Gastrointestinal tissue-based molecular biomarkers: A practical categorization based on the 2019 WHO Classification of Epithelial Digestive Tumours

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Abstract

Molecular biomarkers have become one of the cornerstones of oncological pathology. The method of classification not only directly affects the manner in which patients are diagnosed and treated, but also guide the development of drugs and of artificial intelligence tools. This work aims to organize and update gastrointestinal molecular biomarkers in order to produce an easy-to-use guide for routine diagnostics. For this purpose, we have extracted and re-organized the molecular information of epithelial neoplasms included in the new "WHO Classification of Tumours of the Digestive System" book (5th Edition 2019).

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Introduction

In the era of molecular medicine, with the expansion of digital pathology and the revolution of artificial intelligence (AI), molecular biomarker classifications of cancer are more important than ever before.[1] A rational cancer taxonomy is necessary to standardize diagnoses, make decisions on biomarker/drug development and generate an appropriate background for AI tools.[2] Molecular biomarkers have a prominent role in oncological pathology, with diagnostic, predictive, and/or prognostic value. A single and

unified classification for biomarkers is important to collect relevant information and keep it updated. This represents a challenge for modern (morpho-molecular) pathologists.[3]

The value of biomarkers in routine tissue diagnostics

A "biomarker" is defined as "any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease".[4] Every year, there are between 15,000 to 20,000 new scientific articles on cancer biomarkers.[5] Unfortunately, from every 100 such biomarkers, less than 1% make it into a form that is useful for patient diagnosis or stratification [6], mostly due to a variety of scientific and technical reasons.[7]

As a result, there is no clear-cut evidence in the literature as to which biomarkers are essential for diagnostics and/or therapeutic decision-making. However, the World Health Organization (WHO) Classification of Tumours consensus of international experts represents the best indication of how relevant these biomarkers are in routine diagnostic practice. Our goal is to summarize the use of these biomarkers in the gastrointestinal system (from oesophagus to anal canal), and obtain indications of the specific weight, form and relevance of biomarker analysis in disease taxonomy and clinical decision-making.

Current biomarker classification and proposed subcategorization.

A biomarker is a characteristic that is objectively measured and evaluated as an indicator of normal/pathogenic processes or pharmacologic therapeutic responses.[8] The 5th Edition WHO Tumour Classification of Digestive System Tumours includes diagnostic, predictive, and prognostic molecular biomarkers as the major categories.[9] Diagnostic biomarkers are intended to help pathologists establish a specific diagnosis; predictive markers indicate the probability of benefiting from a specific therapy; and prognostic ones determine the outcome of patients, in the absence of specific treatments.[8]

The decision as to which group a biomarker belongs to represents the first step of assessment. Some biomarkers may fullfill the criteria for more than one category as well. Additionally, it is possible to subdivide categories which lead to, improving their

organization and comprehension. In this manner, for diagnostic biomarkers, the second step is to determine if it is useful in *differential diagnos*is or if it contributes to *cancer classification*. For predictive biomarkers, the following question should be asked: Are there definitive randomised clinical trials or cohort studies that support their efficacy? If the answer is yes, then these would correspond to *established predictive biomarkers*. If the answer is negative, but they are currently under investigation, one may classify them as a "*potentially predictive biomarkers".* If they are not yet associated with any clinical trial, we propose to label them by the term "*pre/clinical predictive biomarkers"*. In this circumstance, it is unlikely that they would be designated within one of the first level groups within the WHO Classification of Tumours, although they often have relevance to the understanding of tumour pathogenesis and may be included under this topic. For prognostic biomarkers, the main question is whether they are prognostic *specific* markers for a certain entity, or whether they are used to create risk *stratification* groups. Biomarkers that do not fit into any of these categories should be classified as "Others". This classification is summarized in figure 1.

However, in order to understand, adequately categorize, and subcategorize these biomarkers, it is necessary to methodically evaluate other attributes associated with them.

