Proposed Protocol for use of MammaPrint Microarray in Clinical Practice

Content:

1. What is MammaPrint

1. MammaPrint is a **RNA based micro-array genomic assay** performed on formalin fixed paraffin embedded (FFPE) tumour tissue. It consists of 70 genes which have been validated in an prospective, randomized trial (MINDACT) as a prognostic and predictive marker in non-metastatic breast cancer independent from traditional clinical parameters (Piccart et al 2016). MammaPrint is also provides an additional 80-gene genomic assay namely BluePrint.
2. **BluePrint** provides the molecular subtype .
    a. Luminal A (Low Risk)
    b. Luminal B (High Risk)
    c. Basal-like
    d. HER2 enriched

2. Purpose of this document

1. To provide a framework to funders wherein breast cancer patients who might benefit from MammaPrint can have fair access to microarray technology in South Africa.
2. To ensure the cost-effective use of MammaPrint as biomarker in early stage breast cancer
    1. If used appropriately it should reduce the use of chemotherapy by ~60% in ER/PR positive breast cancer when compared to those treated according to current clinical guidelines (Grant et al. 2013).
    2. In patients thought not to be in need of chemotherapy, ~38% will gain additional benefit when treated based on the MammaPrint result (Pohl et al. in press).
    3. In HER2 positive patients, Targetprint and Blueprint can quantify the HER2 over expression as well as provide the molecular subtype (Grant et al. 2015; Kotze et al. 2015). Up to 53% of tumours thought to be HER2 positive are of a different molecular subtype (Prat et al. 2014, Fehrenbacher et al 2014).
3. To provide treating clinicians with guidelines and information to select patients appropriately as well as allow timeous access to the test and the results in order to enable individualised treatment whilst maintaining autonomy in decision making.
4. To prevent inappropriate use of MammPrint in patient who will ultimately receive chemotherapy regardless of the result. To this end both the patient and treating phycician should be well informed and willing to apply treatment based on the MammaPrint result.

3. When should it be used?

MammaPrint is most FREQUENTLY used in the **adjuvant setting** in ER/PR positive tumours to decide on the appropriate use of chemotherapy with or without Traztuzamab as well as Stage Ia/b ER/PR negative HER2 positive tumours. Accurate nodal assessment is imperative to identify patients with more advanced nodal disease, therefore a point of caution is needed in patients who have a positive sentinel node where fewer than 3 nodes have been removed and it is suggested that patients in whom a complete axillary dissection is considered, the procedure be performed prior to requesting MammaPrint.

MammaPrint can also be used in the **neo-adjuvant setting** to select patients who should undergo neo-adjuvant chemotherapy with or without Traztuzamab as an alternative to primary surgery or neo-adjuvant endocrine therapy. In order to minimize the risk of clinical information becoming available after surgery, indicating a need for further chemotherapy in spite of an initial low-risk MammaPrint result, it is imperative that adequate sampling of the tumour and regional lymphnodes be done. Four to 5 core needle biopsies or vacume biopsies should be performed during the diagnostic workup as

well as a sentinel node biopsy / axillary node sampling obtaining 3 or more nodes prior to requesting MammaPrint.

4. Who will qualify?

a. **Selection criteria**

<table>
<tr>
<td>To determine adjuvant chemotherapy:
ER/PR positive, HER2 negative tumours:
Node negative tumours ≥10 mm
or
Any tumour size with ≤ 3 nodes involved</td>
<td rowspan="2">CONTRA-INDICATIONS

• ER/PR/HER2 negative breast cancer (TNBC)
• Node-negative ER/PR positive tumours <10 mm
• HER2-positive tumours ≤5 mm
• HER2 negative tumours with 4 or more nodes involved or Extra-nodal extension
• Prior chemotherapy for current tumour
• Confirmed metastatic disease
• Any contra-indication for chemotherapy or Traztuzumab where applicable
• Refusal to apply treatment decision based on MammaPrint</td>
</tr>
<tr>
<td>To determine the appropriate use of Herceptin:
ER/PR positive, HER2-positive Tumours:
Any tumour size
• HER2:CEP17 ratio ≥ 4 but Copy number <12
• HER2:CEP17 ratio < 4 but Copy number ≥12

