Molecular test algorithms for digestive tumours

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SUMMARY

The Belgian Commission of Personalized Medicine has been created to advise the federal government on all matters related to personalised medicine in oncology, including the reimbursement of molecular tests. Here, we propose the Belgian strategy for molecular testing in the digestive tumours within a scientific-based framework. For each tested biomarker, a clinical test level is attached, which is key to establish the relevance of the test and to define the reimbursement. For each digestive tumour type, the different molecular tests are represented as decision trees with its test utility, test level and a brief technical test description.

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INTRODUCTION

Personalised medicine aims to improve care and clinical outcomes of cancer patients by tailoring treatment for individual patients. Furthermore, personalised medicine guarantees optimal use of the health care budget by avoiding ineffective and potentially harmful therapies.[1] Recently, a Belgian strategy to prioritise up-to-date molecular testing and coordinate the use of targeted therapies within a scientific-based frame-

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TABLE 1. Test levels.

1	Standard of care biomarker for diagnosis and/or prognosis*
	Biomarker predictive of response or resistance to a reimbursed drug in Belgium for this indication
2A	Recommended standard of care biomarker for diagnosis and/or prognosis+
	Biomarker predictive of response or resistance to an EMA-approved drug for this indication
2B	Biomarker predictive of response or resistance to a reimbursed drug in Belgium for another indication (clinical trial available in Belgium or EU)
3	Compelling clinical evidence supporting the biomarker for diagnosis and/or prognosis Biomarker predictive of response or resistance to - a non-EMA-approved drug in this indication - a reimbursed drug in Belgium for another indication (clinical trial not available in Belgium or EU) - an EMA-approved drug for another indication Compassionate use of drug

*standard of care: included in guidelines (WHO, etc.) and consensus from experts of the Commission of Personalized Medicine (ComPerMed), +recommended standard of care: clinical evidence and consensus from experts of the ComPerMed. EMA: European Medicines Agency.

work in the cancer treatment in the field of molecular biomarkers has been proposed.[2]

METHODOLOGY

The Belgian Commission of Personalized Medicine (ComPerMed) has set up an expert group to evaluate systematically all molecular biomarker tests currently performed in Belgium in clinical routine for diagnostic, prognostic and/or predictive (therapeutic) purpose for each cancer type. In this manuscript, we describe molecular test algorithms for the most frequent digestive tumours, such as colorectal cancers (CRCs), gastric tumours, gastrointestinal stromal tumours (GISTs) and pancreatic tumours. First, commonly used molecular biomarker tests in Belgium were listed for each digestive tumour type, and an 'a priori' 4-level scale of test relevance has been defined (*Table 1*). For each molecular test performed on the four digestive tumour types, the test utility (diagnostic, prognostic and/or therapeutic utility) and its corresponding test level was discussed until a consensus was reached to ensure the relevance of the test for a specific clinical question. A GIST is a mesenchymal tumour and is included in this category of 'digestive epithelial tumour types since this exercise is not yet done for soft tissue tumours, and most GISTs occur in the gastrointestinal tract. As previously described, only molecular biomarker tests with an assigned level 1 or 2A were retained.[2,3] Some level 2B molecular diagnostic tests were also considered if the expert group predicted that these molecular tests will increase to level 1 or 2A in the near future. In case the test is performed by next generation sequencing (NGS), the genes and regions with level 1 or 2A were also selected.

RESULTS: ALGORITHMS AND KEY MESSAGES

The different molecular tests are represented as decision trees for each digestive tumour type (*Figures 1 to 4*) and will be published on a website in 2018. Test utility, test level and a brief technical test description are annotated for each molecular biomarker test. Tumour incidence, provided by the Belgian Cancer Registry, is also indicated.

