A consolidated working classification of gastric cancer for histopathologists (Review)

SIMONA COSTACHE[1,2*], MARIA SAJIN[1,3], SARAH WEDDEN[4] and CORRADO D'ARRIGO[2*]

[1]University of Medicine and Pharmacy 'Carol Davila', 020021 Bucharest, Romania; [2]Poundbury Cancer Institute, Dorchester DT13BJ, UK; [3]University Emergency Hospital Bucharest, 050098 Bucharest, Romania; [4]Cancer Diagnostic Quality Assurance Services (CADQAS), Dorchester DT13BJ, UK

Abstract. Gastric cancer (GC) remains a disease with poor prognosis despite increasing availability of more effective targeted treatment. This may be in part due to the difficulty in selecting patients for appropriate treatment. Conventional taxonomic classifications of GC are ill-suited to make full use of recent advances in personalised therapy. In the past decade a number of molecular classifications have been proposed to address this; however, to date, there has been little implementation in the diagnostic routine. The lack of harmonisation between these classifications, the complexity and unavailability of some of the tests required plus the demands on time and resources, all contribute to poor uptake in the diagnostic routine. In the present study, these classifications were reviewed and an inclusive working classification that includes their main points, focuses on prognosis and treatment options and can be delivered using four on-slide tests (*in situ* hybridization for Epstein-Barr encoding region and immunohistochemistry for mismatch repair, E-cadherin and p53) is proposed. These tests can be performed on paraffin-embedded tissue and could be available in the majority of histopathology laboratories. The proposed classification also includes reflex testing for specific biomarkers relevant to treatment selection.

Contents

Correspondence to: Dr Simona Costache, Poundbury Cancer Institute, 3 Queen Mother Square, Dorset, Dorchester DT13BJ, UK
E-mail: simona.costache.anapat@gmail.com

*Contributed equally

1. Introduction

Gastric cancer (GC) is an aggressive disease, numerous patients are diagnosed at advanced stages and some are inoperable (1,2). It consists of several subtypes; their relative incidence is influenced by genetic and environmental factors, thus, prevalence of each subtype may vary significantly in different populations (3). Conventional treatment is of limited success (4-8). Recent advances in personalised treatment improve outcomes but, for this to be effective, distinct subtypes need to be recognised. Different morphological and molecular subtypes have been highlighted by numerous classifications, but no unifying classification is currently in use. In the present study, the main classifications were reviewed in order to provide a workable scheme that includes the important elements of each, so they can be delivered with on-slide tests in the diagnostic routine and prognostic and predictive data can be provided. It is important that a working molecular classification is structured to include present companion diagnostic biomarkers necessary for selection for biological therapies. Since new biological therapies will inevitably emerge, this classification needs to be able to accommodate new companion diagnostics. Finally, for a working classification to have a significaticant impact on patients outcomes worldwide, it needs to be implementable in histopathology departments using available resources.

2. The Laurén Classification

The Finnish pathologist Pekka Laurén first recognised GC as a heterogenous group of diseases and, in 1965, published his reductive histological classification consisting of only three subtypes that associated with the biology of the disease (9).

He described two main types: intestinal and diffuse, both with no direct counterpart in previous morphologic classifications. As a general principle, the intestinal type had recognisable morphological counterparts in colorectal cancer whilst the diffuse type did not. The intestinal type is characterised by cells resembling those found in the small intestine. It tends to be slower growing and less aggressive than the diffuse type, which is characterised by cells that infiltrate more widely and are less organised. Laurén also recognised a minor group (~15% of cases) that did not sit within these two types and later termed 'atypical' or 'indeterminate' (9). In 1995, Carneiro and colleagues divided Laurén's indeterminate category into mixed tumours (having at least 5% of both intestinal and diffuse components) and solid tumours (10).

There was little understanding of the molecular and biological significance of loss of cell-to-cell adhesion in 1965. More recently, this has been linked with epithelial-to-mesenchymal transition (EMT). Nevertheless, Laurén recognised that tumours with loss of cell-to-cell adhesion represented a separate entity. Authors' opinion suggest that loss of cell-to-cell adhesion (11,12) is the linchpin of the Laurén classification.

3. The World Health Organisation (WHO) classification

This classification recognises molecular subgroups (see below, intrinsic classifications) but even in its latest (2019) edition, remains fixed on taxonomy and has dozens of different morphological types. It describes five main subtypes of adenocarcinoma: i) tubular (the most common), ii) papillary, iii) poorly cohesive (this includes signet ring cell and other subtypes), iv) mucinous and v) mixed (13). Adenocarcinoma represents 95% of all malignant epithelial tumours of the stomach (14). The World Health Organisation (WHO) classification does not provide sufficient details to drive personalised treatment and, notably, the Laurén classification continues being described, even in the current WHO publication, due to its direct relevance for prognosis and treatment.

4. Intrinsic classifications

The Laurén and WHO classifications are focused on the morphology of the tumour cell compartment. ~20 years ago, the intrinsic properties of the tissue (rather than of the tumour cells alone) were investigated with a view to provide important clues for tumour classification, tumour behaviour and response to treatment. Such classifications represented a significant step forward in improving outcomes for patients with GC. Since then, the intrinsic properties of tumours have been scrutinised by genomics, transcriptomics and proteomics of the whole tumour tissue and intrinsic classifications have been proposed for numerous tumour types, including ovarian, colon, breast, endometrium and lung (15-19).

The two major intrinsic molecular classifications for GC are those of The Cancer Genome Atlas and the Asian Cancer Research Group. Both are large scale, multi-institutional studies that characterise the changes that occur in GC using multi-omics techniques. Both aim at understanding the biology of the disease to identify new therapeutic targets (20-22). The Cancer Genome Atlas (TCGA) and the ACRG classifications are briefly described below.

TCGA intrinsic classification. In 2014, TCGA proposed a classification of gastric carcinoma based on the genetic and molecular characteristics of the tumour (20). It was developed by TCGA research network as part of a large-scale effort to understand the underlying basis of gastric carcinoma. Tissues from 295 cases of primary gastric carcinoma with no prior chemotherapy or radiotherapy were studied using six different molecular platforms, including single nucleotide polymorphism array, somatic copy-number analysis, whole-exome sequencing, mRNA sequencing, miRNA sequencing, array-based DNA methylation profiling and reverse-phase protein arrays. A subset (77%) was also tested by whole genome sequencing (Next-generation sequencing).