All tumours with Equivocal HER2 results
• Single Probe ISH Copy number >4 and ≤ 6
• Dual Probe ISH with HER2:CEP17 ratio < 2 but Copy number >4 and ≤6
ER/PR negative, HER2 positive tumours:
Node negative patients with tumours ≥5mm but < 10mm regardless of FISH result</td>
</tr>
</table>

b. Motivation for special cases

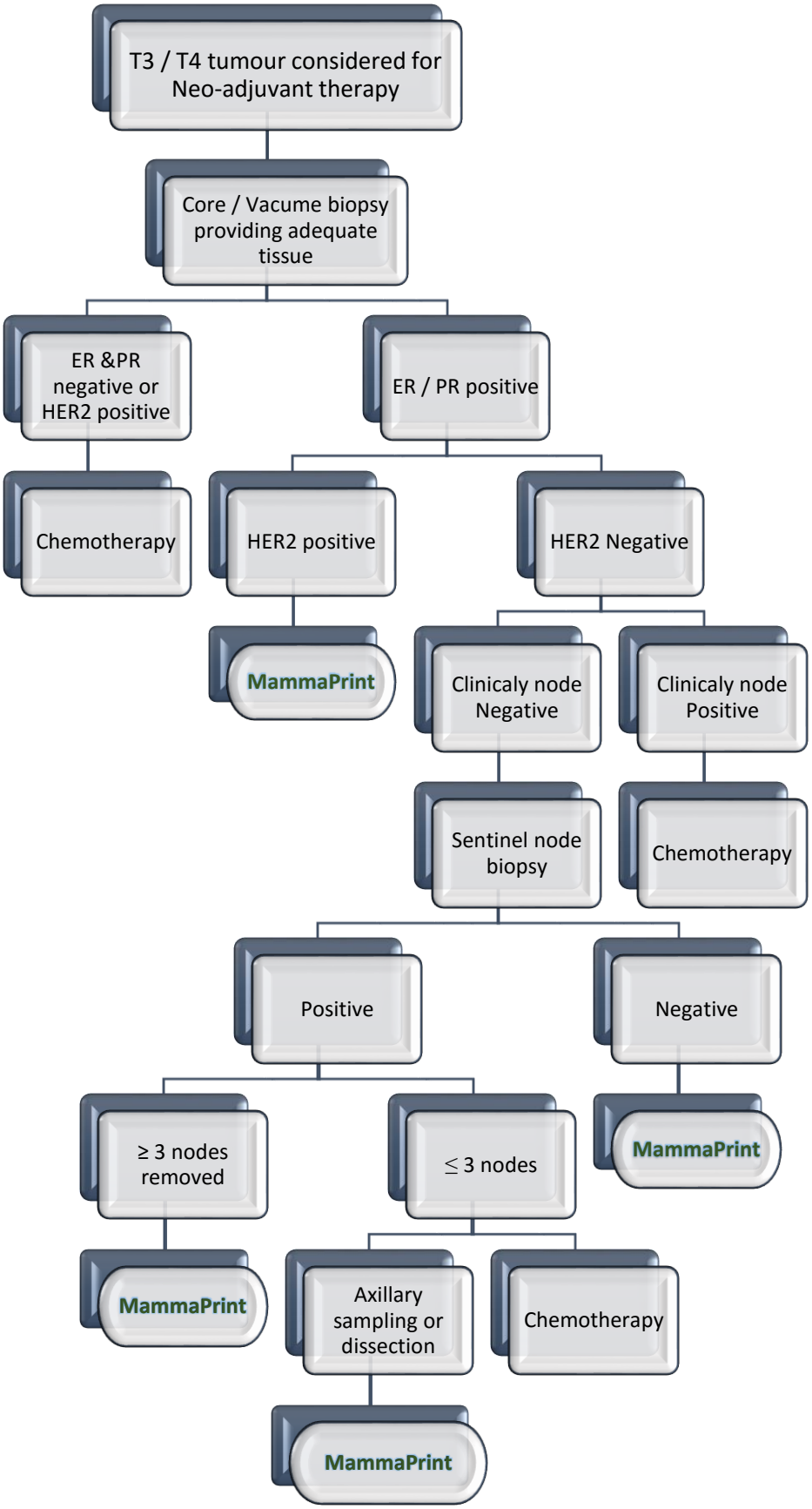
Although careful thought has been put into the development of the decision making protocol, it is expected that there might be a small number of patients who do not fullfill all the criteria, but whom might benefit from MammaPrint or where it would be inadvisable to treat the patient based on the MammaPrint result. In these exeptional circumstances a motivation should be drawn up for deviating from the MammaPrint testing and treatment algorithm provided below. It should include thorough clinical information to be assessed by a MammaPrint Expert Panel appointed by the Medical Scheme.