Variables to consider in the categorization of biomarkers:

1. ***Context: System, organ and entity.***

   Context is a relevant aspect to consider in any biomarker assessment. A specific biomarker can have different attributes depending on the location (system/organ) and the disease (entity) in question. For example, the presence of *EGFR* (*epidermal growth factor receptor*) activating mutations in oesophageal squamous cell carcinoma is an adverse prognostic factor, and EGFR targeted therapies have failed to improve survival.[10-12] The same molecular alterations in non-small cell lung carcinoma (NSCLC) confer a better prognosis and also provide the patient with an opportunity to receive tyrosine kinase inhibitors (TKIs) therapy with a significant chance of improved survival.[13] Activating mutations of *BRAF* (*v-raf murine sarcoma viral oncogene homolog B1*), in colorectal carcinoma (CRC) may confer resistance to anti-EGFR

therapy and helps establish a worse prognosis[14]; while the same alterations (*BRAF V600E* activating mutation) in melanoma predict response to treatment with BRAF inhibitors, such as Vemurafenib.[15] Sometimes, the existence of certain molecular alterations are known, but there are no clinical trials available that support their routine use. On other occasions, support exists for a specific organ, but not for others. An example of this is *ERBB2* (*HER2-human epidermal growth factor receptor 2*) mutation in small intestine adenocarcinoma, which can be detected. In theory patient with cancer harbouring the mutation adds benefit from anti- *ERBB2* therapy, but it does not yet have an established predictive value.[16] On the other hand, alterations in the same biomarker in oesophagus/oesophagogastric junction adenocarcinoma are predictive of response to this targeted therapy.[17]

2. Status: Specific molecular alteration.

Evaluation of the status of a biomarker implies specification of the molecular alterations that gives it clinical utility. Determination of the specific alteration (i.e. activating mutation, translocation, overexpression, etc) conceptually corresponds to the exact molecular phenomenon involved. In this manner, the lack of *KRAS/NRAS* activating mutations in CRC predicts a favourable response to anti-EGFR therapy.[14] In contrast, the presence (not the lack) of activating *KIT* mutations in other malignant neoplasms, such as melanoma or gastrointestinal stromal tumours (GISTs), predict response to imatinib therapy.[18]

3. Level of detection.

The level at which the alteration is detected is also crucial in the evaluation of the biomarker status. The clinical utility of the biomarker can be detected at genetic, transcriptomic and/or at the protein level; specific mutations of *KRAS*, *NRAS* and *BRAF* in CRC are good examples of genetic level detection.[19] Some alterations detected at the transcriptomic level are oncotype Dx in breast cancer[20] and consensus molecular subtypes (CMS) in CRC.[21] At the protein level, examples are c-MET (mesenchymal-epithelial transition factor) in CRC or ALK (anaplastic lymphoma kinase) fusion in NSCLC, both could be detected by immunohistochemistry[22-23]. To add more complexity, a biomarker can be detected at different levels with different

clinical significance, independent of the system/organ where it occurs. An example of this is *ERBB2* in NSCLC in which mutation is not associated with *ERBB2* amplification or overexpression, suggesting a distinct entity and a potential different therapeutic target.[24] Conversely, evaluation of *ERBB2* in gastric/gastroesophageal junction adenocarcinomas and breast carcinomas shows that gene amplification and protein overexpression are both useful in prediction of target therapies.[25-26]

Gastrointestinal system biomarkers update

1. General

A total of 54 different biomarkers are mentioned 98 times across the gastrointestinal tract chapters of the WHO blue book. Figure 2 summarises them, showing if the technology used corresponds to immunohistochemical (IHC) tests, transcriptomic tools or DNA-based mutational assays. Microsatellite instability (MSI) is described 11 times, one for diagnostic, four for prognostic and six for therapeutic purposes. p53/TP53 is used ten times, three times as an IHC test, and seven as a DNA-based mutational analysis tool. *KRAS* study is indicated on six occasions, all of which correspond to DNA mutational analysis with predictive utility, with one use as a potential prognostic biomarker. On the contrary, overexpression of ERBB2 is indicated five times, all using IHC, but considering that some cases will require confirmation by fluorescence in situ hybridisation (FISH). The organ with the most biomarkers mentioned was the small intestine/ampulla, with 31 different markers mentioned, the vast majority being prognostic specific markers with potential for future use. Finally, the large intestine has more established biomarkers for current routine use than any other anatomic site, with 15 markers of diagnostic, established predictive and prognostic use.