This work resulted in the grouping of GC into four major types: i) GC associated with Epstein-Barr virus (EBV), ii) GC with mismatch repair deficiency (dMMR), iii) genomically stable (GS) GC and iv) GC with chromosome instability (CIN).

GC-EBV(+). GC-EBV(+) (9%) is more common in younger individuals, predominately male patients and has an improved prognosis compared with other subtypes. These tumours are mainly in fundus or body (62%), have hypermethylation of CDKN2A (p16^{INK4a}) promoter, have the highest rate of phosphoinosytol-3 kinase (PIK3CA) mutations (80%) and overexpress programmed death-ligand (PD-L)1/2 (15%) due to amplification at 9p24.1, a locus containing genes encoding for JAK2, PD-L1 and PD-L2.

GC-dMMR. GC-dMMR (22%) is typically more aggressive than other subtypes and affects older patients (median age, 72 years). It is characterised by hypermethylation of MLH1 promoter. Mutations in PIK3CA are also common in this subtype (42%).

GC-GS. GC-GS (20%) is characterised by low levels of genetic instability and tends to be slower-growing. It is predominantly of diffuse histology (73%) and is associated with CDH1 (E-cadherin) mutations (37%), RHOA mutations (30%) as well as CLDN18-ARHGAP (Claudin-18) rearrangements (30%). The latter two mutations are mutually exclusive; they affect key molecules in cell-to-cell adhesion and are probably responsible for the diffuse growth pattern.

GC-CIN. GC-CIN (50%) subtype is characterised by amplification of receptor tyrosine kinases, has a high percentage of TP53 mutations (73% of CIN tumours) and corresponds to the intestinal type of Laurén. Numerous genes are affected in this subtype, including VEGFA (7%), ERBB2 (24%), ERBB3 (8%), ERBB1 (10%), FGFR2 (8%) as well as c-Met (8%).

ACRG intrinsic classification. There are significant differences in GC arising in the Asian population, possibly related to a combination of genetic and environmental factors. In 2014, ACRG published a different molecular classification for GC. Initially, they performed whole genome sequencing on tissue from 49 gastric tumours (22), and later added gene expression profiling, genome-wide copy number, microarrays and targeted gene sequencing from a further 251 cases (21).

ACRG divide GC into MMR-proficient (pMMR) and MMR-deficient (dMMR) types. The pMMR GC is further divided into three subtypes. Their proposed four molecular

subtypes have some overlap with TCGA groups and are GC-dMMR, GC with EMT (GC-EMT), CG with intact p53 (GC-p53wt) and GC with functional loss of p53 due to mutation (GC-p53m).

GC-dMMR. The GC-dMMR (23%) group contains tumours that are mainly located in the antrum, are usually diagnosed at early stages (clinical stage I/II), have the lowest frequency of recurrence and, when recurrence occurs, this is usually in liver. These tumours are predominantly intestinal type (60%) and have the best prognosis. In this group, there are GC with mutations in KRAS (23%), PI3K-PTEN-mTOR (42%), ALK (16%) and ARID1A (44.2%). This subtype has an overlap with the GC-dMMR of TCGA classification.

GC-EMT. The GC-EMT (15%) subtype is characterised by loss of CDH1 (E-cadherin) and is predominantly observed in young patients. These tumours correspond to the diffuse type of Laurén, they are diagnosed at late stages (80% are clinical stages III/IV) and therefore have worse prognosis with high recurrence rates (mainly in peritoneal cavity). This subtype has the lowest number of mutational events and has overlaps with the GC-GS of TCGA classification.

GC-p53wt. The GC-p53wt (26%) group is found more frequently in male patients, is mostly of the Laurén's intestinal type and has intermediate prognosis. Numerous of these patients are diagnosed in early stages (clinical I/II). This subtype has a higher mutation rate in APC, ARID1A, KRAS, PIK3CA and SMAD4. In addition, this group contains the highest proportion of integrated EBV and therefore may overlap with the GC-EBV(+) of TCGA classification.

GC-p53m. The GC-p53m (36%) subtype is identified more frequently in male patients and is of Laurén's intestinal type. It is diagnosed at advanced stages and has intermediate prognosis. This group is characterised by high prevalence (60%) of TP53 mutation, is associated with amplification in ERBB2, ERBB1, CCNE1 and CCND1 genes and may overlap with the GC-CIN of TCGA classification.

It is recognised that while there is some overlap with TCGA, a major difference is that the ACRG lacks a category that relies solely on EBV status, whilst TCGA does not have a category reliant solely on p53 status.

5. Singapore-Duke classification

Numerous other classifications of GC have been proposed. Some focus on clinical or surgical parameters, which have less relevance to the pathologist, whilst others target the cellular biology of GC using *in vitro* models. It is worth describing one such classification, the Singapore-Duke classification, which highlights the current gap in knowledge of the intrinsic classifications. This is focused on molecular *in vitro* studies and is based on different biological properties and response to chemotherapy and targeted therapy (23). It identifies three GC subtypes

i) Mesenchymal subtype. The mesenchymal GC has highly activated EMT molecular pathways, low rates of TP53 mutation and low level of CDH1 (E-cadherin) expression. This subtype has cancer stem cell-like properties, corresponds to the Laurén's diffuse type and is more sensitive to PIK3CA and mTOR inhibitors.

ii) Proliferative subtype. The proliferative subtype has high rates of TP53 mutation, more extensive gene amplification, high levels of genomic instability and DNA hypomethylation and corresponds to Laurén's intestinal type.

iii) Metabolic subtype. The metabolic type has low rates of TP53 mutation, expresses genes characteristic of normal gastric mucosa and has no intuitive counterpart in the Laurén's classification. These tumours respond well to 5-fluorouracil associated with surgery (23).

6. The role of on-slide tests: Molecular classifications using surrogate on-slide tests

The precise molecular landscape continues to be in evolution and while the most effective and reliable method for characterising the various subtypes remains the subject of debate, it is now desirable to devise a working molecular classification for GC that can be adopted widely using the current diagnostic histopathology framework. It should be noted that any GC classification is just one of a number of factors that the multidisciplinary teams/tumour boards consider when determining the best treatment for a patient. The stage of the disease, overall health of the patient and availability of different treatment options are all key elements in the decision making process (24).