Experts have agreed that:

- NGS tests provide valuable information on GIST and metastatic CRCs for therapeutic decisions and may help for diagnosis of malignancy in pancreatic cysts. The analysis of genes and/or regions with at least level 1 and 2A is required;
- due to additional clinical information useful for the clinical management, there is a clear added molecular and economical value for replacing individual gene tests by simultaneous testing of gene panels by NGS;
- there is currently insufficient evidence that NGS gene panels containing genes with test level 2B would improve patient outcome compared to the use of smaller panels. However, these larger NGS tests can direct patients to clinical trials or to off-label drugs. These NGS panels are currently not reimbursed by the Belgian reimbursement INAMI/RIZIV agency;
- for the NGS tests, standardised wet lab and bioinformatics pipeline with a common biological and clinical variant interpretation are mandatory[4];

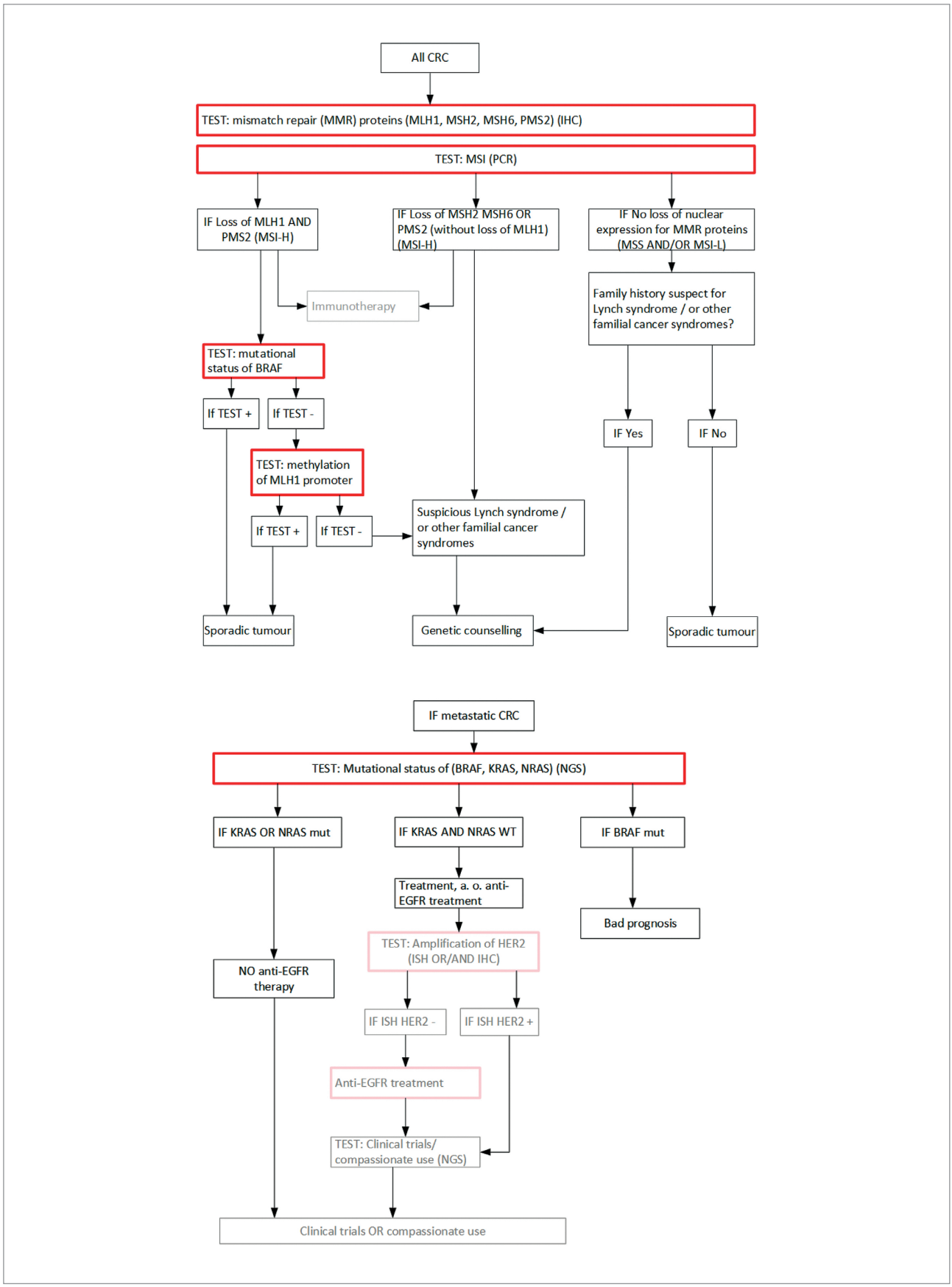

FIGURE 1. Colorectal cancer algorithm.

