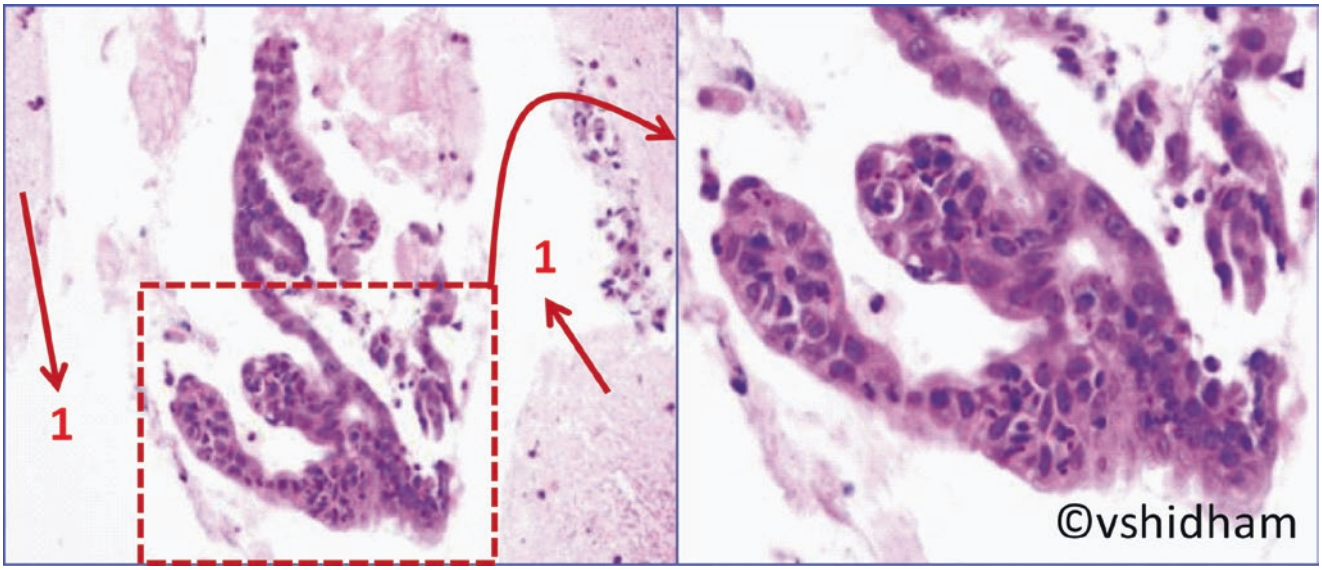

Fig. 2.3 Pancreatic adenocarcinoma (H&E). Cell-block section of FNA biopsy specimen (H&E stain). Note tumor with predominantly neoplastic epithelial component without significant proportion of stroma

recent advances are discussed in detail in the recent review article on CellBlockistry [21a].

Cytological approaches facilitate preoperative tissue diagnosis of lesions, especially those that otherwise may not be accessible with conventional biopsy due to complex locations (most of the pancreatic lesions) or due to potential risks of biopsy-associated complications such as needle tracking. However, due to the relative complexity in interpreting cytopathology material, the availability of expertise may be limited to some special centers.

Onsite adequacy evaluation is an important component to navigate the exact area to be sampled and provide real-time input for retrieval of adequate diagnostic material with triage feedback for appropriate supporting tests such as flow cytometry, microbiology cultures, and cytogenetics. The final goal of onsite adequacy evaluation is to achieve diagnostic material for unequivocal cytopathological interpretation, which for adenocarcinoma and other nonhematopoietic lesions is heavily dependent on evaluation of Papanicolaou (Pap) stained smears. Due to this, it is important to ensure retrieval of diagnostic material on the Pap-stained smears (instead of Diff-Quick [DQ]-stained smears), especially when only a suboptimal scant specimen could be available for final interpretation. In such cases, use of DQ stain initially for onsite adequacy may compromise the final interpretation especially if the lesion turns out to be a well-differentiated adenocarcinoma with scant material, leading to atypical/suspicious type suboptimal final report. Conventionally, wet-fixed smears are needed for Pap staining and air-dried counterpart for DQ staining. However, air-drying of all smears allows application of either Pap stain (after rehydration with post-fixation) or DQ stain electively [22]. Based on published study and long personal experience, using air-dried smears with routine availability of rapid Pap staining protocol during onsite adequacy evaluation is recommended for increasing the chances of final unequivocal cytopathological interpretation of most GI nonhematopoietic lesions [22].

Table 2.3 Cytopathological evaluation of GI lesions

Organ system/lesion	Procedure	Remark
Esophagus	Endoscopic brushing Others – such as: Abrasive balloon [23] Cytosponge in gelatin capsule [24]	Candida, viral cytopathic effect, Barrett's esophagus, dysplasia, carcinoma
Stomach	EUS-FNA	Deeper solid lesions; e.g., GIST
Pancreas	EUS-FNA	Cystic and solid lesions
Pancreatic duct	Endoscopic brushing	Dysplasia, carcinoma
Ampulla of Vater	Endoscopic brushing	Dysplasia, carcinoma
Bile duct	Endoscopic brushing	Dysplasia, carcinoma
Liver	Image-guided FNA	Solid (or cystic) lesions
Lesions – lymph nodes around/ adjacent to tubular GI	EUS-FNA	Cysts and solid lesions
Anal canal	Anal Pap (brushing)	Dysplasia/carcinoma

EUS endoscopic ultrasound, *FNA* fine-needle aspiration, *GIST* gastrointestinal stromal tumor, *GI* gastrointestinal

Commonly used approaches for cytological sampling of lesions in various organ systems are summarized in Table 2.3 [23, 24].

Surgical Pathological (Histopathological) Evaluation

Surgical pathological (histopathological) evaluation of lesions suspicious for cancer identified after clinical examination in concert with different imaging modalities and/or various endoscopic studies with suspicion for malignancy is another modality available in addition to cytopathological methods. Similar to cytopathological evaluation, the role of the biopsy is to distinguish benign lesions from clinically neoplastic mimickers and to rule out or rule in malignancy along with histological typing of the tumor.

Similar to cytopathological evaluation, a variety of approaches may be used to retrieve tissue for surgical pathological (histopathological) evaluation of suspicious lesions. The methodology may range from minimal representative sampling to total resection in various forms and may be categorized mainly into:

1. Diagnostic sampling
   A. Diagnostic biopsies (may be supported by guidance from onsite adequacy evaluation for precise sampling of the lesion by intra-procedural cytology smears)
      (a) Needle core biopsies
      (b) Endoscopic forceps biopsies
      (c) EUS-guided core biopsies
   B. Wedge biopsies
   C. Excisional biopsies
2. Therapeutic excisions
   A. Wide excisions, including endoscopic mucosal resections (EMR) [25]
   B. Radical resections

The major benefit with most of the surgical pathology specimens is the ability to evaluate the tissue architecture and invasion (Fig. 2.2). Even though FNA with good cell-block has numerous benefits as stated previously, there may be a tendency to prefer needle core biopsy over the relatively skill-dependent FNA procedure due to perceived ease in performing core biopsies. Generally, the cytopathological approach has a higher chance of diagnostic outcome as compared to core biopsies with small/tiny tissue for surgical pathology, especially for tumors with a tendency for sclerotic/desmoplastic stroma (e.g., pancreatic ductal carcinoma) [26, 27].

However, the final result with surgical pathology depends on a variety of factors including how the tissue is collected, from where it is collected, how it is fixed and processed, and the final quality of tissue sections with elective application of ancillary tests for final interpretation.

For diagnostic biopsies, it is important to sample the proper area of any lesion. For sampling ulcerated lesions and retrieving representative diagnostic material, the specimens should be taken from all 4 quadrants of the ulcer edge (e.g., for ulcerated carcinomas) and its base (e.g., for ulcerated lymphoma and sarcoma). The surface of the polypoid lesions would be the representative tissue. However, superficial biopsies such as from tubular gut or the ampulla of Vater, extrahepatic bile ducts, and pancreatic ducts may be limited by the difficulty in evaluating the invasion and its depth. For sampling obstructive lesions, an endoscope may not be negotiable and so may be difficult to biopsy. In such cases, brush cytology is an appropriate alternative. Some deeper lesions such as lymphomas, neuroendocrine tumors, GIST, and sarcomas usually have deeper submucosal mural growth pattern. Such lesions may be missed in superficial luminal biopsies and so, in this clinical situation, the same specific biopsy site should be sampled repeatedly to retrieve the representative deeper tissue. Tumors with extensive necrosis may not provide a sample with viable diagnostic component. In such cases, sampling multiple biopsies, especially from the periphery of the lesion, would enhance the possibility of sampling viable diagnostic tissue. In addition, core biopsies may not sample diagnostic material or if it samples diagnostic tissue, it may not be sufficient for precise grading of some lesions such as NETs and GIST. Calculation of Ki67 (MIB1) index (need at least 500 to 1000 tumor cell nuclei) and mitotic figure counting (need up to 50 high power fields) may not be precise on specimens with scant viable tumor components [7, 8].