5. Requisition process

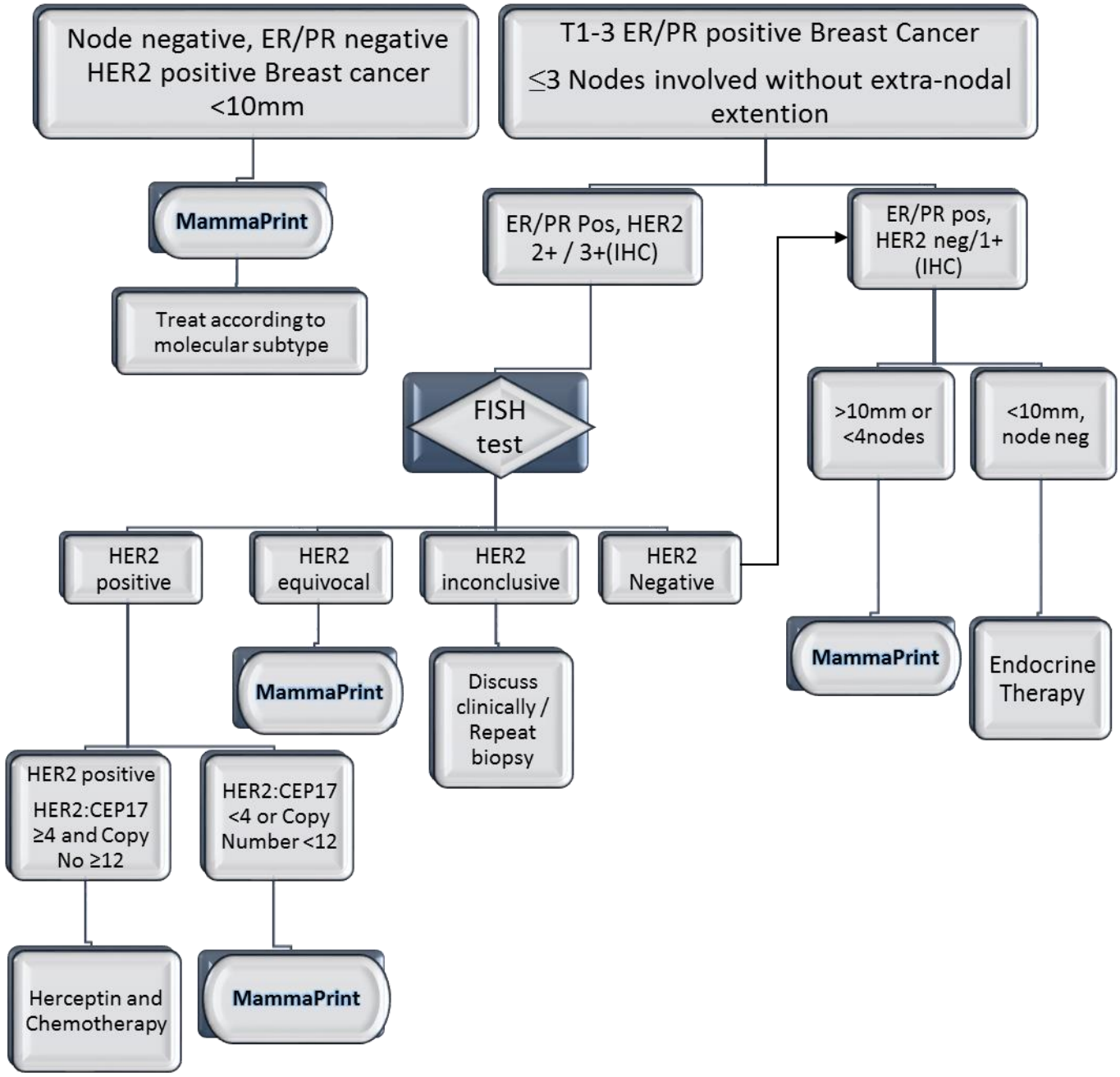
Upon considering MammaPrint, the clinician should be provided with the full protocol to be used for breast cancer patients, including a check list to confirm patient eligibility for testing. The application should be accompanied by all the relevant clinical information such as the histology report and mammography report. A consent form should be signed by both the patient and the treating clinician when requesting reimbursement from their medical scheme, indicating:

