Molecular test algorithms for breast tumours

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SUMMARY

In order to advise the Federal Government on all matters related to personalised medicine in oncology, including the reimbursement of molecular tests, the Commission of Personalized Medicine (ComPerMed) has applied, for the breast tumours, the same methodology as previously applied for the digestive tumours. Meaning, the different molecular tests, represented in the shape of algorithms, are annotated with test levels – which aim to reflect their relevance based on current available data and to define the reimbursement – and are documented with recent literature, guidelines and a brief technical description.

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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 ComPerMed, **Recommended standard of care: clinical evidence AND consensus from experts ComPerMed. *EMA: European Medicines Agency, WHO: World Health Organization, ComPerMed: Commission of Personalized Medicine.*

INTRODUCTION

The treatment of patients with breast cancer has become more challenging with the advent of numerous innovative molecular tests giving the opportunity to the oncologists to tailor their patients' treatments based on prognosis and/or predictors of resistance or response to chemotherapy, endocrine therapy and targeted therapy.

METHODOLOGY

The Commission of Personalized Medicine (ComPerMed) has set up a methodology in order to systematically evaluate and prioritise the molecular tests currently performed in Belgium in clinical routine for each tumour type.[1]

After having achieved a consensus for the digestive tumours, the same methodology has been applied to the breast tumours to ensure the relevance of a molecular test for a specific clinical question.[1]

ALGORITHMS AND KEY MESSAGES

The different molecular tests used in clinical routine in Belgium are represented in algorithms (*Figure 1* and *2*) and are published on the website www.compermed.be. These algorithms will be reviewed annually. Some additional information has been noted, such as tumour incidence (provided by the Belgian Cancer Registry) and, for each molecular test, its utility (diagnostic, prognostic or therapeutic utility) and corresponding test level (*Table 1*) and a brief technical test description. Three test levels were determined, with level 1 representing the highest priority for the qualification of the test by the policy makers. These test levels are linked to the standard of care and to reimbursed treatments in Belgium. Only molecular tests with a test level of 1 or 2A were retained. Some tests with a level 2B were, however, considered if the expert group estimated that they will acquire a test level 1 or 2A in the near future.

In the case of next generation sequencing (NGS) testing, the genes and regions that have to be sequenced were also evaluated.

Experts have agreed that:

- Only test level 1 and 2A molecular tests should be reimbursed by the Belgian reimbursement agency, INAMI/RIZIV.
- Immunohistochemical tests for oestrogen receptor (ER) and progesterone receptor (PgR) and determination of human epidermal growth factor receptor 2 (HER2) status by immunohistochemistry (IHC) and/or *in situ* hybridisation (ISH) are required for early breast cancers as well as for metastatic breast cancers. HER2 testing should follow the new 2018 ASCO-CAP guidelines, which highlights the importance of the quality assurance in HER2 IHC for therapeutic decision making.[2] Testing for ER, PgR and HER2 should be repeated in case of a biopsy in the metastatic setting.
- An official and mandatory government-led external quality assessment (EQA) for the immunohistochemical testing of HER2 overexpression and hormone receptor (ER/PgR) status is currently lacking in Belgium. It would en-