Intraoperative Consult (Including Frozen Sectioning and Imprint/Scrape Cytology Smears)

The final management, especially resection, may need intra-procedural input to guide the surgical treatment. The most common indication is *evaluation of the resection margins* for the tumor. Other benefits of the intraoperative consult include triaging of the fresh specimen for ancillary studies such as cytogenetics, flow cytometry, microbiology culture, and ultrastructural (electron microscopic) studies as indicated based on preliminary morphological evaluation. Some of these ancillary tests may not be possible at a later stage once the tissue is fixed. It is important not to use frozen sections (FS) routinely just for the diagnosis, especially on tiny biopsies and tissues with predominance of fat. Performance of FS without considering this limitation may compromise the morphology required for optimal final interpretation, including interference with some studies such as elective IHC. In case the tissue diagnosis input is a must on such specimens, intra-procedural *imprint/scrape cytology smears* is a better option [27a].

Specimen Handling

For the best interpretation outcome, both cytopathology and surgical pathology specimens have to be collected, handled, and processed properly. All personnel associated in this process should be aware of limitations and precautions with

emphasis on coordination and communication between different entities involved in it for the best outcome. Compromisation may affect the integrity of the specimen needed for the best outcome. Improper fixative, inappropriate fixation time, or prolonged ischemic time (time from excision to putting the specimen in the fixative) may compromise the results of ancillary tests, especially the immunostaining pattern/immunophenotype.

Although *cytopathology specimens* have many benefits as mentioned previously, they also have many challenges due to the complexity in choosing an appropriate collection protocol [20]. Close collaboration with the cytopathology laboratory is needed to achieve the best outcome. The simplest approach would be to submit a fresh specimen to the cytopathology laboratory for immediate processing. Similarly, air-dried direct cytology smears allow more flexibility and may be processed for both Pap and Diff-Quik staining with multiple benefits [22]. If this is not possible, it should follow the protocol standardized for their particular laboratory/institution (Table 2.4) [20, 22].

Table 2.4 Cytopathology specimen submission protocols

Specimen	Specimen submission protocol	Processing
Brushing smear	**Direct smear** (need proper training to smear the specimen on slides) *Smears may be*: **Wet-fixed smear** (immersing the smears in 95% ethyl alcohol before any spread material dries on the slide)	**Papanicolaou (Pap) staining**
	Air-dried smear (the slide with spread specimen is allowed to dry quickly – preferably within 30 seconds)	**Pap staining –** after rehydration with post-fixation [22] **Romanowsky staining** (most commonly used is **Diff-Quik (DQ) staining**) May also be used for **other special stains** such as GMS stain for fungus, etc.
Brushing tip	Tip of the brush with sample is submitted in **cytology fixative** (such as *CytoLyt*® or other *liquid-based cytology (LBC) fixative* for methodologies such as Thinprep® or Surepath™ as recommended by the laboratory)	Direct smear from the sediment or Cytospins™ – both may be stained with Pap or DQ stain LBC smears (Thinprep® or Surepath™) for **Pap staining** Not suitable for cell-block, due to potential compromisation of IHC and other tests.
	In **isotonic medium** such as saline, RPMI, other isotonic such as IsotonicMediumS™ [20a] (should be submitted to cytopathology laboratory for immediate processing without delay – otherwise, the specimen integrity will be compromised)	Direct smear from the sediment or Cytospins™ – both may be stained with Pap or DQ stain LBC smears (Thinprep® or Surepath™) for **Pap staining** If enough sediment – it may be processed **for cell-block** with appropriate method depending on the cellularity of the brushing specimen
Washings/lavages	In **isotonic medium** such as saline, RPMI, other isotonic such as IsotonicMediumS™ [20a] (should be submitted to cytopathology laboratory for immediate processing without delay – otherwise, the specimen integrity will be compromised)	Direct smear from the sediment or Cytospins™ – both may be stained with Pap or DQ stain LBC smears (Thinprep® or Surepath™) for **Pap staining** If enough sediment – it may be processed **for cell-block** with appropriate method depending on the cellularity of the specimen
Serous effusions	Fresh (preferably 100 ml up to 1000 ml) (see reference [20], for more details)	Direct smear from the sediment or Cytospins™ – both may be stained with Pap or DQ stain LBC smears (Thinprep® or Surepath™) for **Pap staining** **For cell-block** with appropriate method depending on the cellularity of the specimen
Fine-needle aspiration (FNA) biopsy (with on-site adequacy evaluation and triage)	**Direct smear** (need proper training to smear the specimen on slides) *Smears may be*: **Wet-fixed smear** (immersing the smears in 95% ethyl alcohol before any spread material dries on the slide)	**Pap staining**
	Air-dried smear (the slide with spread specimen is allowed to dry quickly, preferably within 30 seconds)	**Pap staining –** after rehydration with post-fixation [20] **Romanowsky staining** (most commonly used is **Diff-Quik (DQ) staining**) May also be used for **other special stains** such as GMS stain for fungus, etc.
	Needle rinses in **isotonic medium** such as saline, RPMI, other isotonic such as IsotonicMediumS™ [20a] (should be submitted to cytopathology laboratory for immediate processing without delay – otherwise, the specimen integrity may be compromised). If needed needle rinses may be submitted directly in 10% formalin – but this part cannot be used for cytology preparations, but good for preparation of cell-block	Cytospins™ – both may be stained with Pap or DQ stain LBC smears (Thinprep® or Surepath™) for **Pap staining** **For cell-block** with appropriate method depending on the cellularity of the specimen

GMS Gomori's methenamine silver, *RPMI* Roswell Park Memorial Institute

Small surgical pathology specimens such as core/forceps biopsies in general can be submitted in 10% formalin. Large specimens may be submitted in 10% formalin or as fresh, but fresh specimens must be processed immediately for appropriate final outcome. Fresh unfixed specimen provides the benefit and flexibility of applying different protocols, but not without the risk of compromising tissue integrity if immediate processing cannot be guaranteed. Some specimens may need special attention with preliminary orientation and processing to avoid a sub-optimal outcome. A good example in this category is endoscopic mucosal resection (EMR) specimens. These specimens should be oriented and mounted by pinning onto a paraffin wax block or cork board before submitting in fixative prior to transportation to the laboratory [25].

Application of Various Ancillary Tests

Routine morphological evaluation may not be sufficient for reaching a definitive interpretation, especially with limited biopsy specimen, scantly cellular cytology specimen, or some lesions such as poorly differentiated tumors. Ancillary methods including immunohistochemistry, in situ hybridization (FISH and CISH), other molecular tests, ultrastructural studies (electron microscopy), or histochemistry may be indicated.

The most powerful and practical tool widely used currently is IHC. Other tools have relative limitations and are used sparingly. Electron microscopy needs planning from the beginning of the biopsy procedure when the tissue is still fresh, so that it is appropriately processed with special fixative (glutaraldehyde). In addition, it takes several days to obtain results and is labor intensive. Due to this, the role of electron microscopy has been decreasing steadily with ongoing refinement in IHC. Histochemistry may be performed for neutral and acidic mucins (adenocarcinoma), glycoproteins (adenocarcinoma or hepatocellular carcinoma), neurosecretory granules (neuroendocrine tumors), melanin (primary or metastatic melanoma), and other tumor cell products or associated proteins. But most of these are detected by IHC with better specificity and sensitivity even for detecting some organisms such as *Helicobacter pylori* in gastric biopsies, thus limiting the role of histochemistry in today's practice environment. However, histochemistry is still used for some indications such as for detection of various organisms such as fungi (Periodic acid–Schiff for fungus [PAS-F] and Gomori's methenamine silver [GMS] stain) or acid-fast organisms (various acid-fast bacillus [AFB] stains).

Immunohistochemical Assessment

An increasing number of antibodies that may be applied to FFPE tissue are continuously being added to the ever-expanding spectrum of diagnostic and prognostic immunomarkers. This has facilitated widespread application of immunohistochemistry [28, 29] in routine diagnostic pathology. However, for some lesions, such as lymphomas, there is preference for fresh tissue in isotonic medium for immunolabeling and evaluation by flow cytometry. Although immunophenotyping (either IHC or flow cytometry) is a very powerful tool, it is absolutely essential to understand that it is an ancillary tool and has to be used in the context of a carefully structured differential diagnosis with reference to the clinical details and morphological findings. There are many pitfalls with potential false positivity if this caveat is not taken into consideration. It may be applied for a variety of indications including differential diagnosis of primary site, grading, and increasingly expanding prognostic/therapeutic reasons.

For example, recently, IHC has been made available for evaluation of programmed death ligand 1 (PD-L1) in the tumor cells [30]. Programmed death (PD)-1 (CD279) is a co-inhibitory receptor present on the cell surface of monocytes, T lymphocytes, B lymphocytes, and natural killer cells [31]. It has 2 ligands: PD-L1 (B7-H1) and PD-L2 (B7-DC). Interaction between PD-1 and its ligands down-regulates the T-cell response by inhibiting T-cell receptor signaling. PD-L1 on tumor cells is upregulated. Studies revealed that barricading this interaction with antibodies to PD-1 or PD-L1 reverses this inhibition to regain anti-tumor T-cell activity with therapeutic benefits [31].