The biomarkers discussed below are summarized by category, subcategory and organ in tables 1 and 2.

2. Oesophagus

The routinely used molecular biomarkers in oesophageal lesions include the presence of aberrant immunohistochemical expression of p53, which may be associated with a better diagnostic reproducibility of dysplasia (*differential diagnosis biomarker*) and an increased risk of neoplastic progression (*prognostic risk stratification biomarker*), in the context of Barrett's oesophagus.[27-28] In addition, ERBB2 overexpression and/or *ERBB2 gene* amplification in lower oesophagus/oesophagogastric junction adenocarcinoma carries a predictive value for response to ERBB2-targeted therapy (*established predictive biomarker*).[17]

There are other markers not yet used in routine pathological analysis, but they may be important in the near future. EGFR protein overexpression in oesophageal squamous cell carcinoma is considered an adverse *prognostic specific* factor[10], because targeted therapies have not been successful in improving survival.[11-12] The loss of MMR proteins expression (with the consequent MSI) and overexpression of PD-L1 in oesophagus/oesophagogastric junction adenocarcinoma, are linked to the potential use of immune checkpoints inhibitors. These checkpoint inhibitors are under clinical trial evaluation for immunotherapy [29], as is CTLA4 overexpression (*potentially predictive biomarkers*).[29-30]

Other possible biomarkers are methylation of the *CDKN2A* (*p16* promoter which inhibits its gene expression) and genomic instability (specifically copy number alterations). Both biomarkers have potential value as *prognostic risk stratification biomarkers* in Barrett dysplasia[28], but this is not yet supported by strong retrospective or prospective studies.

3. Stomach

The molecular biomarkers with current use in gastric tumours include ERBB2 overexpression and/or *ERBB2* gene amplification in gastric adenocarcinoma and gastric undifferentiated carcinoma, with *established predictive* value for response to ERBB2-targeted therapy[17,31,32] and the presence of *MALAT1-GLI1* fusion gene for diagnostic confirmation of gastroblastoma, a rare gastric biphasic tumour recently described (*differential diagnosis biomarker*).[33] *TP53* and *RB1 (retinoblastoma gene 1)*

mutations also act as d*ifferential diagnosis biomarkers,* helping to distinguishing gastric neuroendocrine carcinomas (NECs) from G3 neuroendocrine tumours (NETs), in which these genes are more frequently wildtype; this is also applicable for the remainder of the digestive organs.[34]

There is a plethora of markers used in gastric cancer biology, specifically with regard to *prognostic specific* markers, with little direct routine application. These include EGFR and c-MET overexpression[31], MSI[35], EBV(Epstein Barr virus) detection[36], high expression levels of EGF(epidermal growth factor) /TGF-α (transforming growth factor alpha), VEGF-A (vascular endothelial growth factor-A) and CD44, reduced expression of E-cadherin, expression of matrix metalloproteinases (MMP1, MMP7 and MMP10), upregulation of SPC18 (*SEC11A*), and Protocadherin B9 (*PCDHB9*) overexpression.[37-39] MSI and PD-L1 expression are *potential predictive molecular biomarkers* under investigation in clinical trials .[35,40,41]

Finally, for gastric dysplasia, there are biomarkers of *disease progression* that are seldom used routinely today. These are DNA content abnormalities (aneuploidy or elevated 4N fraction)[42]; aberrant p53/*TP53*; mutations of *RNF43, APC (adenomatous polyposis coli), ARID (*AT-rich interactive domain)*1A* and *ARID2* [43,44]; and inactivation by promoter methylation of *p16* and *MLH1 (with consequent MSI)* [45,46].