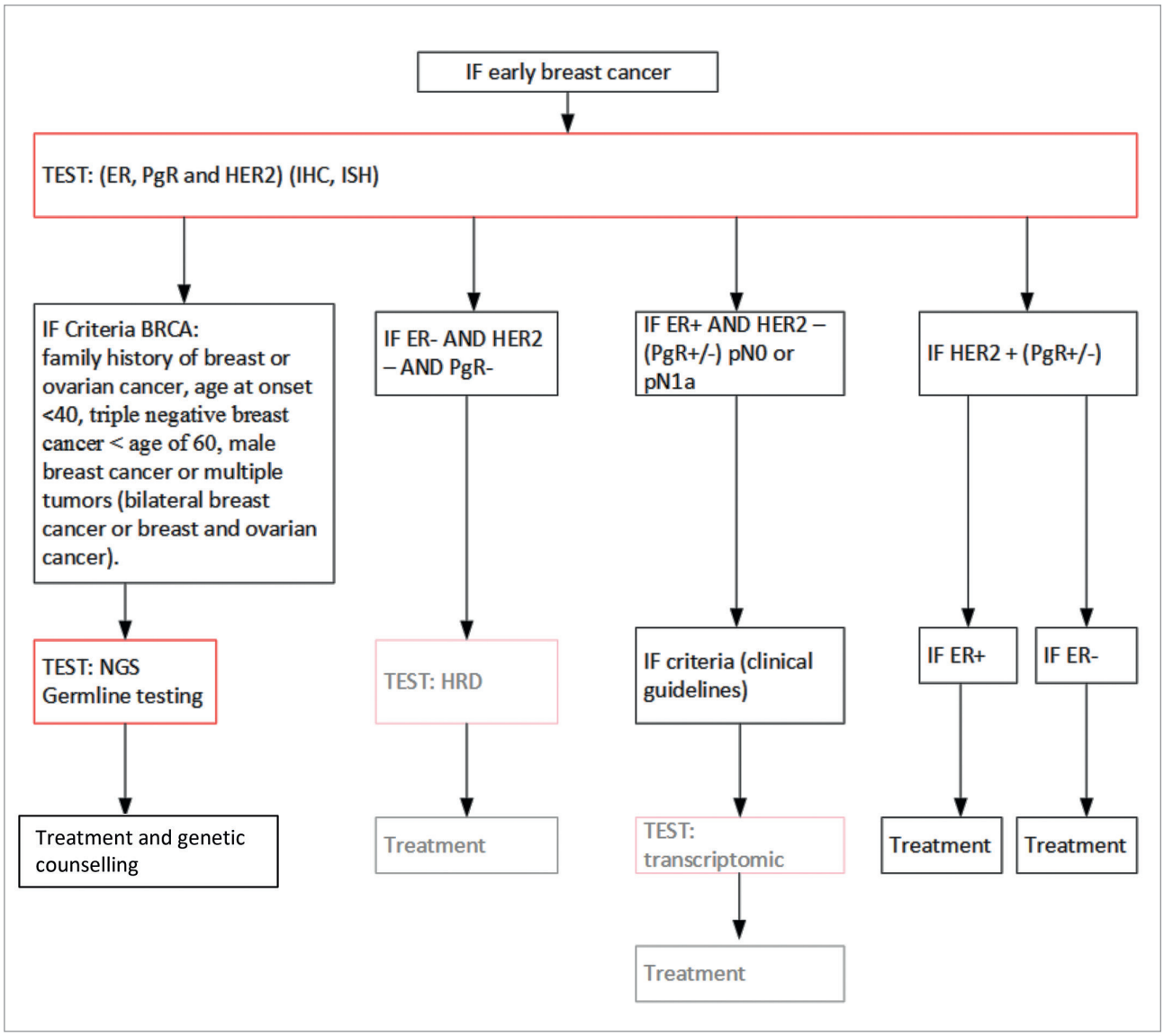

FIGURE 1. Breast algorithm: molecular tests with level 1 or 2A are represented in a red rectangle; molecular tests with a test level >2A are in shadow. *ER: oestrogen receptor, PgR: progesterone receptor, HER2: human epidermal growth factor receptor 2, IHC: immunohistochemistry, ISH: in situ hybridisation, BRCA: breast cancer, NGS: next generation sequencing, pN0: no regional lymph node metastasis, pN1a: regional lymph node metastasis completely resected, HRD: homologous recombination deficiency.*

sure the quality of these tests and standardised interpretation and reporting throughout Belgium. For the moment, commercial EQA schemes are followed by most laboratories. The lack of mandatory EQAs for the HER2 IHC test has led to upfront HER2 ISH testing in many laboratories.

- NGS germline tests on DNA from blood of breast cancer patients in the frame of a genetic counselling are required if the patient fulfils the criteria of the Belgian Society of Human Genetics for germline testing for hereditary breast and ovarian cancer. Recent changes include indication for germline testing in case of diagnosis of breast cancer ≤40 years or triple negative breast cancer ≤60 years, irrespective of familial history.[3]
- For the NGS tests, standardised wet lab and bioinformatics methods, as well as more uniform biological and clinical variant interpretation are mandatory.[4]
- *ESR1* and *PIK3CA* mutational status test by the analysis of DNA extracted from a metastatic biopsy or circulating tumour DNA (ctDNA) by polymerase chain reaction or NGS is recommended in routine management for metastatic ER+/HER2- cancers. The detection of pathogenic mutation in the ligand binding domain of *ESR1* is correlated with secondary resistance to aromatase inhibitors (level