Discussing application of IHC in detail is beyond the scope of this chapter [28]. A few immunomarkers applicable to GI cancers are shown in Table 2.5 [16, 17, 30–32].

Molecular Pathology

The role of molecular tests in GI cancer is continuously increasing. Please refer to the chapter on this topic in this book for more details in addition to other publications on this topic [17, 33–35]. Here, it is important to understand some basic details related to these. The molecular tests may be DNA-based or RNA-based. Recently, the role of microRNA (miRNA) is evolving. DNA is very robust and miRNA is relatively stable. In contrast, RNA is quite unstable and requires special precautions and protocols due to ubiquity of RNAase (RNA-destroying enzyme) present in tissue samples and in the devices/steps at different stages of processing. However, currently, many refinements have been achieved in the application of RNA-based molecular tests performed on FFPE [36]. Thus, like IHC, most of the molecular tests could be performed on FFPE, which in general is the most easily available clinical material for performing elective molecular pathology test at any stage on the archived FFPE tissue. Also, it is important to know the proportion of viable tumor component in the FFPE section in comparison with background nontumor nucleated component. Many tests require a minimum fraction of tumor component for optimum results. One should check with the laboratory performing a particular molecular pathology test

Table 2.5 Application of immunomarkers in gastrointestinal cancers: a few examples

Diagnostic	
Evaluate invasion	
Cytokeratin (CK) (Pan cytokeratin)	Identify single cells in diffusely spreading carcinoma – especially in small biopsies
Differential for primary site	
CK 7 and CK 20 coordinate pattern	Broad scrutiny for primary site identification
BER/EP4	Adenocarcinoma metastases to serous fluid cavities
Organ/site/tumor-specific immunomarkers	
CDX2/ STAB 2/ CDH 17	Colorectal-intestinal, pancreato-biliary, upper GI
Arginase	Hepatocellular carcinoma
Albumin miRNA (CISH)	Hepatocellular carcinoma
Estrogen receptor	Breast, ovary
LCA	Lymphoproliferative lesions
PAX 8	Ovary, kidney
PSA/PAP	Prostrate
MART 1/melan A	Melanoma
Calretinin	Mesothelioma
CD117/PGDF/DOG1	Gastrointestinal stromal tumor (GIST)
TTF-1	Lung, thyroid
Organ/site-specific immunostaining pattern	
pCEA/CD10	Bile canalicular pattern in hepatocytes
CD34	Diffuse sinusoidal immunostaining pattern (hepatocellular carcinoma versus regenerating nodule)
CK 19	Identify small bile ducts in small biopsies in differential diagnosis of regenerating nodule versus hepatocellular carcinoma
Differentiation immunomarkers (with many exceptions)	
Synaptophysin, chromogranin, CD56, INSM1	Neuroendocrine differentiation
Cytokeratins	Broad epithelial differentiation
LCA	Broad hematopoietic differentiation
Vimentin	Broad sarcomatous differentiation
Prognostic	
MIB 1 (Ki 67) (especially dual color-Ki 67- nuclear Brown, with LCA-cytoplasmic-Red)	Grading of neuroendocrine tumors (NET), GIST, lymphoma [9a]
Mismatched repair (MMR) proteins – MLH1, PMS2, MSH2, MSH6 (loss of nuclear immunoreactivity to these immuomarkers)	Hereditary colon adenocarcinoma (Lynch syndrome) [17]
Therapeutic	
Her2/Neu	Gastric and gastroesophageal junction adenocarcinoma
PD-L1	Targeted antibodies [30–32]
CD117	GIST – tyrosine-kinase inhibitor [16]

regarding the minimum tumor proportion required for a specific test in their laboratory. This may be overcome by selectively dissecting out the tumor by various microdissection methodologies. For other molecular pathology tests, there may be specific protocols requiring fresh or frozen tissue or tissue collected in special medium/preservative such as RNAlater® [37]. All of these limitations should be taken into consideration prior to proceeding with any molecular tests on any specimen. The overview for approaching molecular pathology tests on GI cancer specimens is summarized in Fig. 2.4 [17, 30–32, 38–45].

Classification of Gastrointestinal Tumors

GI cancers have been classified traditionally at two levels: macroscopic and microscopic.

Macroscopic Classification

Ultimately, similar to other cancers, microscopic findings in GI cancers decide the final interpretation and classification. But, the macroscopic gross evaluation including tumor configuration, size, and anatomic site is an important step with extended practical application, especially during endoscopic examination. The tumors of tubular GIT may be classified based on the approach used for gastric tumors, which are generally divided into four types: type I (polypoid), type II (fungating), type III (ulcerated), and type IV (infiltrative, also called *linitis plastica*) [46]. Some macroscopic features of ulcerated lesions may help to distinguish a benign ulcer from an ulcerated carcinoma (type III). A small, punched-out, well-circumscribed ulcer with a smooth base and edematous regular margin favors a benign gastric ulcer. In comparison, an irregular ulcer with raised, firm borders with necrotic and hemorrhagic base, typically favors a malignant ulcer [47].

Similar to gastric cancer, colorectal cancer (CRC) can also be classified macroscopically [48]:

1. *Exophytic tumors*: usually large, polypoid lesions (typically in the cecum) are rarely obstructive.
2. *Infiltrative ulcerating tumors*: ulcer with irregular raised edges.
3. *Constricting annular tumors*: functionally obstructive lesion with firm consistency due to desmoplasia resulting in proximal dilatation with typical double-contrast "apple-core" sign.
4. *Diffuse tumors*: similar to linitis plastica of the stomach with infiltrative growth along the bowel wall.

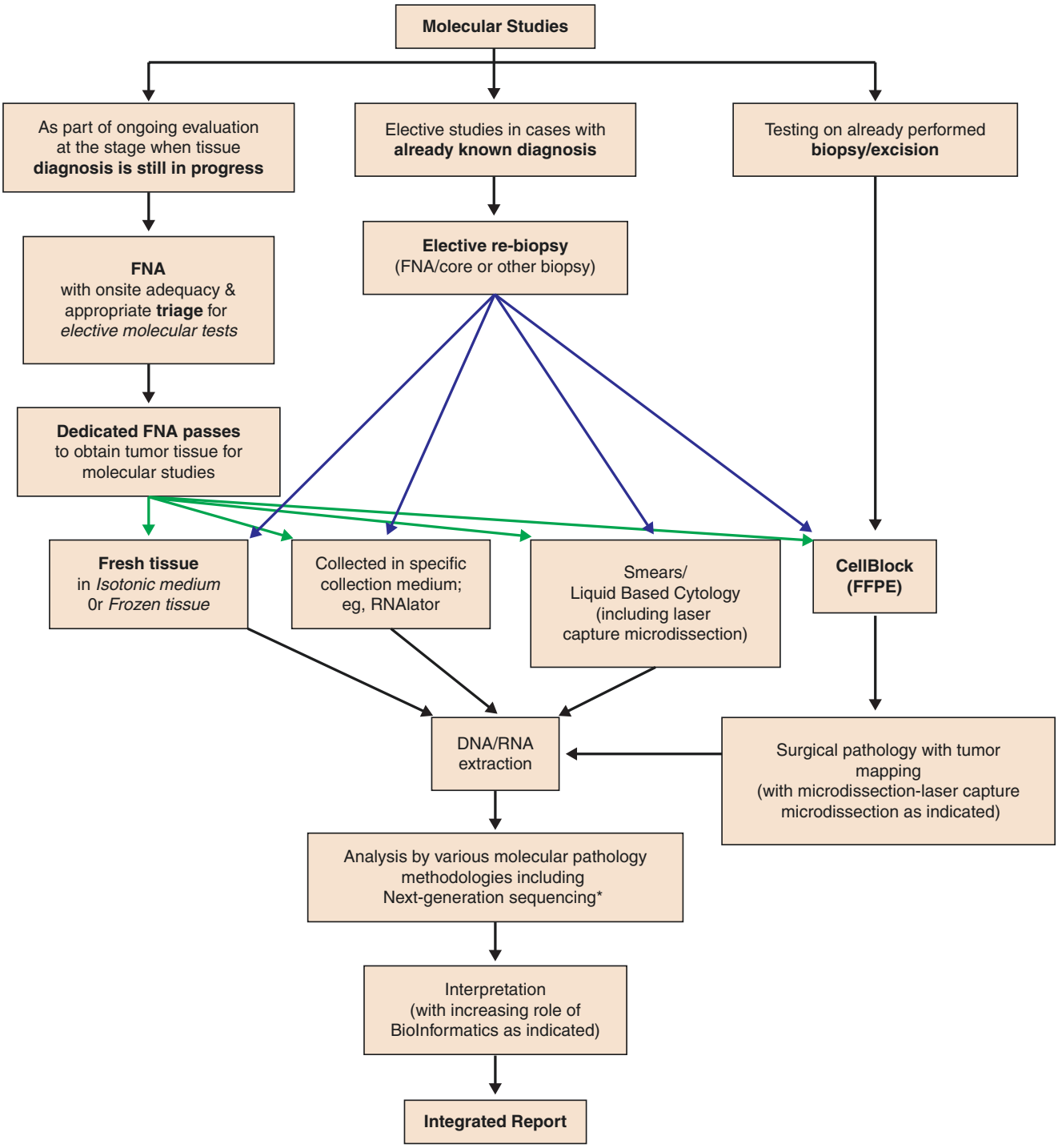

Fig. 2.4 Approach to evaluate commonly used molecular pathology tests and methodologies applicable to GI cancers (∗See references [17, 30–32, 38, 63–70])

Although macroscopic classification does not have a prognostic significance independent of the histological subtype [49], anatomic site does. *Right-sided t*umors – located in the cecum, ascending colon, hepatic flexure, or transverse colon – have a better prognosis as compared to left-sided tumors – located in the splenic flexure, descending colon, or sigmoid colon [50]. This may be related to tendency for microsatellite instability (MSI) in the right colon.