4. *Small intestine and ampulla*

In ampullary and non-ampullary adenocarcinomas, only MSI is a regularly used molecular biomarker. Its indication includes immunotherapy selection (*established predictive biomarker)*, determination of a possible hereditary origin (*differential diagnosis biomarker)* and its use as a *specific prognostic parameter* (early results show that MSI may improve overall survival).[47,48] Markers with potential *specific prognostic* value, but without regular pathological use, include *KRAS* activating mutations in ampullary adenocarcinoma[19]; Chromosome 18 deletion, chromosome 14 gain, whole arm copy number variations, and *CDKN1B* mutations in small intestinal and ampullary neuroendocrine neoplasms.[49-51]

Other biomarkers with potential *prognostic specific or predictive pre-clinical* value in non-ampullary adenocarcinoma are *TP53, IDH (isocitrate dehydrogenase*), *CDH1 (cadherin-1*), *FGFR2 (fibroblast growth factor receptor 2), FLT3* (fms-like tyrosine kinase 3), *NPM1* (*nucleophosmin*) , *PTEN* (*phosphatase and tensin homolog*), *c-MET, AKT1, RET (rearranged during transfection), NOTCH1 (neurogenic locus Notch homolog protein 1), ERBB4 (receptor tyrosine kinase 4/HER-4), CHN2 (beta-chimaerin), KRAS, SMAD4* (*mothers against decapentaplegic homolog 4*), *ERBB2* and *CTNNB1/E-Cadherin*.[16,52-55]

5. *Appendix*

In appendiceal adenocarcinoma, multiple studies have been conducted , but there is insufficient evidence to make firm recommendations in *potentially predictive and pre-clinical biomarkers* (*KRAS*, MSI, *GNAS* [Guanine nucleotide-binding protein G]).[56-58] As in the rest of the digestive system, the presence of *TP53* and *RB1* mutations can help distinguish appendiceal neuroendocrine carcinomas (NECs) from G3 neuroendocrine tumours (NETs), in which these genes are more frequently wildtype.[34]

6. *Colon and rectum*

The molecular markers routinely used in CRC comprise the lack of activating mutations of *KRAS/NRAS* and *BRAF* (extended *RAS* testing), both with *established predictive* value for effective response to anti-EGFR therapy.[14,59] *BRAF* activating mutations have *differential diagnostic* utility in the exclusion of Lynch syndrome and they are associated with an adverse *specific prognosis*.[60,61] MSI is an *established predictive* marker in colorectal adenocarcinoma associated with a significant response to PD-L1 inhibitors in patients who failed conventional therapy, confers a good prognosis to *BRAF* wildtype patients (*specific prognostic marker*) and is useful in Lynch syndrome diagnosis. [62]

In addition, two different methods are being used for colorectal adenocarcinoma molecular *diagnostic classification*: genomic-scale analysis (hypermutated or non-hypermutated colorectal cancers)[63] and transcriptomic profiling (consensus molecular subtypes for colorectal cancer).[21] Both have potential for sub-type based targeted therapies.

There are other markers in CRC that are not yet used currently, but show some promising results. c-MET overexpression has *potential predictive* value for response to c-MET inhibitors.[23] *PIK3CA* (*Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha*) activating mutations are associated with a worse *specific prognosis*, negative predicted response to anti-EGFR therapy, and a successful adjuvant response to acetylsalicylic acid.[64,65(p3)] Immune related markers like Immunoscore, represent a potential *prognostic stratification* tool [66,67] and gene expression signatures have a more restricted *prognostic* use, specifically for determining the risk of recurrence after surgery. [68,69]