Molecular stratification of GC can provide insights about the underlying biology, which may have important implications for treatment decisions. However, it is not currently possible to deliver worldwide molecular classification using the tools employed by TCGA or ACRG. Such tools are costly, not widely available, and there is a lack of capacity for the rapid turnaround time required for critically-ill patients. A fall-back position is to devise a series of on-slide tests that histopathologists can implement more widely and can be performed on formalin-fixed paraffin-embedded (FFPE) tissue.

Setia *et al* (25) provided an excellent example of such an approach. In 2016, they published a GC classification using only on-slide tests [immunohistochemical (IHC) and *in situ* hybridization (ISH)]. Initially, they evaluated 15 biomarkers using FFPE tissue from a cohort of 146 cases of GC and ultimately condensed GC into five clusters using only four on-slide tests. The authors adopted a hierarchical approach, similar to that of the molecular classification of endometrial carcinoma now ratified by the WHO (26).

Cluster 1. GC-EBV(+) (5%). This group is associated with marked lymphoid infiltrate, high PD-L1 expression and has an improved survival rate.

Cluster 2. GC-dMMR (16%). This is characterised by loss of MLH1 and PMS2 in 96% of cases, has lower rate of nodal metastasis and improved survival rate.

Cluster 3. GC-E-cadherin(-) (21%). These tumours have aberrant E-cadherin expression and contain predominately tumour of Laurén's diffuse type. This group is associated with low rate of p53 mutation compared with the other clusters. It is further divided into Cluster 3A (40%) showing complete loss of E-cadherin and Cluster 3B (60%) showing granular cytoplasmic staining of E-cadherin. Cluster 3B contains more elderly patients than 3A.

Cluster 4. GC-p53m (51%). This has aberrant p53 expression, contains predominantly tumours of Laurén's intestinal type, is associated with high Her2 expression, has more often
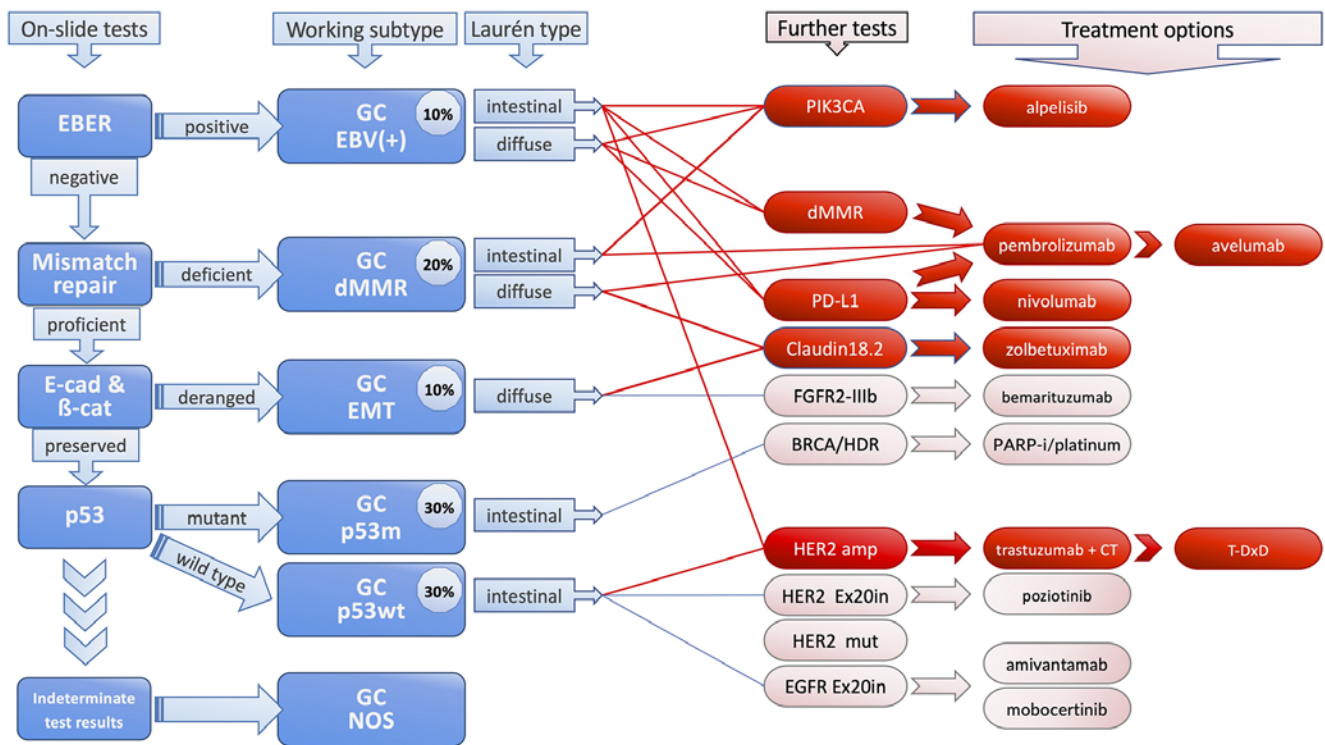
Figure 1. Proposed working classification. On the left side, the five on-slide tests required for classification are presented. The working classification comprises of 6 subtypes. The gastric cancer 'not otherwise specified' subtype is reserved for all cases with indeterminate test results. Further companion diagnostics may be selected after the Laurén's types have been considered. The therapeutic options which are more widely available at present are in red, while the others (in pink with blue lines) are mostly in pipelines. EBER, Epstein-Barr encoding region; E-cad, E-cadherin; β-cat, beta-catenin; EBV, Epstein-Barr virus; dMMR, mismatch repair deficiency; EMT, epithelial-mesenchymal transition; p53m, mutant p53; p53wt, wild-type p53; NOS, not otherwise specified; T-DxD, trastuzumab deruxtecan.

carcinoma within lymphatics and lymph node positivity. Based on IHC expression of MUC and CD10, Cluster 4 is subdivided into four subgroups.

Cluster 5. GC-p53wt (7%). This has normal p53 expression, includes tumours that lack EBV or dMMR and has no defect of E-cadherin expression. All these tumours are of the Laurén's intestinal type.