With the increasing role of endoscopy, macroscopic classification has evolved to categorize early neoplasia (type 0) of the digestive tract [51–53]. This classification distinguishes polypoid/protruded (type 0–I); nonpolypoid/nonprotruded, nonexcavated (type 0–II); and nonpolypoid, and excavated

(type 0–III) lesions. Type 0–II lesions are subdivided by the absence (type 0–IIa-elevated and type 0–IIb-flat) or presence (type 0–IIc) of a depression. This morphological macroscopic terminology applies to esophagus, stomach, and colon with increasing clinical relevance in the era of endoscopy [51]. But macroscopic features of GI cancers have limited diagnostic, predictive, and prognostic significance. Absolute dependence of staging on imaging findings without meticulous grossing of resection specimen is discouraged. Generally, malignant tumors are nonencapsulated with irregular infiltrative borders. They are usually large and solid with foci of necrosis/hemorrhages. As standard of practice, microscopic surgical pathology examination with tissue diagnosis is critical for appropriate management.

Microscopic Classification

CAP and other professional bodies have recommended internationally accepted terminology and diagnostic criteria established by the WHO for consistency and uniformity in pathological reporting (Table 2.6) [6, 54].

Table 2.6 Pathological classifications of various GI tumors (WHO 2000) [6]

Esophageal tumors
Epithelial tumors
Squamous cell papilloma 8052/0
Intraepithelial neoplasia
Squamous
Glandular (adenoma)
Carcinoma
Squamous cell carcinoma 8070/3
Verrucous (squamous) carcinoma 8051/3
Basaloid squamous cell carcinoma 8083/3
Spindle cell (squamous) carcinoma 8074/3
Adenocarcinoma 8140/3
Adenosquamous carcinoma 8560/3
Mucoepidermoid carcinoma 8430/3
Adenoid cystic carcinoma 8200/3
Small cell carcinoma 8041/3
Undifferentiated carcinoma 8020/3
Others
Carcinoid tumor 8240/3
Nonepithelial tumors
Leiomyoma 8890/0
Lipoma 8850/0
Granular cell tumor 9580/0
Gastrointestinal stromal tumor 8936/1
Benign 8936/0
Uncertain malignant potential 8936/1
Malignant 8936/3
Leiomyosarcoma 8890/3
Rhabdomyosarcoma 8900/3
Kaposi sarcoma 9140/3
Malignant melanoma 8720/3
Others – lymphoma
Secondary tumors
Melanoma

Table 2.6 (continued)

Gastric tumors
Epithelial tumors
Intraepithelial neoplasia – adenoma 8140/0
Carcinoma
Adenocarcinoma 8140/3
Intestinal type 8144/3
Diffuse type 8145/3
Papillary adenocarcinoma 8260/3
Tubular adenocarcinoma 8211/3
Mucinous adenocarcinoma 8480/3
Signet-ring cell carcinoma 8490/3
Adenosquamous carcinoma 8560/3
Squamous cell carcinoma 8070/3
Small cell carcinoma 8041/3
Undifferentiated carcinoma 8020/3
Others
Endocrine neoplasms of the stomach
1. Carcinoid – well-differentiated endocrine neoplasm
1.1 ECL-cell carcinoid
1.2 EC-cell, serotonin-producing carcinoid
1.3 G-cell, gastrin-producing tumor
1.4 Others
2. Small cell carcinoma – poorly differentiated endocrine neoplasm
3. Tumor-like lesions
Hyperplasia
Dysplasia
Nonepithelial tumors
Leiomyoma 8890/0
Schwannoma 9560/0
Granular cell tumor 9580/0
Glomus tumor 8711/0
Leiomyosarcoma 8890/3
GI stromal tumor 8936/1
Benign 8936/0
Uncertain malignant potential 8936/1
Malignant 8936/3
Kaposi sarcoma 9140/3
Others
Malignant lymphomas
Marginal zone B-cell lymphoma of MALT-type 9699/3
Mantle cell lymphoma 9673/3
Diffuse large B-cell lymphoma 9680/3
Others
Secondary tumors (breast, melanoma, etc.)

Table 2.6 (continued)

Small intestinal tumors
Epithelial tumors
Adenoma 8140/0
Tubular 8211/0
Villous 8261/0
Tubulovillous 8263/0
Intraepithelial neoplasia 2 (dysplasia) associated with chronic inflammatory diseases
Low-grade glandular intraepithelial neoplasia
High-grade glandular intraepithelial neoplasia
Carcinoma
Adenocarcinoma 8140/3
Mucinous adenocarcinoma 8480/3
Signet-ring cell carcinoma 8490/3
Small cell carcinoma 8041/3
Squamous cell carcinoma 8070/3
Adenosquamous carcinoma 8560/3
Medullary carcinoma 8510/3
Undifferentiated carcinoma 8020/3
Carcinoid (well-differentiated endocrine neoplasm) 8240/3
Gastrin cell tumor, functioning (gastrinoma) 8153/1 or nonfunctioning
Somatostatin cell tumor 8156/1
EC-cell, serotonin-producing neoplasm 8241/3
L-cell, glucagon-like peptide and PP/PYY-producing tumor
Mixed carcinoid–adenocarcinoma 8244/3
Gangliocytic paraganglioma 8683/0
Nonepithelial tumors
Lipoma 8850/0
Leiomyoma 8890/0
Gastrointestinal stromal tumor 8936/1
Leiomyosarcoma 8890/3
Angiosarcoma 9120/3
Kaposi sarcoma 9140/3
Others
Malignant lymphomas
Immunoproliferative small intestinal disease 9764/3 (includes α[alpha]-heavy-chain disease)
Western type B-cell lymphoma of MALT 9699/3
Mantle cell lymphoma 9673/3
Diffuse large B-cell lymphoma 9680/3
Burkitt lymphoma 9687/3
Burkitt-like/atypical Burkitt lymphoma 9687/3
T-cell lymphoma 9702/3
Enteropathy associated 9717/3
Unspecified 9702/3
Others
Secondary tumors
Polyps
Hyperplastic (metaplastic)
Peutz–Jeghers
Juvenile

Table 2.6 (continued)

Tumors of the appendix
Epithelial tumors
Adenoma 8140/02 (cystic counterpart – cystadenoma)
Tubular 8211/0
Villous 8261/0
Tubulovillous 8263/0
Serrated 8213/0
Carcinoma
Adenocarcinoma
8140/3 (cystic counterpart – cystadenocarcinoma)
Mucinous adenocarcinoma 8480/3
Signet-ring cell carcinoma 8490/3
Small cell carcinoma 8041/3
Undifferentiated carcinoma 8020/3
Carcinoid (well-differentiated endocrine neoplasm) 8240/3
EC-cell, serotonin-producing neoplasm 8241/3
L-cell, glucagon-like peptide
And PP/PYY-producing tumor
Others
Tubular carcinoid 8245/1
Goblet cell carcinoid (mucinous carcinoid) 8243/3
Mixed carcinoid–adenocarcinoma 8244/3
Nonepithelial tumors
Neuroma 9570/0
Lipoma 8850/0
Leiomyoma 8890/0
Gastrointestinal stromal tumor 8936/1
Leiomyosarcoma 8890/3
Kaposi sarcoma 9140/3
Others
Malignant lymphoma
Secondary tumors
Hyperplastic (metaplastic) polyp

(continued)

Table 2.6 (continued)

Tumors of the colon and rectum
Epithelial tumors
Adenoma 8140/0
Tubular 8211/0
Villous 8261/0
Tubulovillous 8263/0
Serrated 8213/0
Intraepithelial neoplasia 2 (dysplasia) associated with chronic inflammatory diseases
Low-grade glandular intraepithelial neoplasia
High-grade glandular intraepithelial neoplasia
Carcinoma
Adenocarcinoma 8140/3
Mucinous adenocarcinoma 8480/3
Signet-ring cell carcinoma 8490/3
Small cell carcinoma 8041/3
Squamous cell carcinoma 8070/3
Adenosquamous carcinoma 8560/3
Medullary carcinoma 8510/3
Undifferentiated carcinoma 8020/3
Carcinoid (well-differentiated endocrine neoplasm) 8240/3
EC-cell, serotonin-producing neoplasm 8241/3
L-cell, glucagon-like peptide and PP/PYY-producing tumor
Others
Mixed carcinoid–adenocarcinoma 8244/3
Others
Nonepithelial tumors
Lipoma 8850/0
Leiomyoma 8890/0
Gastrointestinal stromal tumor 8936/1
Leiomyosarcoma 8890/3
Angiosarcoma 9120/3
Kaposi sarcoma 9140/3
Malignant melanoma 8720/3
Others
Malignant lymphomas
Marginal zone B-cell lymphoma of MALT type 9699/3
Mantle cell lymphoma 9673/3
Diffuse large B-cell lymphoma 9680/3
Burkitt lymphoma 9687/3
Burkitt-like/atypical Burkitt lymphoma 9687/3
Others
Secondary tumors
Polyps
Hyperplastic (metaplastic)
Peutz–Jeghers
Juvenile