7. *Anal canal*

The molecular biomarkers with clinical utility are p16 expression and polymerase chain reaction (PCR) determination of high-risk human papilloma virus (HPV) genotypes (usually 16) in anal squamous dysplasia. The *risk of progression* from a

high-risk lesion to squamous cell carcinoma (SCC) is influenced by HPV genotype, immune status and other factors. [70-72]

Other markers with *potential and/or pre-clinical predictive* utility include PD-L1 expression in SCC[73,74]; *KRAS* and *NRAS* lack of activating mutations and MSI in anal adenocarcinoma.[75]

Conclusion

Despite the significant knowledge on the molecular basis of cancers of the digestive tract, there are relatively few biomarkers with established clinical utility, and most target common tumour types. Our review follows the new WHO approach to molecular markers that is easily identifiable and also readily revisited when new information becomes available in the future. This systematic approach to the characterisation of new molecular markers may be used for the future taxonomy of cancers, which are also likely to benefit from computational pathology, especially within the next 5-year cycle of the WHO Classification of Tumours.

Individual contributions

Quezada-Marín J - conceptualization of biomarker classification, biomarker content preparation, co-lead manuscript preparation.

Lam AK - biomarker content preparation, manuscript preparation.

Ochiai A - biomarker content preparation, manuscript preparation.

Odze R - biomarker content preparation, manuscript preparation.

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Goldblum JR - biomarker content preparation, manuscript preparation.

Cree IA - biomarker content preparation, co-lead manuscript preparation.

Salto-Tellez M - conceptualization of the paper; conceptualization of the biomarker classification, biomarker content preparation, lead manuscript preparation and final editing.

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Table 1. Pre-invasive molecular biomarkers by category, subcategory and evaluated organ of the gastrointestinal tract.

Category	Subcategory	Oesophagus	Stomach	Small intestine/ampulla	Appendix	Colorectal	Anal canal
Diagnostic	Differential Diagnosis	p53 (BD)	..	..	..	..	..
	Cancer classification	..	..	..	..	..	..
Predictive	Established	..	..	..	..	..	..
	Potential	..	..	..	..	..	..
	Pre-clinical	..	..	..	..	..	..
Prognostic	Specific	..	..	..	..	..	..
	Risk Stratification	p53 (BD)	DNA CAb (GD)	..	..	..	p16 (ASD)
		CDKN2A (BD)	p53/*TP53* (GD)	..	..	..	HPV (ASD)
		Gen Inst (BD)	*RNF43* (GD)	..	..	..	..
		..	p16 (GD)	..	..	..	..
		..	*APC* (GD)	..	..	..	..
		..	*ARID1A* (GD)	..	..	..	..
		..	*ARID2* (GD)	..	..	..	..
		..	MSI (GD)	..	..	..	..

Table 2. Invasive molecular biomarkers by category, subcategory and evaluated organ of the gastrointestinal tract.