More recently, others have used the same portfolio of on-slide tests and the same subclassification. For example, Ramos *et al* (27) demonstrated that such an approach is viable in a prospective study. Importantly, they highlighted the potential difficulty in classifying tumours when expression of these four markers is heterogeneous. They raised the issue of sampling bias, thus recognising the importance of correctly interpreting mixed profiles. Ahn *et al* (28) tested a retrospective cohort of GC patients using tissue microarrays (TMAs) and showed similar correlation with prognosis. Zhao *et al* (29) used retrospective tissue in TMAs stained by IHC for mismatch repair proteins (PMS2, MLH1, MSH2 and MSH6), E-cadherin and p21 to classify CG into four subtypes, which associate with different prognoses.

7. Additional companion diagnostic biomarkers useful in GC

The adoption of a working classification would be greatly helped by providing clear links to specific treatments and recommendations on when additional tests should be performed, with a particular emphasis on biomarkers relevant for treatment selection. Some of these biomarkers are already in clinical routine use and others not yet in mainstream use for GC.

Her2. The frequency of HER2 mutation in GC has been reported to be as high as 7.7% in a previous study (30); however, a lower average of ~4.5% is more often reported (31-33). HER2 gene amplification represents a major proportion of these mutations, probably ~50%, and results in Her2 protein overexpression. In these patients, humanised anti-Her2 monoclonal antibodies, including trastuzumab, can be used successfully either to block Her-2 function, or to approach a toxic payload, including trastuzumab deruxtecan (1,34,35). Other HER2 mutations are putative resistance mechanisms to trastuzumab in Her2-positive GC. Some HER2 mutations within the active tyrosine kinase (TK)-domain, such as Ex20 insertions, are targetable by specific TK inhibitors (TKIs) (36). While a small amount of data is available on the exact incidence of HER2 activating mutations in GC (37), they are likely to represent at least 25% of total HER2 mutations (38).

EGFR. This protein is overexpressed in 27-64% of GC and can be targeted by humanised anti-EGFR monoclonal antibodies, including cetuximab or nimotuzumab (39,40). Overexpression is not always associated with gene amplification. Sensitising and resistance mutations in EGFR are known to exist in other tumour sites however no data is available on their incidence in GC.
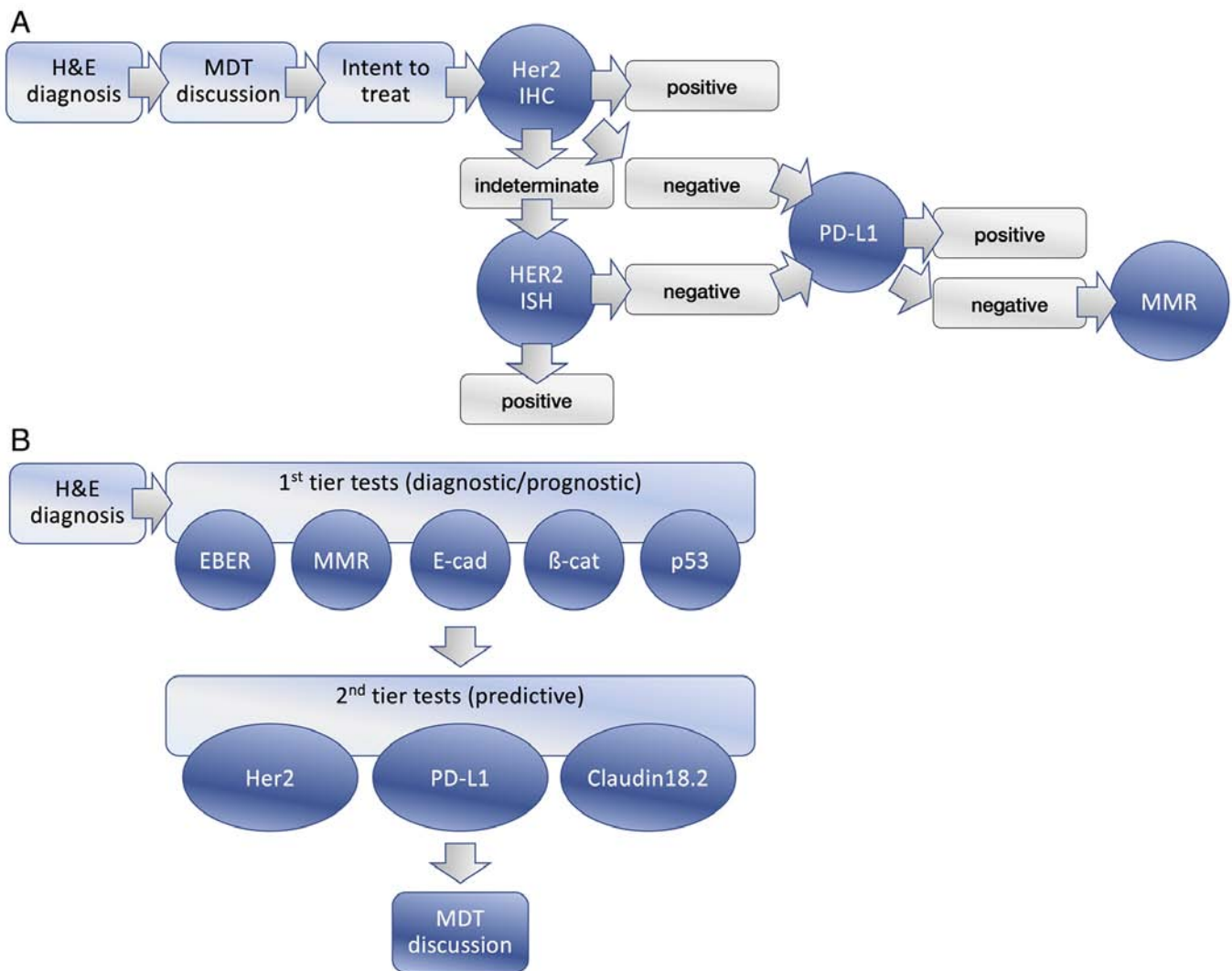
Figure 2. Laboratory workflow for on-slide tests. (A) Current mandatory laboratory workflow, slow TAT. Current step by step approach often used is demonstrated. This leads to slow TAT due to the need of interpretation of each test before the order of the next one. (B) Modified laboratory workflow, rapid TAT. After histological diagnosis of gastric cancer, all tier 1 tests are requested at the same time; tumours are classified using the hierarchical approach; second tier tests for the prediction of response to specific treatments are then reflexed accordingly. This approach on other tumour types allowed the consistent report of these datasets within two to four days TAT. TAT, turnaround time; H&E, haematoxylin and eosin; MDT, multidisciplinary team; EBER, Epstein-Barr encoding region; E-cad, E-cadherin; β-cat, beta-catenin.