Tumors of the anal canal
Epithelial tumors
Intraepithelial neoplasia 1 (dysplasia)
Squamous or transitional epithelium
Glandular
Paget disease 8542/3
Carcinoma
Squamous cell carcinoma 8070/3
Adenocarcinoma 8140/3
Mucinous adenocarcinoma 8480/3
Small cell carcinoma 8041/3
Undifferentiated carcinoma 8020/3
Others
Carcinoid tumor 8240/3
Malignant melanoma 8720/3
Nonepithelial tumors
Secondary tumors

Table 2.6 (continued)

Tumors of the liver and intrahepatic bile ducts
Epithelial tumors
Benign
Hepatocellular adenoma (liver cell adenoma) 8170/01
Focal nodular hyperplasia
Intrahepatic bile duct adenoma 8160/0
Intrahepatic bile duct cystadenoma 8161/0
Biliary papillomatosis 8264/0
Malignant
Hepatocellular carcinoma (liver cell carcinoma) 8170/3
Intrahepatic cholangiocarcinoma 8160/3
(peripheral bile duct carcinoma)
Bile duct cystadenocarcinoma 8161/3
Combined hepatocellular and cholangiocarcinoma 8180/3
Hepatoblastoma 8970/3
Undifferentiated carcinoma 8020/3
Nonepithelial tumors
Benign
Angiomyolipoma 8860/0
Lymphangioma and lymphangiomatosis 9170/0
Hemangioma 9120/0
Infantile hemangioendothelioma 9130/0
Malignant
Epithelioid hemangioendothelioma 9133/1
Angiosarcoma 9120/3
Embryonal sarcoma (undifferentiated sarcoma) 8991/3
Rhabdomyosarcoma 8900/3
Others
Miscellaneous tumors
Solitary fibrous tumor 8815/0
Teratoma 9080/1
Yolk sac tumor (endodermal sinus tumor) 9071/3
Carcinosarcoma 8980/3
Kaposi sarcoma 9140/3
Rhabdoid tumor 8963/3
Others
Hematopoietic and lymphoid tumors
Secondary tumors
Epithelial abnormalities
Liver cell dysplasia (liver cell change)
Large cell type (large cell change)
Small cell type (small cell change)
Dysplastic nodules (adenomatous hyperplasia)
Low grade
High grade (atypical adenomatous hyperplasia)
Bile duct abnormalities
Hyperplasia (bile duct epithelium and peribiliary glands)
Dysplasia (bile duct epithelium and peribiliary glands)
Intraepithelial carcinoma (carcinoma in situ) 8500/211
Miscellaneous lesions
Mesenchymal hamartoma
Nodular transformation
(nodular regenerative hyperplasia)
Inflammatory pseudotumor

Table 2.6 (continued)

Tumors of the gallbladder and extrahepatic bile ducts
Epithelial tumors
Benign
Adenoma 8140/0
Tubular 8211/0
Papillary 8260/0
Tubulopapillary 8263/0
Biliary cystadenoma 8161/0
Papillomatosis (adenomatosis) 8264/0
Intraepithelial neoplasia (dysplasia and carcinoma in situ)
Malignant
Carcinoma
Adenocarcinoma 8140/3
Papillary adenocarcinoma 8260/3
Adenocarcinoma, intestinal type 8144/3
Adenocarcinoma, gastric foveolar type
Mucinous adenocarcinoma 8480/3
Clear cell adenocarcinoma 8310/3
Signet-ring cell carcinoma 8490/3
Adenosquamous carcinoma 8560/3
Squamous cell carcinoma 8070/3
Small cell carcinoma 8041/3
Large cell neuroendocrine carcinoma 8013/3
Undifferentiated carcinoma 8020/3
Biliary cystadenocarcinoma 8161/3
Carcinoid tumor 8240/3
Goblet cell carcinoid 8243/3
Tubular carcinoid 8245/1
Mixed carcinoid–adenocarcinoma 8244/3
Others
Nonepithelial tumors
Granular cell tumor 9580/0
Leiomyoma 8890/0
Leiomyosarcoma 8890/3
Rhabdomyosarcoma 8900/3
Kaposi sarcoma 9140/3
Others
Malignant lymphoma
Secondary tumors

Table 2.6 (continued)

Tumors of the exocrine pancreas
Epithelial tumors
Benign
Serous cystadenoma 8441/0
Mucinous cystadenoma 8470/0
Intraductal papillary-mucinous adenoma 8453/0
Mature teratoma 9080/0
Borderline (uncertain malignant potential)
Mucinous cystic neoplasm with moderate dysplasia 8470/1
Intraductal papillary-mucinous neoplasm with moderate dysplasia 8453/1
Solid-pseudopapillary neoplasm 8452/1
Malignant
Ductal adenocarcinoma 8500/3
Mucinous noncystic carcinoma 8480/3
Signet-ring cell carcinoma 8490/3
Adenosquamous carcinoma 8560/3
Undifferentiated (anaplastic) carcinoma 8020/3
Undifferentiated carcinoma with osteoclast-like giant cells 8035/3
Mixed ductal-endocrine carcinoma 8154/3
Serous cystadenocarcinoma 8441/3
Mucinous cystadenocarcinoma 8470/3
– Noninvasive 8470/2
– Invasive 8470/3
Intraductal papillary-mucinous carcinoma 8453/3
– Noninvasive 8453/2
– Invasive (papillary-mucinous carcinoma) 8453/3
Acinar cell carcinoma 8550/3
Acinar cell cystadenocarcinoma 8551/3
Mixed acinar-endocrine carcinoma 8154/3
Pancreatoblastoma 8971/3
Solid-pseudopapillary carcinoma 8452/3
Others
Nonepithelial tumors
Secondary tumors

Traditionally, tumor classification is based on type of tissue differentiation and is termed *histogenetic classification*, which categorizes different tumors with reference to various morphological features including: (1) site of primary tumor, (2) differentiation/histogenesis, (3) architectural phenotype, and (4) degree of differentiation (grade).

1. **Site of Primary Tumor:** Neoplasms of epithelium may be benign (*papillomas/adenomas*) or malignant (*carcinomas*). Similarly, those of connective tissue may be benign (various *–omas*) or malignant (*sarcomas*). Although generally there is good concordance between the type of normal tissue and type of neoplasm, some tumors with discordant differentiation may be seen in odd tissues. For example, carcinomas with total (as squamous cell carcinoma) or partial (adenosquamous carcinoma) squamous differentiation may be seen in organs such as the colon, rectum, and pancreas, which normally do not have squamous epithelium.
2. **Differentiation/Histogenesis:** Carcinomas demonstrating glandular growth pattern are *adenocarcinomas* versus *squamous cell carcinomas* with squamous differentiation.

Other than in the esophagus and anus (which in a significant proportion in these sites are *squamous cell carcinoma*), most of the GI carcinomas are *adenocarcinomas*.

Adenocarcinomas may be subdivided morphologically into various subtypes such as *usual type* (with glands of variable size, shapes, and maturity in the background of variable proportion of desmoplastic stroma); *mucinous type* (adenocarcinomas comprising of more than 50% component producing abundant secretory mucin (the term "adenocarcinoma with mucinous differentiation" may be used for tumors with marginal proportion of mucinous component >10% but <50%); *signet-ring cell type* (adenocarcinomas showing at least 50% signet-ring cells with cytoplasmic mucin vacuole pushing the nucleus).

Benign/malignant neoplasms of connective tissue, adipose tissue, smooth muscle, skeletal muscle, vessels, cartilage, and bone are broadly labeled respectively as fibroma/fibrosarcoma, lipoma/liposarcoma; leiomyoma/leiomyosarcoma; rhabdomyoma/rhabdomyosarcoma, angioma/angiosarcoma; chondroma/chondrosarcoma; and osteoma/osteosarcoma.

The tumors of hematopoietic and lymphoid tissues are *leukemias* and *lymphomas*. In the adult population, the majority of malignant neoplasms of tubular GIT are *carcinomas* followed by lymphomas and sarcomas, which are relatively the predominant tumor in the pediatric population.