CRC: colorectal cancer, wt: wild type, mut: mutation, EGFR: epidermal growth factor receptor, NGS: next generation sequencing.

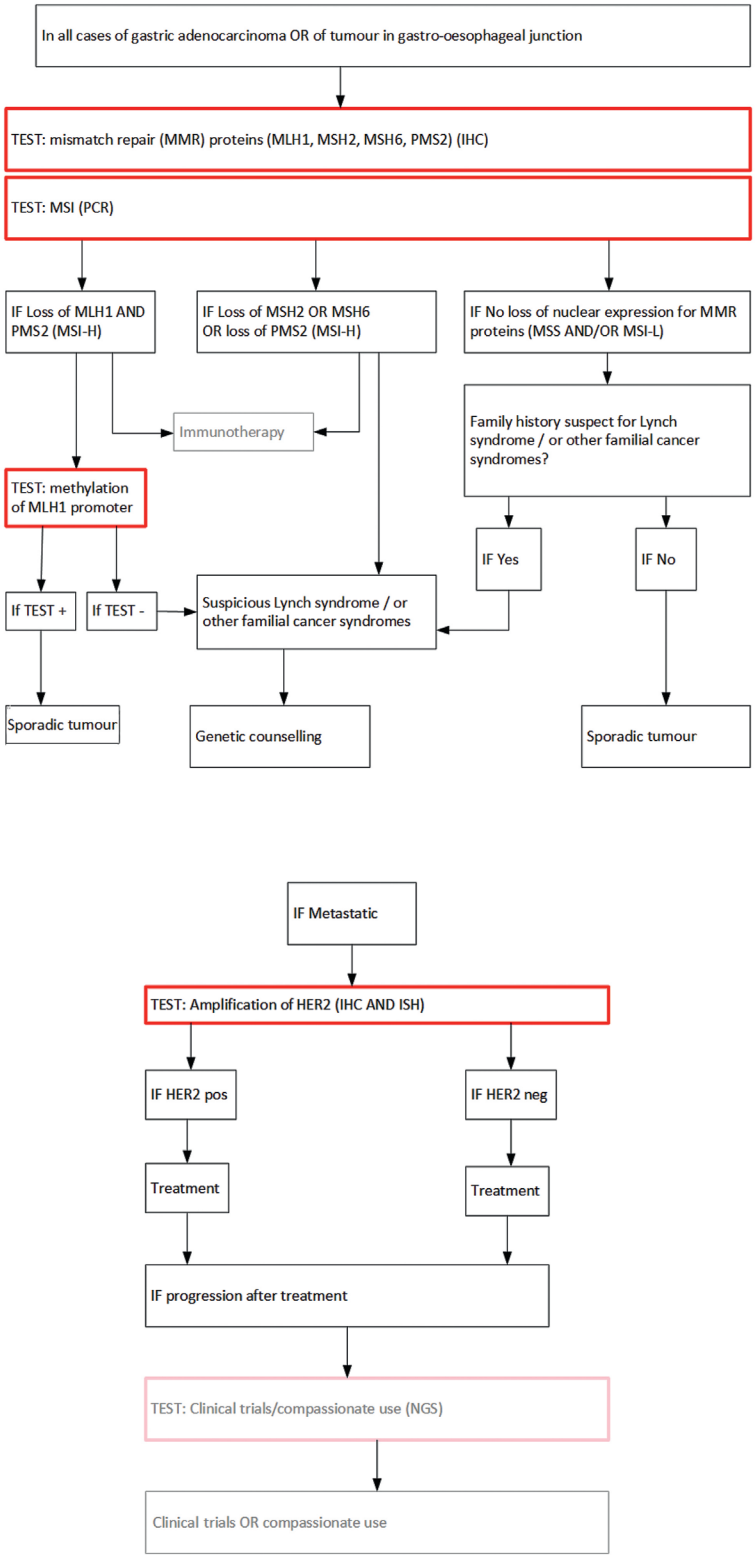

FIGURE 2. Gastric cancer algorithm.