PD-L1. PD-L1 selects for eligibility to immune checkpoint inhibitors (anti-PD-1, anti-PD-L1 or anti-CTLA-4) such as pembrolizumab, durvalumab, nivolumab or ipilimumab (41-43).

PIK3CA. Mutations in the catalytic domain of PIK3CA are the third most frequent mutations in GC. These mutations are associated with more aggressive behaviour and are present in 9-12% of non-hypermutated and 32% of hypermutated tumours (44). PIK3CA mutations are associated with GC-EBV(+) and GC-dMMR clusters. The most common mutation is H1047R in Ex20, which has a predilection for the GC-dMMR cluster (45). Tumour harbouring PIK3KA activating mutation can be targeted by TKIs.

KRAS. In TCGA study, KRAS mutations occur in 23% of all cases, although other studies report different penetrance, from 4 to 23% (21,46). Regardless of their incidence, there are strong correlations between KRAS mutations and the GC-dMMR cluster. At present, the only targetable KRAS mutation is G12C; this has a low prevalence, between 0.33% or 2/595 patients and 0.6% or 9/1401 patients (47-49).

ALK. The incidence of ALK rearrangement in GC is low and possibly <1% (50,51) and may be associated with dMMR. Carcinomas with ALK translocation respond well to TKIs.

MET. Amplification of MET is probably frequent in GC (52,53). Its prevalence differs in various studies, from 50% *in vitro* (52,54) to 20% *in vivo* (54-57). However, in a small cohort of 38 locally advanced GC, polysomy of Ch 7 rather than gene amplification was the reason for the increased number of MET genes per cell (58).

Claudin18.2. Part of a large family of transmembrane proteins involved in tight junctions, claudin18.2 arises from differential splicing of mRNA and is overexpressed is some GC cases, particularly those of diffuse Laurén type (59,60). It is targeted by antibody-drug conjugates, bispecific antibodies (zolbetuximab) and cell therapies such as chimeric antigen receptor T-cells (61). The mechanism of action of zolbetuximab is either via antibody-dependent cellular cytotoxicity or

complement-dependent cytotoxicity. The identification of GC with overexpression of claudin18.2 is likely to become soon mandatory (62).

8. An inclusive working classification

Recent advances in personalised treatment have provided renewed pressure to abandon traditional taxonomic classifications in favour of molecular classifications. GC has unfulfilled needs with a large proportion of patients potentially eligible for treatments that could improve outcomes significantly. It is important to define parameters for a classification of GC that enables improved access to such treatments and harmonises diagnostic categories and nomenclature. The longevity of the Laurén classification is testament to the strength inherent to the taxonomic approach and should be retained. The majority of the classifications using on-slide tests only inform some of the oncological treatments; there is a need for additional tests to select the appropriate therapy. A working classification should therefore incorporate all the currently required biomarkers and make provision for any future companion diagnostics.

In the present study, minor modifications of the nomenclature were proposed in order to reflect in an improved way current knowledge underlying these subgroups; some annotation regarding the potential therapeutic approaches was provided and possible additional companion diagnostic tests that should be considered were indicated. This proposal is summarised in Fig. 1. Through acquired knowledge from other tumour sites (e.g. endometrium), it is considered that for all cases with controversial or uninterpretable test results an indeterminate category should be added (63). The present study's classification used EBV-ISH, MMR status by IHC, E-cadherin and beta-catenin IHC and p53 IHC, which are all tests that can be delivered by most histopathology laboratories.

An important consideration for large-scale implementation is cost and impact on laboratory capacity. While a step-wise approach would have reduced the number of tests required, it would have a negative impact on TAT and would have increased the indirect costs of the laboratory to the extent that these would have exceeded any savings in reagents. It was demonstrated in other tumour types that performance of all the necessary tests up fronted results in considerable savings and allowed clinicians to have all the data within a few days (64). A first tier of on-slide biomarkers would be likely to provide prognostic data and instruct a second tier of predictive biomarkers for therapy (Fig. 2). As part of the proposed evolution of this classification, the feasibility of its implementation on a cohort of GC using FFPE have been assessed.

9. Discussion

There are a number of GC classifications based on different tests. Some of the diagnostic categories have considerable overlap, however this is not always clear, since they use different terminology. The authors consider that a first step towards widespread implementation of a working classification is the characterization of a terminology that associates the subgroups with the test results. The 'cascade' approach

that has been used successfully in the WHO classification of endometrial carcinoma was adopted (26).

The modified working classification proposed here has a major focus on treatment options to help histopathologists as well as oncologists. Similar to the classification of endometrial carcinoma, there is aspiration that working molecular classification will become established in the diagnostic routine. The limited capacity for further companion diagnostic tests is well known; the working classification recognises associations between diagnostic groups and specific targeted treatments and therefore is focused on further tests regarding subgroups that are more likely to benefit. In this classification, it is relatively easy for further tests to be added as new targeted treatments become available. In Fig. 1, thin blue lines were used for treatment options that may emerge from clinical trials to exemplify how new tests can be added to the model.

Recent studies using artificial intelligence revealed that systems can be built to recognise molecular changes from H&E sections (65,66). Therefore, it is plausible that pathologists may eventually be able to recognise various molecular subtypes of GC from unique morphological features without the adjunctive help of the molecular data. This would certainly be useful for all those clinicians who do not have access to molecular tools. As demonstrated in other tumour sites, subclassification by molecular changes using surrogate on-slide markers, is a step towards this (63).

The proposed classification of the present study is a pragmatic approach to aid current GC patients. A major limitation is the relatively small number of available on-slide tests when compared with the multi-omics approach. However, whilst other classifications that use multi-omics may be more accurate in identifying relevant subgroups, their widespread implementation is currently not possible.