3. **Architectural Phenotype:** Like other tumors, GI tumors may be classified based on growth pattern and microscopic architecture, which also provides important histogenetic clues while interpreting the tumor biopsies or resection specimens. Architectural pattern of epithelial tumors may be *tubular* (branching tubules of variable sizes); *papillary* (finger-like projections with fibrovascular central cores); *solid* or *trabecular* (seen in medullary carcinoma of the colon, neuroendocrine tumors, and hepatocellular carcinoma). Some tumors may show a *cystic* pattern (seen in the pancreas but relatively uncommon in tubular GI tumors, as mucinous carcinomas and endothelial tumors such as lymphangioma or hemangioma). Even solid tumors including stromal tumors/sarcomas, lymphomas, and carcinomas with central necrosis may present as cystic lesions, especially at imaging level. However, in general, growth pattern of GI tumors has little prognostic significance [55, 56]. Recently, a polyp with serrated glandular architecture has been linked as a precursor lesion for colorectal carcinomas [57].

A few examples suggesting applications of growth patterns for tumor classifications include *Lauren classification*, categorizing gastric cancers into different types: *Intestinal, diffuse*, mixed, and *indeterminate/unclassified* [58], in which diffuse growth pattern with highly unfavorable prognosis has macroscopic linitis plastica appearance with signet-ring cells at the microscopic level [59]. Colon cancers with *tumor budding* in the form of single cells or groups of less than 4 tumor cells at the invasive margin have worse prognosis and are associated with a diffuse growth pattern [60–65].

4. **Degree of Differentiation (Grade):** Tumor grade reflects the biological properties of the tumor. In general high-grade tumors are associated with aggressive biological behavior. The clinical significance of grading may be different for each tumor category. As an example, carcinomas or sarcomas with lower grade may be biologically less aggressive and amenable to surgical excision as compared to higher grade counterparts. On the other hand, low-grade lymphomas, although more indolent and slow growing than high-grade lymphomas, are difficult to be cured by medical therapy.

Although there are various approaches in grading tumors, the most commonly applied is the degree of resemblance of the tumor morphology to its non-neoplastic counterpart. Several microscopic features are taken into consideration for grading a tumor, including the anatomic site of origin of the tumor, the class of the tumor (i.e., carcinoma, sarcoma, or lymphoma), and the histological subtype within the class. The simplest approach applied for grading includes degree of gland formation in adenocarcinomas versus degree of keratinization in squamous cell carcinomas [56]. Most grading systems assign the grade based on the most poorly differentiated area. Some consider average of grades in different areas of the tumor. Arbitrarily most pathologists grade GI cancers into 4 grades: *Well differentiated* (grade 1), *moderately differentiated* (grade 2), *poorly differentiated* (grade 3), and *undifferentiated* (grade 4). Due to this subjective judgment left to the individual observer, there may not be reproducible outcome with significant degree of interobserver variability [66]. Despite these limitations, grading has some prognostic significance in most gastrointestinal malignancies [55, 56]. In addition, if the grade of the primary tumor is known, it may help while evaluating the interpretation of metastases at later stage during comparative review.

The CAP-suggested grading system is based on a semiquantitative approach for improved reproducibility and considers the proportion of neoplastic glands in the tumor: *grade X* (grade cannot be assessed); *grade 1* (well differentiated) – more than 95% glands; *grade 2* (moderately differentiated) – 50–95% glands; *grade 3* (poorly differentiated) – 5–49% glands; and *grade 4* (undifferentiated) – fewer than 5% glands [56]. Further simplification of this grading system has suggested a 2-tiered system for improved reproducibility [49]. *Higher grade tumors* demonstrate adverse prognosis independent of the stage. However, some poorly differentiated colorectal adenocarci-

nomas, such as those with MSI, may have better prognoses [67]. This simple approach has to be modified for some subtypes of carcinoma (e.g., medullary carcinoma of the colon is left ungraded; signet-ring carcinoma is defined as poorly differentiated or high-grade).

Other tumors including neuroendocrine tumors, sarcomas, and lymphomas have a special grading system based on different parameters such as proliferation index (mitotic figures or Ki-67 index estimation), necrosis, and other features.

Staging of Malignant Gastrointestinal Tumors

Staging is one of the best but simplest time-tested approaches for stratifying malignant neoplasms for prognostic grouping and is very important for planning the therapeutic management of the case. A staging system based on TNM classification standardized by the AJCC and UICC is recommended by CAP [12, 13, 15]. It has been used all over North America by national, regional, and local tumor registries and is also accepted internationally.

General Principles of the TNM Staging

TNM staging is based on classification and grouping of: "T" for the primary tumor status, "N" for regional lymph node status, and "M" for distant metastatic disease status (Table 2.7) [15, 68]. Final AJCC stage is assigned progressively from stage I through stage IV based on various combinations of staging in each category standardized for the individual organ system (see TNM staging of colon cancer as example in Table 2.1, Fig. 2.5) [15]. Lymphoma has a special staging system without applying the TNM approach for most lymphomas, except some types such as primary cutaneous lymphoma [15]. Although in general AJCC staging criteria are practiced, some ongoing approaches continue to evolve and claim better prognostic correlation [69].

More features are added to include other details: prefix "p" refers to the *pathological* classification; prefix "c" for the *clinical* classification. Prefix "r" is used for *recurrent tumors* following curative therapy (subject to the documentation of disease free interval) (Table 2.8) [12, 13, 15].

"R" classification is for *residual tumor* after primary therapy (e.g., curative surgical resection):

Table 2.7 TNM staging: general guidelines [15]

Topic	Rules
Microscopic confirmation	Microscopic confirmation is necessary for TNM classification, including clinical classification (with rare exception)
	In rare clinical scenarios, patients who do not have any biopsy or cytology of the tumor may be staged. This is recommended in rare clinical situations, only if cancer diagnosis is NOT in doubt. In the absence of histological confirmation, survival analysis may be performed separately from staged cohorts with histological confirmation. Separate survival analysis is not required if clinical findings support a cancer diagnosis and specific site
	Example: Lung cancer diagnoses by CT scan only, that is, without a confirmation biopsy[a]
Time frame/staging window for determining clinical stage	Information gathered about the extent of the cancer is part of clinical classification: From date of diagnosis before initiation of primary treatment or decision for watchful waiting or supportive care to one of the following time points, whichever is shortest: 4 months after diagnosis To the date of cancer progression if the cancer progresses before the end of the 4-month window; data on the extent of the cancer are only included before the date of observed progression
Time frame/staging window for determining pathological stage	Information including clinical staging data and information from surgical resection and examination of the resected specimens – if surgery is performed before the initiation of radiation and/or systemic therapy – from the date of diagnosis: Within 4 months after diagnosis To the date of cancer progression if the cancer progresses before the end of the 4-month window; data on the extent of the cancer are included only before the date of observed progression And includes any information obtained about the extent of cancer up through completion of definitive surgery as part of primary treatment if that surgery occurs later than 4 months after diagnosis and the cancer has not clearly progressed during the time window *Note*: Patients who receive radiation and/or systemic therapy (neoadjuvant therapy) before surgical resection are not assigned a pathological category or stage, and instead, they are staged according to post-neoadjuvant therapy criteria
Time frame/staging window for staging post-neoadjuvant therapy or post-therapy	After completion of neoadjuvant therapy, patients should be staged as follows: yc: post-therapy clinical yp: post-therapy pathological The time frame should be such that the post-neoadjuvant surgery and staging occur within a time frame that accommodates disease-specific circumstances, as outlined in the specific chapters and in relevant guidelines *Note*: Clinical stage should be assigned before the start of neoadjuvant therapy

(continued)

Table 2.7 (continued)

Topic	Rules
Progression of disease	If there is documented progression of cancer before therapy or surgery, only information obtained before the documented progression is used for clinical and pathological staging Progression does not include growth during the time needed for the diagnostic workup, but rather a major change in clinical status Determination of progression is based on managing physician judgment and may result in a major change in the treatment plan
Uncertainty among T, N, or M categories, and/or stage groups: rules for clinical decision making	If uncertainty exists regarding how to assign a category, subcategory, or stage group, the lower of the **two possible** categories, subcategories, or groups is assigned for T, N, or M Prognostic stage group/stage group Stage groups are for patient care and prognosis based on data. Physicians may need to make treatment decisions if staging information is uncertain or unclear *Note*: Unknown or missing information for T, N, M, or stage group is never assigned the lower category, subcategory, or group
Uncertainty rules do not apply to cancer registry data	If information is not available to the cancer registrar for documentation of a subcategory, the main (umbrella) category should be assigned (e.g., T1 for a breast cancer described as <2 cm in place of T1a, T1b, or T1c) If the specific information to assign the stage group is not available to the cancer registrar (including subcategories or missing prognostic factor categories), the stage group should not be assigned but should be documented as unknown
Prognostic factor category information is unavailable	If a required prognostic factor category is unavailable, the category used to assign the stage group is: X If the prognostic factor is unavailable, default to assigning the anatomic stage using clinical judgment
Grade	The recommended histological grading system for each disease site and/or cancer type, if applicable, is specified in each chapter and should be used by the pathologist to assign grade The cancer registrar will document grade for a specific site according to the coding structure in the relevant disease site chapter
Synchronous primary tumors in a single organ: (*m*) suffix	If multiple tumors of the same histology are present in one organ: The tumor with the highest T category is classified and staged The (*m*) suffix is used An example of a preferred designation is: pT3(m) N0 M0 If the number of synchronous tumors is important, an acceptable alternative designation is to specify the number of tumors. For example, pT3(4) N0 M0 indicates four synchronous primary tumors *Note*: The (*m*) suffix applies to multiple invasive cancers. It is not applicable for multiple foci of in situ cancer or for a mixed invasive and in situ cancer
Synchronous primary tumors in paired organs	Cancers occurring at the same time in each of paired organs are staged as separate cancers. Examples include breast, lung, and kidney **Exception**: For tumors of the thyroid, liver, and ovary, multiplicity is a T-category criterion, and thus, multiple synchronous tumors are not staged independently
Metachronous primary tumors	Second or subsequent primary cancers occurring in the same organ or in different organs outside the staging window are staged independently and are known as metachronous primary tumors Such cancers are not staged using the y prefix
Unknown primary or no evidence of primary tumor	If there is no evidence of a primary tumor, or the site of the primary tumor is unknown, staging may be based on the clinical suspicion of the organ site of the primary tumor, with the tumor categorized as T0. The rules for staging cancers categorized as T0 are specified in the relevant disease site chapters **Example**: An axillary lymph node with an adenocarcinoma in a woman, suspected clinically to be from the breast, may be categorized as T0 N1 (or N2 or N3) M0 and assigned Stage II (or Stage III) **Examples of exception**: The T0 category is not used for head and neck squamous cancer sites, as such patients with an involved lymph node are staged as unknown primary cancers using the "Cervical Nodes and Unknown Primary Tumors of the Head and Neck" system (T0 remains a valid category for human papillomavirus [HPV]-associated and Epstein–Barr virus [EBV]-associated oropharyngeal and nasopharyngeal cancers)
Date of diagnosis	It is important to document the date of diagnosis, because this information is used for survival calculations and time periods for staging The date of diagnosis is the date a physician determines the patient has cancer. It may be the date of a diagnostic biopsy or other microscopic confirmation or of clear evidence on imaging. This rule varies by disease site and shares similarities with the earlier discussion on microscopic confirmation