MSI: microsatellite instability, PCR: polymerase chain reaction, IHC: immunohistochemistry, ISH: in situ hybridisation, pos: positive, neg: negative, NGS: next generation sequencing.

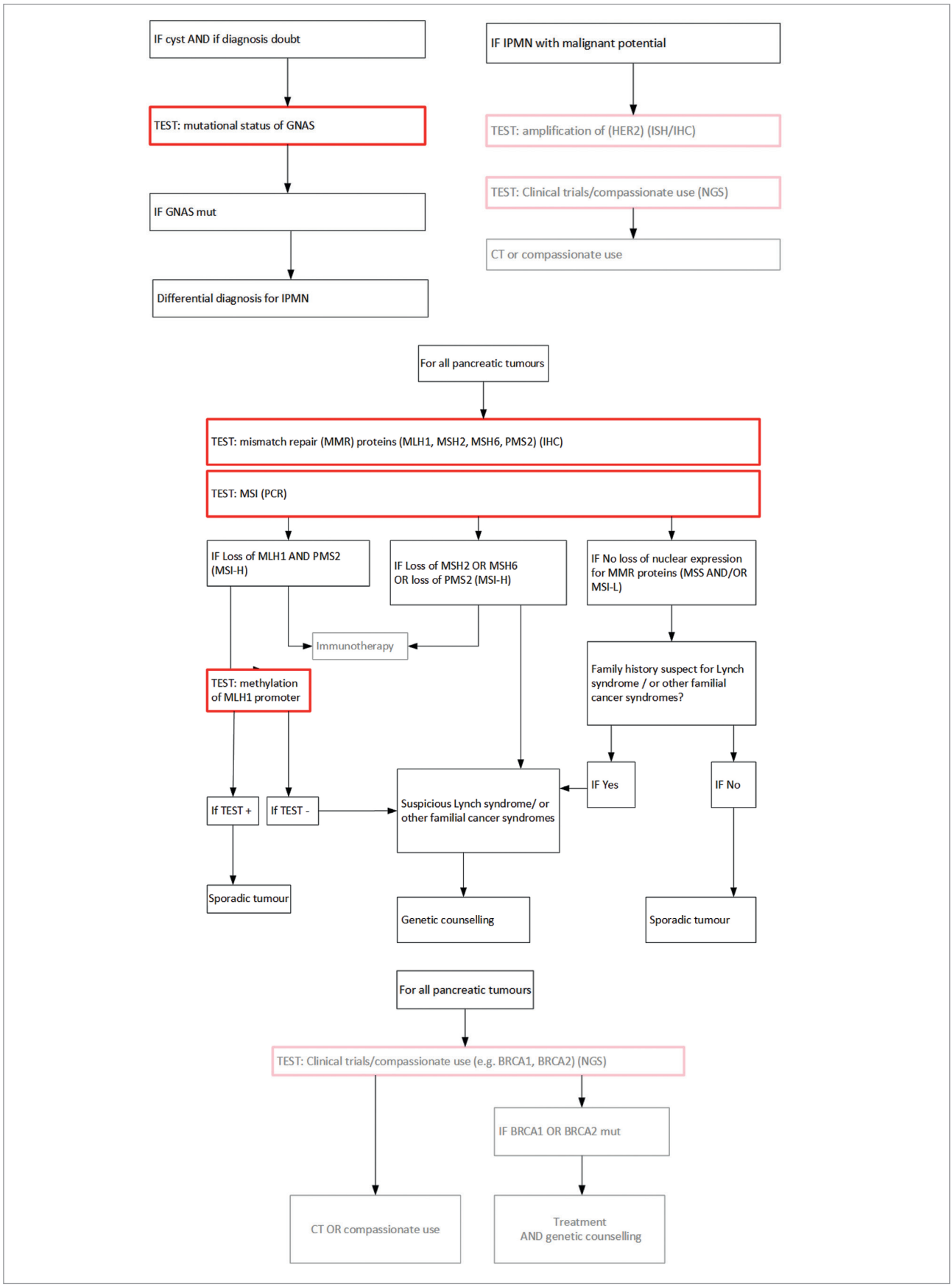

FIGURE 3. Pancreatic cancer algorithm.

mut: mutation, IPMN: intraductal papillary mucinous neoplasms, IHC: immunohistochemistry, ISH: *in situ* hybridisation, NGS: next generation sequencing, CT: clinical trial, MSI: microsatellite instability.