1. An understanding of MammaPrint and the implications of the result.
2. Consent for genomic testing.
3. An undertaking to use / omit chemotherapy and/or Trastuzumab based on the MammaPrint result.
4. That failure to treat according to the MammaPrint result without due clinical motivation will result in the patient being responsible for payment of the test.
5. Seeking further clinical opinions after the test has been requested, would not absolve the patient from the provisions of point 4.
6. Agreement that the genetic test result may be made available to the medical scheme in the event that authorisation for reimbursement is based on a requirement for data review.

6.1 Clinical decision making diagram – Neo-adjuvant use



6.2 Clinical decision making diagram – Adjuvant us



7 Background

MammaPrint microarray testing consists of two components reimbursed by at least 20 medical schemes in South Africa and Namibia based on the following benefits:

a. **MammaPrint**: The 70-gene expression profile determines high/low risk of distant metastasis independent of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) status.
b. **BluePrint**: 80-gene expression profile subdivides breast tumours into Luminal A, Luminal B, HER2-enriched and Basal-like intrinsic subtypes to identify active treatment pathways.

Although MammaPrint provides prognostic risk assessment the addition of molecular subtyping identifies the true processes which are driving tumour proliferation. The molecular subtypes (Luminal A and B, Basal-like and HER2 enriched) help to distinguish driver pathways from passenger pathways, allowing more appropriate treatment selection by:

1) subdividing hormone-positive breast cancer into the Luminal A and Luminal B molecular subtypes
2) identifying endocrine treatment resistant ER-positive tumours lacking ER-alpha function (basal-like), and
3) subdividing HER2-positive tumours into Luminal and HER2-enriched subtypes

Breast cancer patients are treated in an environment of imperfect information subject to ever expanding understanding of the tumour biology. The ability to determine genetic changes in cells that drive proliferation led to the development of drugs such as Trastuzumab (Herceptin) that specifically target these changes. Biological agents like Trastuzumab are expensive and still have significant risks. In order to optimize the benefit gained by these agents, accurate determination HER2 status is essential. Currently all newly diagnosed breast cancer are tested for ER, PR and HER2 overexpression using IHC. If HER2 is found to be 2+ or 3+, a dual probe FISH is usually requested to quantify the level of overexpression/amplification, but even this is fraught with inaccuracies due to the use of CEP17 as a surrogate for chromosome 17 polysomy. Borley et al. (2014) have shown that a FISH HER2:CEP17 ratio of <4 and copy number under 12 have a poor response to Trastazamab.

By adding molecular subtyping using BluePrint, identifying true HER2 positive tumours is now possible. Whitworth et al. (2014) demonstrated that Blueprint enables further refinement of treatment decision-making in IHC/FISH HER2-positive patients in a similar way that MammaPrint or Oncotype DX informs selection of chemotherapy in ER/PR positive tumours. Notably, 22% of the more than 400 breast cancer patients studied were reclassified into different subgroups compared with conventional assessment, shown to result in an improved distribution of response rates in the relevant treatment groups. The study performed by Yao et al. (2015) validated the rational-based method applied in the development of Blueprint, leading to a functional subtyping profile.

In a recent study from Cape Town it was found that only 3 of 19 ER/PR/HER2 positive tumours were of the HER2-enriched subtype while 6 were Luminal A and 10 were Luminal B. In 5 of the patients, FISH tests were repeated and 4 were found to be contradictory (Myburgh et al 2016) . This confirms other reports about the overestimation of clinical Her2-positivity when molecular subtyping was used as control and the poor response to Herceptin in Luminal A and B patients.

Since FDA approval was obtained for MammaPrint in 2007 with use of fresh tumour biopsies, and again in 2015 for use of FFPE, we accumulated real-world observational data on more than 250 breast cancer patients referred for microarray testing. Application of the data in ethically approved research projects have made meaningful contributions to our collective understanding of all the aspects that need to be taken into account for development of a Clinical Practice Guideline for Breast Cancer genetic testing using

the MammaPrint microarray platform. This includes the requirement for clear communication to patients of sometimes uncertain risks and benefits that determine their choice to receive systemic therapy.