Original and primary source for this information is the *AJCC Cancer Staging Manual, Eighth Edition* (2017) published by Springer International Publishing

[a]Author's note: Recommend pathology reporting using CAP cancer protocols [68]

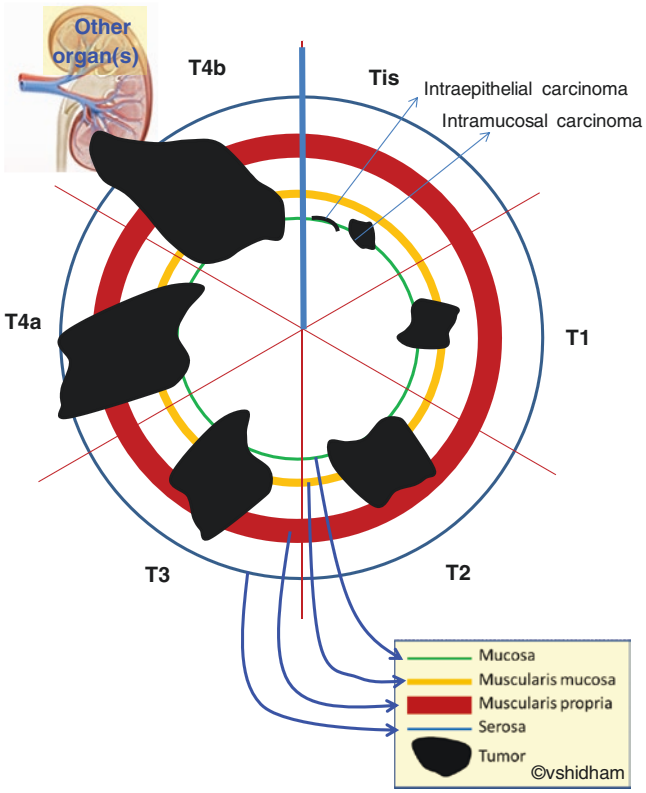

Fig. 2.5 T staging of colon carcinoma as example (see Table 2.1) [13]

- **R0** – *negative* for residual disease after definitive therapy (after curative surgical resection or total remission without detectable residual tumor)
- **R1**– residual tumor with *microscopically positive* resection margin
- **R2** – residual tumor with *macroscopically positive* resection margin

R classification is not usually followed by most institutions; instead, the report includes information on *resection margins*.

T Category

T staging (Table 2.9) for tubular GI cancer is assigned based on the depth of invasion of the primary tumor into various layers with incremental status as it invades from superficial to deeper layers (Tables 2.1 and 2.2, Fig. 2.5) [15]. For some tumors, for example liver tumors, it is based on other features such as size, vascular invasion, and multifocality.

Carcinoma in situ (pTis) includes *intraepithelial carcinoma* (when malignant cells are still restricted superficial to the basement membrane and have not invaded beyond it) and *intramucosal carcinoma* (in which tumor cells invade lamina propria without invading muscularis mucosa into submucosa). However, use of these terminologies may be confusing if applied randomly. In the colon, both *intraepithelial*

Table 2.8 Staging classifications/designator rules [15]

Classification	Designation	Details
Clinical	cTNM or TNM	Criteria: used for all patients with cancer identified before treatment It is composed of diagnostic workup information, until first treatment, including: Clinical history and symptoms Physical examination Imaging Endoscopy Biopsy of the primary site Biopsy or excision of a single regional node or sentinel nodes, or sampling or regional nodes, with clinical T Surgical exploration without resection Other relevant examinations *Note*: Exceptions exist by site, such as complete excision of primary tumor for melanoma
Pathological	pTNM	Criteria: used for patients if surgery is the first definitive therapy It is composed of information from: Diagnostic workup from clinical staging combined with Operative findings Pathology review of resected surgical specimens
Post-therapy or post-neoadjuvant therapy	ycTNM or ypTNM	For purposes of post-therapy or post-neoadjuvant therapy, *neoadjuvant therapy* is defined as systemic and/or radiation therapy given before surgery; primary radiation and/or systemic therapy is treatment given as definitive therapy without surgery
		yc The yc classification is used for staging after primary systemic and/or radiation therapy, or after neoadjuvant therapy and before planned surgery **Criteria**: First therapy is systemic and/or radiation therapy
		yp The yp classification is used for staging after neoadjuvant therapy and planned post-neoadjuvant therapy surgery **Criteria**: First therapy is systemic and/or radiation therapy and is followed by surgery.

(continued)

Table 2.8 (continued)

Classification	Designation	Details
Recurrence or retreatment	rTNM	This classification is used for assigning stage at time of recurrence or progression until treatment is initiated. **Criteria**: Disease recurrence after disease-free interval or upon disease progression if further treatment is planned for a cancer that Recurs after a disease-free interval Progresses (without a disease-free interval)
		rc Clinical recurrence staging is assigned as rc
		rp Pathological staging information is assigned as rp for the rTNM staging classification. This classification is recorded in addition to and does not replace the original previously assigned clinical (c), pathological (p), and/or post-therapy (yc, yp) stage classifications, and these previously documented classifications are not changed
Autopsy	aTNM	This classification is used for cancers not previously recognized that are found as an incidental finding at autopsy and not suspected before death (i.e., this classification does not apply if an autopsy is performed in a patient with a previously diagnosed cancer) **Criteria**: No cancer suspected prior to death Both clinical and pathological staging information is used to assign a TNM

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Table 2.9 Summary of TNM classification rules based on the *AJCC Cancer Staging Manual, Eighth Edition* (2017) [15]

T stage	N stage	M stage
Determined by *site-specific features* based on *size* and/or local *extension*	Determined by disease-specific rules based on number and location of *positive regional nodes*	Determined by positive biopsy of the metastatic site (pM1)
cT: *Clinical assessment* of T based on physical examination, imaging endoscopy, and biopsy and surgical exploration *without resection*	Minimum number of lymph nodes to be examined for staging defined by site and disease type However, N staging is performed based on pathological evaluation of sampled nodes even if minimum number could not be sampled	cM – clinical M classification is based only on history and examination Imaging of distant organ sites NOT required to assign cM0
pT: Pathological assessment of T based on microscopic evaluation of the resected tumor (or biopsy only if it assigns the highest T stage)	Pathological assessment of the primary tumor (pT) is must to assign pathological assessment of nodes (pN) except with unknown primary (T0)	pM0 – pathological M0 is NOT a valid category and may not be assigned. If a biopsy of suspected metastatic site is negative, it should be staged as cM0
pT generally based on single resection. If resected as >1 specimen, reasonable estimation is required to assess combined size/extension Disease-specific rules may apply	Pathological status of lymph node or sentinel node(s) without pT but with only clinical T (cT) is classified as clinical nodal status (cN)	Case with pathological T and N may be grouped as pathological TNM using clinical M designator (cM0 or cM1) (e.g., pT1 pN0 cM0 = pathological stage I)
Tumor size recorded in *whole millimeters* (smaller fractions are rounded to the nearest whole millimeter: 1 through 4 rounded down, and 5 through 9 rounded up)	Pathological status of a single node or nodes in the highest N category is classified as pN even in the absence of pathological information on other nodes	Case with pathological M1 (pM1) may be grouped as clinical and pathological Stage IV regardless of "c" or "p" status of T and N (e.g., cT1 cN1 pM1 = clinical or pathological stage IV)
Case may be classified by pT or pN without resection if microscopically *confirmed by biopsy*	*Sentinel lymph node* biopsy is denoted with (sn), e.g., pN0(sn), pN1(sn)	ITC in metastatic sites (e.g., bone marrow), circulating tumor cells (CTC), or disseminated tumor cells (DTC) classified as cM0(i+)
	Lymph nodes with only *isolated tumor cells* (ITC) are staged as pN0 (disease-specific rules apply, e.g., melanoma) Standard definition of ITC is cluster of tumor cells smaller than 0.2 mm in greatest dimension. These are usually not detected by HE but by special technique such as IHC	Serous effusion fluids positive for malignant cells is equivalent to distant metastasis
	Direct extension of primary tumor into regional node is classified as node positive and is part of pN	"MX" is eliminated in AJCC (2010) seventh edition
	Tumor nodule with smooth contour in regional node area classified as positive node	
	When size is the criterion for N category, stage *by size of metastasis*, **not size of node** when reported (unless specified in disease-specific in disease-specific rules)	