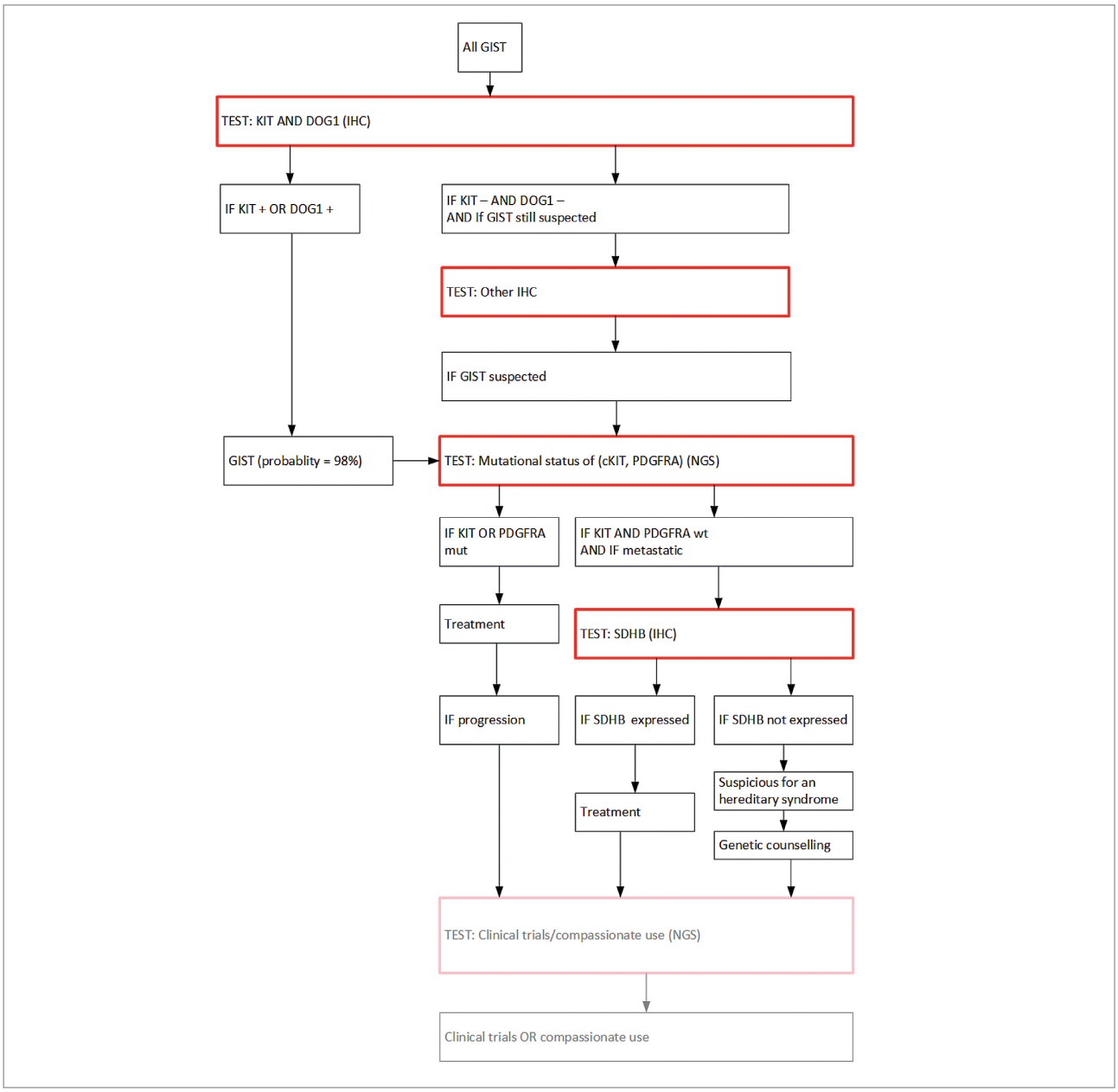

FIGURE 4. Gastrointestinal stromal tumour algorithm.
GIST: gastrointestinal stromal tumour, IHC: immunohistochemistry, NGS: next generation sequencing, mut: mutation, wt: wild type, CT: clinical trial.

