Signal Detection

A new biomarker has been discovered for a specific, aggressive type of breast cancer that had previously eluded diagnosis until late stages of the disease and thereby compromised treatment

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Breast cancer is the most common cancer in women worldwide, with nearly 1.7 million new cases diagnosed in 2012 and a five year prevalence of 6.23 million. About 400,000 cases per year take place in Europe, 315,000 in the US and 460,000 in the rest of the world (1). This represents about 12% of all new cancer cases and 25% of all cancers in women.

There are three major types of breast cancer, characterised by the presence of key biomarkers: oestrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor (HER2). A fourth type, characterised by the lack of all three biomarkers, is informally known as triple-negative.

However, there is another type of breast cancer, which is very aggressive, extremely difficult to diagnose at presentation and 'hidden' among ER, PR, HER2 and triple-negative expressing tumours. It used to be referred to as the 'aggressive type' and has only recently been named basal-like breast cancer (BLBC) (2-4) (see Figure 1).

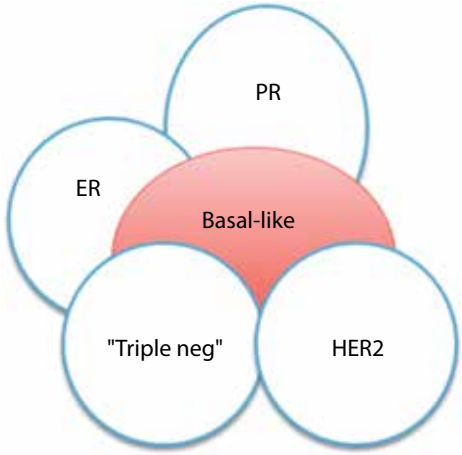

Figure 1: Types of breast cancer, including 'hidden' basal-like type

What is BLBC?

BLBC is a hormone-independent, aggressive type of breast cancer with a high rate of metastasis to the brain and lungs. It affects up to 30-45% of breast cancer patients, depending on ethnicity (5-7). BLBC is not synonymous with triple-negative breast cancer, nor is its occurrence limited to this cancer sub-type.

BLBC is very difficult to identify at the time of the initial diagnosis of breast cancer. It is usually detected two to five years later when patients return with an unexpectedly worse course of disease – and usually with metastasis to the brain or lungs. At this point, effective therapy has been compromised (4,6,8). About 50% of BLBC patients develop metastasis and 30% die in five years (8). Additionally, about 90% of BRCA1 gene mutation carriers are likely to develop BLBC. Furthermore, approximately 20-30% of BLBC tumours also express ER/PR and/or HER2 marker. This confuses the clinical picture, leading to misdiagnosis (8-10).

Until recently, BLBC lacked biomarkers capable of guiding diagnosis, prognosis and therapy, as ER, PR and HER2 biomarkers cannot detect BLBC. The 'gold standard' was based on a 306-gene microarray, unsuitable for large-scale clinical use. Other biomarkers explored in research settings included cytokeratins (CK5/6, CK14 and CK17), either alone or in combination with P-cadherin, caveolin 1 and 2, c-kit or p53 (11-13). However, this approach has not yet progressed to clinical use.

As such, BLBC patients are poorly diagnosed at onset and are treated with ineffective therapies. Therefore, the need for accurate and timely diagnosis of BLBC status in patients diagnosed with breast cancer is unmet.

The Role of FOXC1

The discovery and clinical validation of new cancer biomarkers is a complex process. Biomarker validation first requires discovery in a basic research setting, followed by clinical validation in a number of patient studies and, later, manufacturing and regulatory approval, according to guidelines in Europe or the FDA in the US. Even though quite a few candidate biomarkers have been reported in literature, the last diagnostic test for breast cancer based on a single biomarker was HER2, which was introduced in 1998.

FOXC1 is a 70kD nuclear transcription factor known to play a key role in regulating cell growth, proliferation and differentiation in mesodermal tissue development. It is not expressed in adult or normal breast tissues.

The role of FOXC1 as a biomarker specifically for BLBC was discovered in 2010 by a group of oncologists at the John Wayne Cancer Institute in Santa Monica, California, as part