Central data collection and audit

Although results from international trials are available on the use of genomic assays, there is a paucity of data on South African patients and protocols. It is important that clinical and genomic data be collected centrally to allow periodic review and research. Ethics approval for continuation of the protocol used to obtain informed consent for genomic breast cancer research as part of two postgraduate studies will be applied for on an annual basis.

Personalised Decision making

Although careful thought has been put into the development of the decision making protocol, it is expected that there might be a small number of patients who do not fullfill all the criteria, but whom might benefit from MammaPrint or where it would be inadvisable to treat the patient based on the MammaPrint result. A special motivation path is therefore suggested for such situations falling into three broad catagories:

1. Eligibility for MammaPrint

   In these cases the patient does not comply with the proposed inclusion and exclusion criteria for MammaPrint. Due to special clinical circumstances the treating clinician feels that MammaPrint would help in decision making, a special motivation for the test can be written.

2. Treatment of bordeline cases

   In 1-2% of cases the 70-gene MammaPrint test might give a result which is very close to the cut-off point between high and low risk whilst revealing a Luminal type tumour. Small variations in genetic expression have been described within the same tumour. In these cases that are clearly marked as “borderline” in the reports the clinician should be allowed to motivate for the use of chemotherapy.

3. Unexpected additional clinical information

   In such cases, the decision making protocol would have been followed, but after the MammaPrint results were obtained additional clinical information comes to light, neccesitating the use of chemotherapy. A full clinical report detailing the reasons for deviation from the expected treatment path should be written to prevent the patient from becoming responsible for the cost.

   In these exeptional circumstances discussion at a multidisciplinary tumour meeting or contacting experienced local researchers on MammaPrint is advised. If a motivation is drawn up for deviation from the MammaPrint testing and treatment algorithm provided below, it should include thorough clinical information to be assessed by the Medical Scheme MammaPrint Expert Panel.

6. Selected References

Borley A, Mercer T, Morgan M. Impact of HER2 copy number in IHC2+/FISH-amplified breast cancer on outcome of adjuvant trastuzumab treatment in a large UK cancer network. Br J Cancer 2014; 110: 2139-2143.

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7. ASCO 2013 guidelines for HER2 reporting

ASCO guidelines for HER2 reporting - 2013
Must report **HER2 test result as positive** for HER2 if: • IHC 3+ based on circumferential membrane staining that is complete, intense • ISH positive based on: Single-probe average HER2 copy number ≥6.0 signals/cell Dual-probe HER2/CEP17 ratio ≥2.0 with an average HER2 copy number ≥4.0 signals per cell Dual-probe HER2/CEP17 ratio ≥2.0 with an average HER2 copy number <4.0 signals/cell Dual-probe HER2/CEP17 ratio <2.0 with an average HER2 copy number ≥6.0 signals/cell Must report **HER2 test result as equivocal** and order reflex test (same specimen using the alternative test) or new test (new specimen, if available, using same or alternative test) if: • IHC 2+ based on circumferential membrane staining that is incomplete and/or weak/moderate and within >10% of the invasive tumour cells or complete and circumferential membrane staining that is intense and within ≤10% of the invasive tumour cells • ISH equivocal based on: Single-probe ISH average HER2 copy number ≥4.0 and <6.0 signals/cell Dual-probe HER2/CEP17 ratio <2.0 with an average HER2 copy number ≥4.0 and <6.0 signals/cell Must report **HER2 test result as negative** if a single test (or both tests) performed show: • IHC 1 as defined by incomplete membrane staining that is faint/barely perceptible and within > 10% of the invasive tumour cells • IHC 0 as defined by no staining observed or membrane staining that is incomplete and is faint/barely perceptible and within ≤ 10% of the invasive tumour cells • ISH negative based on: Single-probe average HER2 copy number <4.0 signals/cell Dual-probe HER2/CEP17 ratio <2.0 with an average HER2 copy number <4.0 signals/cell Must report **HER2 test result as indeterminate** if technical issues prevent one or both tests (IHC and ISH) from being reported as positive, negative, or equivocal.