Category	Subcategory	Oesophagus	Stomach	Small intestine/ampulla	Appendix	Colorectal	Anal canal
Diagnostic	Differential Diagnosis	··	*MALAT1-GLI1 (GB)*	MSI (AAdC-NAAdC)	*TP53 (NEN)*	*BRAF(AdC)*	*TP53 (NEN)*
		··	*TP53 (NEN)*	*TP53 (NEN)*	*RB1 (NEN)*	*TP53 (NEN)*	*RB1 (NEN)*
		··	*RB1 (NEN)*	*RB1 (NEN)*	··	*RB1 (NEN)*	··
		··	··	··	··	MSI (CRC)	··
	Cancer classification	··	··	··	··	CMS (AdC)	··
		··	··	··	··	GC (AdC)	
Predictive	Established	ERBB2 (AdC)	ERBB2 (AdC-UC)	MSI (AAdC-NAAdC)	··	*KRAS (AdC)*	··
		··	··	··	··	*NRAS (AdC)*	··
		··	··	··	··	*BRAF(AdC)*	··
		··	··	··	··	MSI (AdC)	··
		··	··	··	··	*PIK3CA (AdC)*	··
	Potential	MSI (AdC)	MSI (AdC)	··	··	*c-MET (AdC)*	PD-L1 (SCC)
		PDL1 (AdC)	PDL1 (AdC)	··	··	··	··
		CTLA4 (AdC)		··	··	··	··
	Pre-clinical	EGFR (SCC)	··	*BRAF (NAAdC)*	*KRAS (ApAC)*	··	*KRAS (AdC)*
		··	··	*KRAS (NAAdC)*	MSI (ApAC)	··	*NRAS (AdC)*
		··	··	*IDH1 (NAAdC)*	*GNAS (ApAC)*	··	MSI (AdC)
		··	··	*ERBB2 (NAAdC)*	··	··	··
Prognostic	Specific	··	EGFR (AdC)	MSI (AAdC-NAAdC)	··	*BRAF(AdC)*	··
		··	c-MET(AdC)	*Chr 18 del (NEN)*	··	MSI (AdC)	··
		··	ERBB2 (AdC)	*Chr 14 gain (NEN)*	··	*PIK3CA (AdC)*	··
		··	MSI (AdC)	*WACNV (NEN)*	··	··	··
		··	EBV (AdC)	*CDKN1B (NEN)*	··	··	··
		··	EGF/TGF-α (AdC)	*KRAS (AAdC)*	··	··	··
		··	VEGF-A (AdC)	*TP53 (NAAdC)*	··	··	··
		··	CD44 (AdC)	*KRAS (NAAdC)*	··	··	··
		··	E-cadherin (AdC)	*CHN2 (NAAdC)*	··	··	··
		··	MMP1 (AdC)	*SMAD4 (NAAdC)*	··	··	··
		··	MMP7 (AdC)	*KIT (NAAdC)*	··	··	··
		··	MMP10 (AdC)	*HER4 (NAAdC)*	··	··	··
		··	SPC18 (AdC)	*CDH1 (NAAdC)*	··	··	··
		··	PCDH B9 (AdC)	*FGFR2 (NAAdC)*	··	··	··
		··	··	*FLT3 (NAAdC)*	··	··	··
		··	··	*NPM1 (NAAdC)*	··	··	··
		··	··	*PTEN (NAAdC)*	··	··	··
		··	··	*c-MET (NAAdC)*	··	··	··
		··	··	*AKT1 (NAAdC)*	··	··	··
		··	··	*RET (NAAdC)*	··	··	··
		··	··	*NOTCH1 (NAAdC)*	··	··	··
		··	··	*CTNNB1/E Cadh (NAAdC)*	··	··	··
		··	··	*ERBB2 (NAAdC)*	··	··	··
		··	··	··	··	IS (AdC)	··

	Risk Stratification	..	..	..	..	GES (AdC)	..

Legends (tables and figures):

Figure 1. Proposed categorization of molecular biomarkers.
The subdivision in specific categories and subcategories improves the organization, facilitate their comprehension and allows an adequate update of molecular biomarkers.

Figure 2. Gastrointestinal molecular biomarkers frequency by detection technology.
Each square represents an individual count of a molecular biomarker in gastrointestinal system. The technologies of detection are: IHC test (Green); Transcriptomic tool (Blue); DNA-based mutational assays (Red).

* In most cases MSI is studied by DNA-based mutational assays. However, in some organs like large intestine IHC is useful as a diagnostic biomarker of Lynch Syndrome while DNA-based mutational assays are used for predictive and prognostic analysis (Red/green square).