Availability of data and materials

Not applicable.

Authors' contributions

SC searched the literature for similar work and articles. SC, MS, SW and CD contributed to writing the manuscript. All authors read and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Johnston FM and Beckman M: Updates on management of gastric cancer. Curr Oncol Rep 21: 67, 2019.
2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A and Bray F: Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA A Cancer J Clin 71: 209-249, 2021.
3. Rawla P and Barsouk A: Epidemiology of gastric cancer: Global trends, risk factors and prevention. Prz Gastroenterol 14: 26-38, 2019.
4. Wagner AD, Syn NL, Moehler M, Grothe W, Yong WP, Tai BC, Ho J and Unverzagt S: Chemotherapy for advanced gastric cancer. Cochrane Database Syst Rev 8: CD004064, 2017.
5. Park SR, Chun JH, Kim YW, Lee JH, Choi IJ, Kim CG, Lee JS, Bae JM and Kim HK: Phase II study of low-dose Docetaxel/Fluorouracil/Cisplatin in metastatic gastric carcinoma. Am J Clin Oncol 28: 433-438, 2005.
6. Sun DS, Jeon EK, Won HS, Park JC, Shim BY, Park SY, Hong YS, Kim HK and Ko YH: Outcomes in elderly patients treated with a single-agent or combination regimen as first-line chemotherapy for recurrent or metastatic gastric cancer. Gastric Cancer 18: 644-652, 2015.
7. Narahara H, Iishi H, Imamura H, Tsuburaya A, Chin K, Imamoto H, Esaki T, Furukawa H, Hamada C and Sakata Y: Randomized phase III study comparing the efficacy and safety of irinotecan plus S-1 with S-1 alone as first-line treatment for advanced gastric cancer (study GC0301/TOP-002). Gastric Cancer 14: 72-80, 2011.
8. Cunningham D, Allum WH, Stenning SP, Thompson JN, Van De Velde CJH, Nicolson M, Scarffe JH, Lofts FJ, Falk SJ, Iveson TJ, et al: Perioperative chemotherapy versus surgery alone for resectable gastroesophageal cancer. N Engl J Med 355: 11-20, 2006.
9. Laurén P: The two histological main types of gastric carcinoma: Diffuse and So-called Intestinal-type carcinoma: An attempt at a Histo-clinical classification. Acta Pathol Microbiol Scand 64: 31-49, 1965.
10. Carneiro F, Seixas M and Sobrinho-Simões M: New elements for an updated classification of the carcinomas of the stomach. Pathol Res Pract 191: 571-584, 1995.
11. Lei ZN, Teng QX, Tian Q, Chen W, Xie Y, Wu K, Zeng Q, Zeng L, Pan Y, Chen ZS, et al: Signaling pathways and therapeutic interventions in gastric cancer. Signal Transduct Target Ther 7: 358, 2022.
12. Monster JL, Kemp LJS, Gloerich M and Van Der Post RS: Diffuse gastric cancer: Emerging mechanisms of tumor initiation and progression. Biochim Biophys Acta Rev Cancer 1877: 188719, 2022.
13. Organisation mondiale de la santé and Centre international de Recherche Sur le cancer: Digestive system tumours. 5th edition. International agency for research on cancer, Lyon, 2019.
14. Ajani JA, D'Amico TA, Bentrem DJ, Chao J, Cooke D, Corvera C, Das P, Enzinger PC, Enzler T, Fanta P, et al: Gastric cancer, version 2.2022, NCCN clinical practice guidelines in oncology. J Natl Compr Canc Netws 20: 167-192, 2022.
15. Davidson B and Tropé CG: Ovarian cancer: Diagnostic, biological and prognostic aspects. Womens Health (Lond) 10: 519-533, 2014.
16. Roepman P, Schlicker A, Tabernero J, Majewski I, Tian S, Moreno V, Snel MH, Chresta CM, Rosenberg R, Nitsche U, et al: Colorectal cancer intrinsic subtypes predict chemotherapy benefit, deficient mismatch repair and epithelial-to-mesenchymal transition. Int J Cancer 134: 552-562, 2014.
17. Dai X, Li T, Bai Z, Yang Y, Liu X, Zhan J and Shi B: Breast cancer intrinsic subtype classification, clinical use and future trends. Am J Cancer Res 5: 2929-2943, 2015.
18. Talhouk A and McAlpine JN: New classification of endometrial cancers: The development and potential applications of genomic-based classification in research and clinical care. Gynecol Oncol Res Pract 3: 14, 2016.
19. West L, Vidwans SJ, Campbell NP, Shrager J, Simon GR, Bueno R, Dennis PA, Otterson GA and Salgia R: A novel classification of lung cancer into molecular subtypes. PLoS One 7: e31906, 2012.
20. The Cancer Genome Atlas Research Network: Comprehensive molecular characterization of gastric adenocarcinoma. Nature 513: 202-209, 2014.
21. Cristescu R, Lee J, Nebozhyn M, Kim KM, Ting JC, Wong SS, Liu J, Yue YG, Wang J, Yu K, et al: Molecular analysis of gastric cancer identifies subtypes associated with distinct clinical outcomes. Nat Med 21: 449-456, 2015.
22. Wong SS, Kim KM, Ting JC, Yu K, Fu J, Liu S, Cristescu R, Nebozhyn M, Gong L, Yue YG, et al: Genomic landscape and genetic heterogeneity in gastric adenocarcinoma revealed by whole-genome sequencing. Nat Commun 5: 5477, 2014.
23. Chia NY and Tan P: Molecular classification of gastric cancer. Ann Oncol 27: 763-769, 2016.
24. Alsina M, Arrazubi V, Diez M and Tabernero J: Current developments in gastric cancer: From molecular profiling to treatment strategy. Nat Rev Gastroenterol Hepatol 20: 155-170, 2023.
25. Setia N, Agoston AT, Han HS, Mullen JT, Duda DG, Clark JW, Deshpande V, Mino-Kenudson M, Srivastava A, Lennerz JK, et al: A protein and mRNA expression-based classification of gastric cancer. Modern Pathol 29: 772-784, 2016.
26. Organisation mondiale de la santé and Centre international de Recherche Sur le cancer: Female genital tumours. 5th edition. International agency for research on cancer, Lyon, 2020.
27. Ramos MFKP, Pereira MA, Amorim LC, Mello ES, Faraj SF, Ribeiro U, Hoff PMG, Cecconello I and Castria TB: Gastric cancer molecular classification and adjuvant therapy: Is there a different benefit according to the subtype? J Surg Oncol 121: 804-813, 2020.
28. Ahn S, Lee SJ, Kim Y, Kim A, Shin N, Choi KU, Lee CH, Huh GY, Kim KM, Setia N, et al: High-throughput Protein and mRNA Expression-based classification of gastric cancers can identify clinically distinct subtypes, concordant with recent molecular classifications. Am J Surg Pathol 41: 106-115, 2017.
29. Zhao C, Feng Z, He H, Zang D, Du H, Huang H, Du Y, He J, Zhou Y and Nie Y: Protein expression-based classification of gastric cancer by immunohistochemistry of tissue microarray. PLoS One 15: e0238836, 2020.
30. Park S, Ahn S, Kim DG, Kim H, Kang SY and Kim KM: High frequency of juxtamembrane domain ERBB2 mutation in gastric cancer. Cancer Genomics Proteomics 19: 105-112, 2022.
31. Robichaux JP, Elamin YY, Vijayan RSK, Nilsson MB, Hu L, He J, Zhang F, Pisegna M, Poteete A, Sun H, et al: Pan-cancer landscape and analysis of ERBB2 mutations identifies poziotinib as a clinically active inhibitor and enhancer of T-DM1 activity. Cancer Cell 36: 444-457.e7, 2019.
32. Karczewski KJ, Francioli LC, Tiao G, Cummings BB, Alföldi J, Wang Q, Collins RL, Laricchia KM, Ganna A, MacArthur DG, et al: The mutational constraint spectrum quantified from variation in 141,456 humans. Nature 581: 434-443, 2020.
33. Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, Jacobsen A, Byrne CJ, Heuer ML, Larsson E, et al: The cBio cancer genomics portal: An open platform for exploring multidimensional cancer genomics data. Cancer Discovery 2: 401-404, 2012.
34. Gravalos C and Jimeno A: HER2 in gastric cancer: A new prognostic factor and a novel therapeutic target. Ann Oncol 19: 1523-1529, 2008.
35. Bang YJ, Van Cutsem E, Feyereislova A, Chung HC, Shen L, Sawaki A, Lordick F, Ohtsu A, Omuro Y, Satoh T, et al: Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): A phase 3, open-label, randomised controlled trial. Lancet 376: 687-697, 2010.
36. Wilding B, Scharn D, Böse D, Baum A, Santoro V, Chetta P, Schnitzer R, Botesteanu DA, Reiser C, Kornigg S, et al: Discovery of potent and selective HER2 inhibitors with efficacy against HER2 exon 20 insertion-driven tumors, which preserve wild-type EGFR signaling. Nat Cancer 3: 821-836, 2022.
37. Zhu Y, Zhu X, Wei X, Tang C and Zhang W: HER2-targeted therapies in gastric cancer. Biochim Biophys Acta Rev Cancer 1876: 188549, 2021.
38. Kahraman S and Yalcin S: Recent advances in systemic treatments for HER-2 positive advanced gastric cancer. Onco Targets Ther 14: 4149-4162, 2021.
39. Adua D, Di Fabio F, Rojas Llimpe FL, Pini S and Pinto C: Long-term survival in an advanced gastric cancer patient treated with cetuximab in association with FOLFIRI: A case report. J Gastrointest Oncol 5: E13-E17, 2014.