- copy number variants as well as chromosomal rearrangements can be detected by some but not all routinely available NGS panels;
- hotspot mutations in circulating tumour DNA (ctDNA) can be detected by polymerase chain reaction or by NGS. For digestive tumours, the preferred specimens are tissue from metastatic lesions. In their absence, primary tumour tissue is an acceptable alternative. If no tissue can be obtained, analysis of ctDNA can serve as an alternative for determination of mutation status;
- a mutational analysis of *BRAF* in all CRCs showing microsatellite instability-high or loss of *MLH1* and *PMS2* is recommended to distinguish somatic tumours from tumours that arise in a hereditary background (Lynch syndrome);
- a mutation analysis of *KRAS, NRAS* and *BRAF* in metastatic CRCs is required;
- *HER2* amplification testing for metastatic CRCs is emerging but not yet recommended for routine management outside a clinical trial setting;
- testing of amplification of *HER2* in metastatic gastric cancers is required;
- *HER2* amplification testing in pancreatic intraductal pap-

KEY MESSAGES FOR CLINICAL PRACTICE

For an optimal digestive tumour management, it is essential to establish clear molecular test workflows:

It is recommended for colorectal cancer (CRC) management to perform:
- **expression of mismatch repair protein analysis and/or microsatellite instability for all CRCs;**
- **next generation sequencing tests for mutation analysis of *KRAS, NRAS* and *BRAF* for all metastatic CRCs;**
- **a mutational analysis of *BRAF* for all CRCs showing microsatellite instability-high or loss of *MLH1* and *PMS2,* and if BRAF is wild type, a MLH1 promoter methylation analysis.**

It is recommended for gastric cancer management to perform:
- **expression of mismatch repair protein analysis and/or microsatellite instability for all gastric cancers;**
- **a HER2 amplification test for all metastatic gastric cancers;**
- **a MLH1 promoter methylation analysis for all gastric cancers showing microsatellite instability-high or loss of *MLH1* and *PMS2.***

It is recommended for pancreatic cancer management to perform:
- **a mutational analysis of GNAS for differential diagnosis between pancreatic intraductal papillary mucinous neoplasms and cystadenoma.**

It is recommended for gastrointestinal stromal tumour (GIST) management to perform:
- **a protein expression analysis of KIT and DOG1 for all GISTs;**
- **next generation sequencing tests for mutational analysis of KIT and PDGFRA for all GISTs;**
- **a protein expression analysis of SDHB if KIT and PDGFRA are wild type for all metastatic GISTs.**

illary mucinous neoplasms (IPMN) with malignant potential is not recommended for routine management outside a clinical trial setting;
- GNAS mutation analysis is recommended for differential diagnosis between pancreatic IPMN and cystadenoma;
- expression of mismatch repair (MMR) protein analysis and/or microsatellite instability (MSI) should be performed for all CRCs, gastric cancers and pancreatic cancers for diagnosis of hereditary syndromes and/or for prediction of response to immunotherapy. MMR can be identified by immunohistochemistry and MSI by PCR or NGS[5];
- *KIT* and *PDGFRA* mutational analysis is required in all metastatic GISTs and also when considering imatinib in an adjuvant setting.

CONCLUSION

In conclusion, we have emphasised that the molecular alterations to be tested within each testing-flow for each tumour-type suggested here are a starting point to do a similar analysis in other tumour types, with the caveat that some of the proposed tests are not yet reimbursed by the Belgian reimbursement agency INAMI/RIZIV (e.g., the MSI-testing in gastric cancer). Therefore, it is crucial that, for an optimal implementation of this guideline in daily clinical practice, the INAMI/RIZIV ensures corresponding changes in clinical practice such as the availability of drugs for which a clear indication exists (e.g., MSI in gastric cancer). If this is not the case, these flows will merely result in higher expenses for pathology laboratories without any added value for the patient.

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