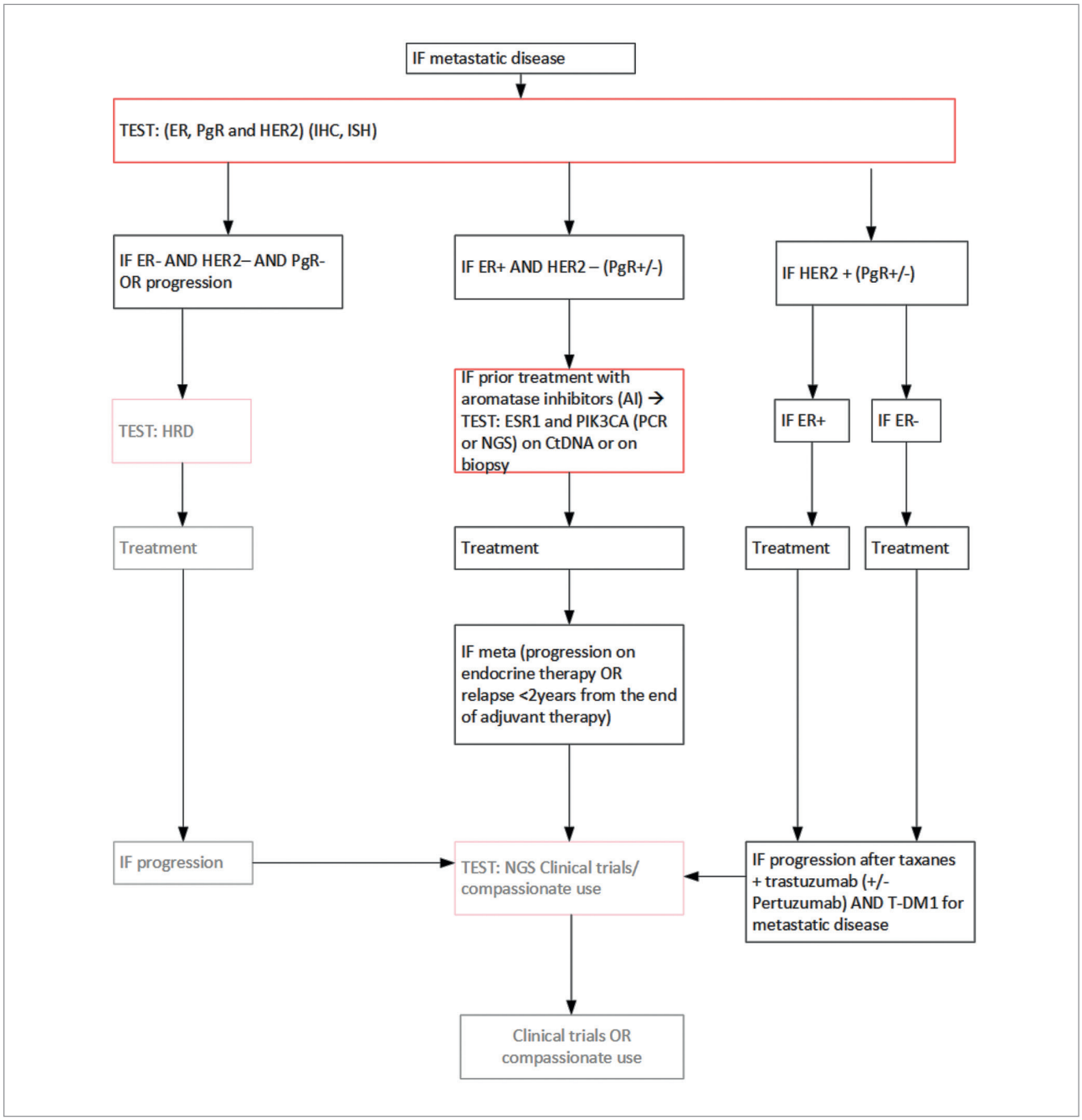

FIGURE 2. Breast algorithm: molecular tests with level 1 or 2A are represented in a red rectangle; molecular tests with a test level >2A are in shadow. *ER: oestrogen receptor, PgR: progesterone receptor, HER2: human epidermal growth factor receptor 2, IHC: immunohistochemistry, ISH: in situ hybridisation, HRD: homologous recombination deficiency, PCR: polymerase chain reaction, NGS: next generation sequencing, CtDNA: circulating tumour DNA, T-DM1: trastuzumab emtansine.*

2A).[5,6] Detection of hotspot *PIK3CA* mutation, and other genetic alterations in the PIK3CA/AKT/mTOR-pathway can be predictive for a therapeutic effect of PIK3CA, AKT and/or mTOR-inhibitors (level 3).