40. Satoh T, Lee KH, Rha SY, Sasaki Y, Park SH, Komatsu Y, Yasui H, Kim TY, Yamaguchi K, Fuse N, *et al*: Randomized phase II trial of nimotuzumab plus irinotecan versus irinotecan alone as second-line therapy for patients with advanced gastric cancer. Gastric Cancer 18: 824-832, 2015.

41. Kang YK, Boku N, Satoh T, Ryu MH, Chao Y, Kato K, Chung HC, Chen JS, Muro K, Kang WK, *et al*: Nivolumab in patients with advanced gastric or gastro-oesophageal junction cancer refractory to, or intolerant of, at least two previous chemotherapy regimens (ONO-4538-12, ATTRACTION-2): A randomised, double-blind, placebo-controlled, phase 3 trial. Lancet 390: 2461-2471, 2017.

42. Muro K, Chung HC, Shankaran V, Geva R, Catenacci D, Gupta S, Eder JP, Golan T, Le DT, Burtness B, *et al*: Pembrolizumab for patients with PD-L1-positive advanced gastric cancer (KEYNOTE-012): A multicentre, open-label, phase 1b trial. Lancet Oncol 17: 717-726, 2016.

43. Janjigian YY, Bendell J, Calvo E, Kim JW, Ascierto PA, Sharma P, Ott PA, Peltola K, Jaeger D, Evans J, *et al*: CheckMate-032 study: Efficacy and safety of nivolumab and nivolumab plus ipilimumab in patients with metastatic esophagogastric cancer. J Clin Oncol 36: 2836-2844, 2018.

44. Kim JW, Lee HS, Nam KH, Ahn S, Kim JW, Ahn SH, Park DJ, Kim HH and Lee KW: PIK3CA mutations are associated with increased tumor aggressiveness and Akt activation in gastric cancer. Oncotarget 8: 90948-90958, 2017.

45. Te Paske IBAW, Garcia-Pelaez J, Sommer AK, Matalonga L, Starzynska T, Jakubowska A; Solve-RD-GENTURIS group, van der Post RS, Lubinski J, Oliveira C, *et al*: A mosaic PIK3CA variant in a young adult with diffuse gastric cancer: Case report. Eur J Hum Genet 29: 1354-1358, 2021.

46. Polom K, Das K, Marrelli D, Roviello G, Pascale V, Voglino C, Rho H, Tan P and Roviello F: KRAS mutation in gastric cancer and prognostication associated with microsatellite instability status. Pathol Oncol Res 25: 333-340, 2019.

47. Salem M, El-Refai S, Sha W, Grothey A, Puccini A, George T, Hwang J, Kadakia K, Musselwhite L, Van Cutsem E, *et al*: O-3 Characterization of KRAS mutation variants and prevalence of KRAS-G12C in gastrointestinal malignancies. Ann Oncol 32: S218, 2021.