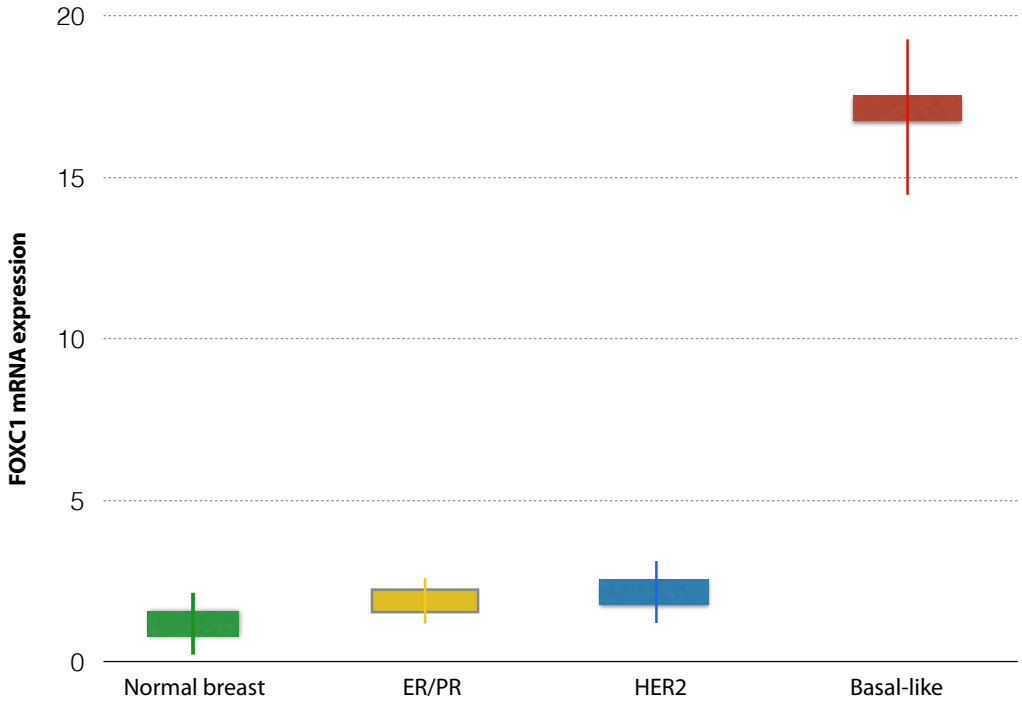

Figure 2: FOXC1 expression in breast cancer sub-types

of functional transcriptomic analysis of gene expression in microarray data sets from published clinical trials. The degree of correlation of each individual gene with the basal-like subtype based on messenger RNA (mRNA) expression was used to identify genes highly specific to BLBC. The following parameters were evaluated:

- Full clinical annotation (eg age, metastasis profile, survival)
- Biomarker expression: ER, PR, HER2, Ki-67, p54, CK5, CK14, CD109 and p-Cadherin
- Gene-expression profile from published microarray data

About 5,096 breast cancer patients from 13 published clinical studies were evaluated. From these results, the FOXC1 transcription factor emerged as a top-ranking biomarker for BLBC (7,14,15). Figure 2 shows that the expression of FOXC1 is specifically associated with BLBC as opposed to HER2 and ER/PR breast cancer or normal breast tissue. The team sought to distill down the complex information obtained from expensive, high-throughput platforms, such as gene expression profiling, to a simpler, inexpensive and more widely used platform, like immunohistochemistry, without compromising accuracy. While this is highly desirable in the field of cancer diagnostics, it had never been attempted before.

To verify these results, five retrospective clinical studies were performed by immunohistochemistry (IHC) staining of formalin-fixed, paraffin-embedded tissues from 1,343 breast cancer patients with known clinical history. The goal from the start was to develop a simple diagnostic test based on a single biomarker that could be run by any pathology laboratory utilising established procedures, irrespective of resource-challenged environments.

The clinical validation study was divided into two phases. FOXC1 IHC test prototype was first evaluated in 897 breast cancer patients with a polyclonal anti-FOXC1 antibody (7). The proprietary monoclonal anti-FOXC1 antibody B2E3 was then raised and validated in two clinical studies, with 451 patients, sponsored by the National Cancer Institute in the US (15). Results of the IHC staining score were compared to gene-expression in the same tumour samples using the PAM50 test, a polymerase chain reaction-based determination of 50 genes in breast cancer tissue (15). These findings have been independently verified by a clinical study with 81 BRCA1-positive breast cancer patients (16).

For suitable interpretation of FOXC1 IHC staining, a scoring system of zero to eight, specific to FOXC1, was developed (15).

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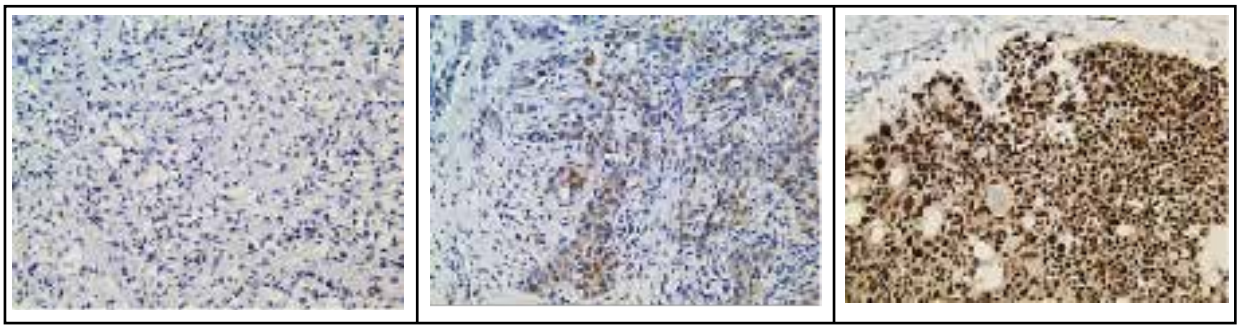

Modified Allred score 0 | Modified Allred score 4 | Modified Allred score 8

Figure 3: FOXC1 IHC staining of breast cancer tissue

This is based on a modification of the Allred scoring system developed for ER receptors, a standard of care for breast cancer diagnosis (17,18). Figure 3 shows the IHC staining of breast cancer tissue for FOXC1 expression.

These studies demonstrated that the presence of FOXC1 is capable of detecting BLBC with sensitivity and specificity via standard IHC staining of breast tissue slides.

From these results, a new test for BLBC was developed. Verification and validation lots of the test components were produced and tested under ISO13485 requirements. The Declaration of Conformity was filed with MHRA less than six years after the role of FOXC1 in BLBC was first reported.

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