For more detailed updated rules, refer to *AJCC Cancer Staging Manual, Eighth Edition* (2017) published by Springer International Publishing. Used with permission of the American Joint Committee on Cancer (AJCC), Chicago, Illinois

carcinoma and *intramucosal carcinoma* are equivalent and have been used interchangeably.

Tumor invading an adjacent organ in contiguity (e.g., colonic carcinoma invading liver or even other segment of tubular GIT) is part of T staging and is not distant metastasis [15]. Similarly, sideways horizontal spread of tumor to the adjacent segment of tubular GIT (e.g., cecal carcinoma spreading along the lumen to adjacent ascending colon and/or adjacent terminal ileum) is also part of pT staging and not distant metastasis [15]. On the other hand, penetration of tumor through a lymph node capsule into a regional lymph node is considered nodal metastasis for N staging.

For multiple primary tumors of tubular GIT, T stage is assigned as per the highest category. However, multiplicity of tumor assigns it a specific T stage in the liver [15].

T staging for some special tumors such as GIST and NET have a special approach. It is based on the size of the tumor in GIST [15] and on the extent of invasion with tumor size in NET (Table 2.2) [15].

N Category

N staging is assigned based on status of regional lymph nodes evaluated conventionally by examining HE-stained sections (Table 2.9) [15]. If lymph nodes are grossly positive, only a representative section is submitted for confirmation. However, grossly negative or equivocal lymph nodes are submitted entirely [49]. The number of lymph nodes that could be evaluated from any resection specimen depends on a variety of factors including anatomic nature of specimen, the length of the resected segment, type of surgical procedure, chemo/radiation therapy status prior to resection, and/or technical skill/diligence on the part of the dissector grossing the specimen. The number of lymph nodes sampled from a node-negative colorectal cancer specimen has been suggested to be at least 12 lymph nodes [70, 71]. At least 1 positive or negative lymph node is needed for assigning pathological N (pN) staging.

Discontinuous spread or *tumor deposits* (TD) in subserosa, mesentery, and nonperitonealized pericolic or perirectal tissues, although not nodal metastases, are considered under N category. These should be distinguished from totally replaced lymph nodes (which are counted as lymph nodes) or venous invasion with extravascular spread (considered as V1/V2).

Positivity of nonregional lymph nodes for tumor is considered distant metastasis and is not part of pN staging, but belongs to pM staging [12, 13, 15].

M Category

Metastasis to any distant organ or tissue including any nonregional lymph node is considered for M staging (Table 2.9) [15]. Presence of isolated tumor cells in the bone marrow, peritoneal seeding, and positive serous fluid cytology are also considered metastases [15].

Satellite lesions (skip lesions) present as multiple tumor foci in adjacent bowel along the mucosa or submucosa are not distant metastases [15]. These must be distinguished from synchronous primary tumors.

Additional Features

There are a few additional features (Table 2.8 [15]) that should be communicated in the final surgical pathology report of excised GI cancer specimens (Table 2.10) [10, 12, 13, 15, 49, 55, 56, 60–65, 72]. Although these features are not reported specifically as an individual category, currently they are a routine part of the CAP cancer protocol in the final pathology report (see colon cancer CAP protocol as example in Table 2.11) [10].

Table 2.10 Additional features to be communicated in final surgical pathology report of excision specimens [10]

Feature	Remarks
L category (*lymphatic invasion by tumor*) [12, 15]	Lymphatic invasion is considered adverse prognostic factor in almost all gastrointestinal carcinomas [49, 55, 56, 72]. *L0: Lack of lymphatic invasion* *L1: Positive for lymphatic invasion*
V category (*venous invasion by tumor*) [12, 15]	Invasion by malignant cells into the large vessels within the tumor mass (*intramural venous invasion*) or in the adjacent vessel visible even on gross or on imaging (*extramural venous invasion*) is independent adverse prognostic factor for many GI cancers, especially gastric carcinomas, pancreatic carcinomas, colorectal carcinomas, hepatocellular carcinomas, and gastrointestinal sarcomas [55]. *V0: Lack of venous invasion* *V1: Microscopic venous invasion* *V2: Macroscopic venous invasion* CAP recommendation: Submit *at least 3 tissue blocks (preferably, 5 blocks)* from the deepest portion of the tumor [37]. Some studies recommend routine *elastic stain* for venous invasion detection [73].
PN category (*perineural invasion*)	Perineural invasion has also been regarded stage-independent adverse prognostic factor especially in some GI cancers such as pancreas and colon [72]. However, studies supporting this unequivocally are quantitatively and qualitatively limited.
Morphology of tumor periphery	Pattern of growth along the periphery of the tumor has been reported to be independent prognostic feature [49, 55, 56, 74]. Colonic adenocarcinoma variant such as *medullary carcinoma* with pushing borders usually has a favorable prognosis even though it has higher grade histomorphology [10]. *Tumor budding* associated with poor prognosis in colon adenocarcinoma is defined as isolated single cells or tiny groups of tumor cells (up to four) invading the stroma [60–65].

Although not reported specifically as individual category, currently these features are routine part of CAP cancer protocol in the final pathology report

Table 2.11 Recommended reporting protocol of various resections of cancers[a] using colon/rectal cancer as an example standardized by the College of American Pathologists (CAP) [10]

Colon and rectum ***(resection, including transanal disk excision of rectal neoplasms)***
Specimen:
Procedure:
+ Specimen length
Tumor site
+ Tumor location
Above or below peritoneal reflection
Tumor size
Greatest dimension: ___ cm
+ Additional dimensions: ___ × ___ cm
Macroscopic tumor perforation
+ Macroscopic intactness of mesorectum
Histological type
Histological grade
+ Histological features suggestive of microsatellite instability
+ Intratumoral lymphocytic response (tumor-infiltrating lymphocytes)
+ Peritumor lymphocytic response (Crohn-like response)
+ Tumor subtype and differentiation (select all that apply)
+ Mucinous tumor component (specify percentage)
+ Medullary tumor component
+ High histological grade (poorly differentiated)
Microscopic tumor extension
Margins
Proximal margin
Distal margin
Circumferential (radial) or mesenteric margin
Mucosal margin (noncircumferential transanal disk excision) (required only if applicable)
Other margin(s) (required only if applicable): Specify margin(s)
Treatment effect (applicable to carcinomas treated with neoadjuvant therapy)
Lymph-vascular invasion
Perineural invasion
Tumor deposits (discontinuous extramural extension)
+ Type of polyp in which invasive carcinoma arose
Pathological staging (pTNM)
TNM descriptors (required only if applicable) (select all that apply)
m (multiple primary tumors)
r (recurrent)
y (post-treatment)
Primary tumor (pT)
Regional lymph nodes (pN)
Number of lymph nodes examined
Number of lymph nodes involved
Distant metastasis (pM)
+ Additional pathological findings
+ Ancillary studies (please see *the CAP Colorectal Biomarker Template)*
If any biomarker is under testing and pending, it should be mentioned under the comments.
+ Comment(s)
+ This information is *optional* because it may be clinically important but is not yet validated and may not be of practical application for regular application in patient management.

[a]All templates are available at CAP website site [10] and show detailed options to be selected along with detailed instructions under various notes

Conclusion

Morphological evaluation with ancillary tests such as immunophenotyping and histochemistry is, and will continue to be, the most critical pivotal component in the management of GI cancers. The current advances in molecular pathology have increased its role and have become an integral part of management in addition to conventional AJCC staging [10].

In future, increasing insight into the molecular biology of all GI cancers including overexpression and/or repression of various genes as well as epigenetic changes would establish a better understanding with ongoing advances in achieving improved tumor classification, diagnosis, prognosis, and targeted personalized therapies [17, 33–35]. Generally, both conventional pathological examination and new molecular tests are required for proper evaluation of any GI cancer for diagnostic and therapeutic decisions. Application of any new biomarkers cannot be justified until the findings demonstrate a convincing positive impact on clinical management. The ongoing advances would improve the understanding in molecular biology of various GI cancers and develop treatment algorithms with targeted therapies tailored for individual patient care as personalized medicine evolves [75].

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