- Tests for the detection of homologous recombination deficiency (HRD), also referred to as *BRCA*ness tests, are emerging for the selection of patients who may require platinum-based chemotherapy or PARP inhibitors.[7-9] The best methodology to assess HRD status has yet to be determined, and several options are available:
  - NGS based platforms investigating mutations in a broad panel of genes involved in the HRD pathway;
  - commercially available assays based on MLPA and array-CGH technology;
  - if feasible, whole genome or exome sequencing to detect mutational signatures linked to HRD.

KEY MESSAGES FOR CLINICAL PRACTICE

For an optimal management of breast cancer, it is essential to establish clear molecular test workflows.

It is recommended for breast cancer management to perform:
- **Oestrogen receptor (ER), progesterone receptor and human epidermal growth factor receptor 2 (HER2) analysis for early breast and metastatic breast cancers.**
- **Next generation sequencing germline tests on DNA from blood of breast cancer patients, if the patient fulfils specific criteria, in early and advanced breast cancer. This should be proposed in the frame of a genetic consultation. The test has prognostic, predictive and preventive utility, in addition to familial genetic counselling.**
- **ESR1 and PIK3CA mutational status test for the determination of endocrine resistance mechanisms and subsequent therapeutic strategy.**

The following tests are emerging:
- **large next generation sequencing panel tests for metastatic breast cancers that have progressed after therapy to direct patients to clinical trials;**
- **tests for the detection of homologous recombination deficiency for therapy selection;**
- **multigene signatures to select patients with ER+/HER2- tumours who may forego chemotherapy.**

- There is currently insufficient evidence to classify other (emerging) prognostic and/or predictive biomarkers as level 1 or level 2A. Phase II-III clinical trials with several biomarker-specific drugs are now ongoing, and some of these already completed recruitment (e.g., ipatasertib in NCT03337724, alpelisib in NCT03056755 and NCT02437318, taselisib in NCT02340221, neratinib in NCT01953926). Other biomarkers demonstrated predictive value in phase III trials (e.g., somatic *BRCA* mutations predictive for carboplatin in NCT00532727). The classification of biomarkers in advanced breast cancers could then change rapidly. NGS with gene panels including these biomarkers can direct metastatic breast cancers patients to clinical trials or to ongoing compassionate use programs of the Federal Agency for Medicines and Health Product FAMHP (e.g., alpelisib for patients with a *PIK3CA* mutation). These molecular tests are currently not reimbursed by the reimbursement agency INAMI/RIZIV.[10]
- Multigene signatures (e.g., MammaPrint, Oncotype DX, Prosigna) are useful to select patients with ER+/HER2- tumours who may forego chemotherapy without relevant impact on survival.[11] However, the identification of the right target population is still a matter of debate, and, because these tests are performed in centralised foreign laboratories, their price is still high, which should be counterbalanced to the treatment-related side-effects and costs, sick-leave costs and psychosocial burden of adjuvant chemotherapy.

CONCLUSION

In conclusion, we have emphasised the importance of a clear workflow for an optimal clinical management of patients with breast cancer. Moreover, national quality control assessments of molecular tests linked with their reimbursements are crucial to ensure their quality (e.g., national EQA for HER2 IHC to be organised and national benchmark for NGS testing that is ongoing). Also, a more homogenous interpretation and annotation of the molecular test results as well as more homogenous test reporting throughout Belgium would increase Belgian healthcare quality.

Some of the molecular tests proposed here are not yet reimbursed by the Belgian reimbursement agency INAMI/RIZIV (e.g., multigene signature). As soon as the test levels increase for a particular biomarker, it is crucial that INAMI/RIZIV quickly updates the nomenclature. If not, these will result in higher expenses for laboratories.

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