BD, Barret dysplasia; p53, Tumour suppressor protein p53; CDKN2A, Cyclin-dependent kinase Inhibitor 2A gene; Gen Inst, Genomic Instability; AdC, Adenocarcinoma; ERBB2, Human epidermal growth factor receptor 2; MSI, Microsatellite instability; PD-L1, Programmed Death-ligand 1; CTLA4, Cytotoxic T-Lymphocyte Antigen 4; SCC, Squamous cell carcinoma; EGFR, Epidermal growth factor receptor; DNA CAb, deoxyribonucleic acid content abnormalities; GD, Gastric dysplasia; RNF43, Ring Finger Protein 43 gene; APC, Adenomatous polyposis coli gene; ARID1A, AT-rich interactive domain-containing protein 1A gene; ARID2, AT-rich interactive domain-containing protein 2 gene; MLH1, MutL homolog 1 gene; p16, Protein p16; GB, Gastroblastoma; MALAT1-GLI1, MALAT1-GLI1 fusion gene; UC, Undifferentiated carcinoma; TP53, Tumour suppressor protein 53 gene; RB1, Retinoblastoma 1 gene; c-MET, tyrosine-protein kinase Met receptor; EBV, Epstein-Barr virus, EGF/TGF-α, epidermal growth factor/ transforming growth factor alpha; VEGF-A, Vascular endothelial growth factor A; CD44, CD44 antigen; MMP1, Matrix metalloproteinase-1; MMP7, Matrix metalloproteinase-7; MMP10, Matrix metalloproteinase-10; SPC18, Septal Pore Cap Protein 18 gene; PCDH B9, Protocadherin B9; KRAS, Kirsten Rat Sarcoma Viral

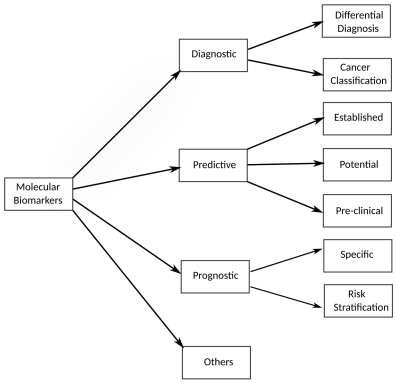
Oncogene Homolog; IDH1, Isocitrate Dehydrogenase 1 gene; Chr 18 del, Chromosome 18 deletion; Chr 14 gain, Chromosome 14 gain; WACNV, Whole arm copy number variation; CDK1B, Cyclin Dependent Kinase Inhibitor 1B gene; CHN2, Chimerin 2 gene; SMAD4, SMAD family member 4 gene; KIT, KIT proto-oncogene, receptor tyrosine kinase; HER4, Human epidermal growth factor receptor 4 gene; CDH1, Cadherin 1 gene; FGFR2, fibroblast growth factor receptor 2 gene; FLT3, fms-like tyrosine kinase 3 gene; NPM1, Nucleophosmin 1 gene; PTEN, phosphatase and tensin homologue gene; MET, tyrosine-protein kinase Met gene; AKT1, AKT serine-threonine kinase 1 gene; RET, ret proto-oncogene; NOTCH1, Notch homolog 1 translocation-associated gene; CTNNB1, catenin beta 1 gene; NEN, Neuroendocrine neoplasms; AAdC, Ampullary adenocarcinoma; NAAdC, Non-ampullary adenocarcinoma; ApAC, appendiceal adenocarcinoma; GNAS, Guanine nucleotide-binding protein G gene; BRAF, Serine/threonine-protein kinase B-raf gene; CMS, consensus molecular groups; GC, genomic classification; NRAS, NRAS proto-oncogene GTPase; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha gene; IS, Immunoscore; GES, gene expression signatures; ASD, Anal squamous dysplasia; HPV, human papilloma virus.

Table 1:

Abbreviations are the same used in figure 2.

Table 2:

Abbreviations are the same used in figure 2.



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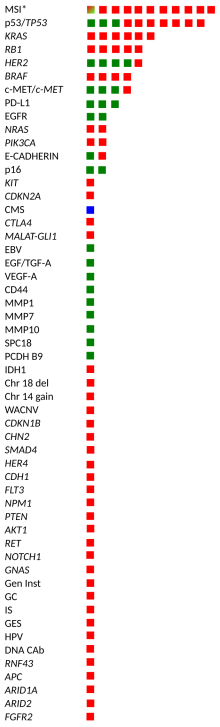

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