48. Osterlund E, Ristimäki A, Kytölä S, Kuopio T, Heervä E, Muhonen T, Halonen P, Kallio R, Soveri LM, Sundström J, *et al*: KRAS-G12C mutation in one Real-life and three population-based Nordic cohorts of metastatic colorectal cancer. Front Oncol 12: 826073, 2022.

49. Chida K, Kotani D, Masuishi T, Kawakami T, Kawamoto Y, Kato K, Fushiki K, Sawada K, Kumanishi R, Shirasu H, *et al*: The prognostic impact of KRAS G12C mutation in patients with metastatic colorectal cancer: A multicenter retrospective observational study. Oncologist 26: 845-853, 2021.

50. Ambrosini M, Del Re M, Manca P, Hendifar A, Drilon A, Harada G, Ree AH, Klempner S, Mælandsmo GM, Flatmark K, *et al*: ALK inhibitors in patients with ALK Fusion-positive GI cancers: An international data set and a molecular case series. JCO Precis Oncol 6: e2200015, 2022.

51. Chon HJ, Kim HR, Shin E, Kim C, Heo SJ, Lee C, Park JK, Noh SH, Chung HC and Rha SY: The Clinicopathologic features and prognostic impact of ALK positivity in patients with resected gastric cancer. Ann Surg Oncol 22: 3938-3945, 2015.

52. Smolen GA, Sordella R, Muir B, Mohapatra G, Barmettler A, Archibald H, Kim WJ, Okimoto RA, Bell DW, Sgroi DC, *et al*: Amplification of MET may identify a subset of cancers with extreme sensitivity to the selective tyrosine kinase inhibitor PHA-665752. Proc Natl Acad Sci USA 103: 2316-2321, 2006.

53. Bachleitner-Hofmann T, Sun MY, Chen CT, Tang L, Song L, Zeng Z, Shah M, Christensen JG, Rosen N, Solit DB, *et al*: HER kinase activation confers resistance to MET tyrosine kinase inhibition in MET oncogene-addicted gastric cancer cells. Mol Cancer Ther 7: 3499-3508, 2008.

54. Kuniyasu H, Yasui W, Kitadai Y, Yokozaki H, Ito H and Tahara E: Frequent amplification of the c-met gene in scirrhous type stomach cancer. Biochem Biophys Res Commun 189: 227-232, 1992.

55. Seruca R, Suijkerbuijk RF, Gärtner F, Criado B, Veiga I, Olde-Weghuis D, David L, Castedo S and Sobrinho-Simões M: Increasing levels of MYC and MET co-amplification during tumor progression of a case of gastric cancer. Cancer Genet Cytogenet 82: 140-145, 1995.

56. Tsujimoto H, Sugihara H, Hagiwara A and Hattori T: Amplification of growth factor receptor genes and DNA ploidy pattern in the progression of gastric cancer. Virchows Archiv 431: 383-389, 1997.

57. Sakakura C, Mori T, Sakabe T, Ariyama Y, Shinomiya T, Date K, Hagiwara A, Yamaguchi T, Takahashi T, Nakamura Y, *et al*: Gains, losses, and amplifications of genomic materials in primary gastric cancers analyzed by comparative genomic hybridization. Genes Chromosom Cancer 24: 299-305, 1999.

58. Janjigian YY, Tang LH, Coit DG, Kelsen DP, Francone TD, Weiser MR, Jhanwar SC and Shah MA: MET expression and amplification in patients with localized gastric cancer. Cancer Epidemiol Biomarkers Prev 20: 1021-1027, 2011.

59. Xu B, Chen F, Zhang X, Wang Z, Che K, Wu N, Yu L, Fan X, Liu B and Wei J: Antigen-Specific T cell immunotherapy targeting Claudin18.2 in gastric cancer. Cancers 14: 2758, 2022.

60. Grizzi G, Venetis K, Denaro N, Bonomi M, Celotti A, Pagkali A, Hahne JC, Tomasello G, Petrelli F, Fusco N, *et al*: Anti-Claudin treatments in gastroesophageal adenocarcinoma: Mainstream and upcoming strategies. J Clin Med 12: 2973, 2023.

61. Qi C, Gong J, Li J, Liu D, Qin Y, Ge S, Zhang M, Peng Z, Zhou J, Cao Y, *et al*: Claudin18.2-specific CAR T cells in gastrointestinal cancers: Phase 1 trial interim results. Nat Med 28: 1189-1198, 2022.

62. Sahin U, Türeci Ö, Manikhas G, Lordick F, Rusyn A, Vynnychenko I, Dudov A, Bazin I, Bondarenko I, Melichar B, *et al*: FAST: A randomised phase II study of zolbetuximab (IMAB362) plus EOX versus EOX alone for first-line treatment of advanced CLDN18.2-positive gastric and gastro-oesophageal adenocarcinoma. Ann Oncol 32: 609-619, 2021.

63. Alexa M, Hasenburg A and Battista MJ: The TCGA molecular classification of endometrial cancer and its possible impact on adjuvant treatment decisions. Cancers 13: 1478, 2021.

64. Wedden S, Miller K, Frayling IM, Thomas T, Chefani A, Miller K, Hamblin A, Taylor JC and D'Arrigo C: Colorectal cancer stratification in the routine clinical pathway: A district general hospital experience. Appl Immunohistochem Mol Morphol 27: e54-e62, 2019.

65. Fremond S, Andani S, Barkey Wolf J, Dijkstra J, Melsbach S, Jobsen JJ, Brinkhuis M, Roothaan S, Jurgenliemk-Schulz I, Lutgens LCHW, *et al*: Interpretable deep learning model to predict the molecular classification of endometrial cancer from haematoxylin and Eosin-stained Whole-slide images: A combined analysis of the PORTEC randomised trials and clinical cohorts. Lancet Digital Health 5: e71-e82, 2023.

66. Fell C, Mohammadi M, Morrison D, Arandjelović O, Syed S, Konanahalli P, Bell S, Bryson G, Harrison DJ and Harris-Birtill D: Detection of malignancy in whole slide images of endometrial cancer biopsies using artificial intelligence. PLoS One 18: e0282577, 2023.