

## Breast cancer - frontiers beyond surgery

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Breast cancer is the commonest female cancer world over, comprising 16% of all female cancers. According to The World Health Organisation, in 2004 an estimated 519,000 women died due to breast cancer. The highest age-standardised incidence of new female breast cancer cases was in North America (99.4 per 100,000) and the lowest was in African countries [1]. The incidence increases linearly with age up to menopause, after which the increase is less marked, and almost absent in developing countries [2]. Mortality rates that remained relatively stable between 1960 and 1990 in most of Europe and the Americas, have declined appreciably to 25-30% in northern Europe [3]. Survival rates are highest (80%) in North America, Sweden and Japan but are below 40% in low income countries [4], with developing countries contributing to 69% of all breast cancer deaths [1]. The low survival rates in less developed countries can be explained by the lack of screening programmes leading to late presentation, as well as inadequate facilities for diagnosis and treatment. In Sri Lanka, breast cancer is the commonest female cancer comprising 25.4% of all Sri Lankan female cancers, and 14.3% of all cancers and is the commonest site specific cancer [5].

### The changing face of surgery

Surgery plays a central role in the locoregional control of solid organ tumours both in the curative and palliative setting. Relative to other forms of cancer therapy, it is a rapid and efficient method of cytoreduction in malignancies. However, as with any modality of therapy the associated morbidity and mortality of surgery at times outweighs the benefits of treatment. The surgical management of solid organ malignancies including breast carcinoma has evolved over the decades due to the convergence of a multitude of factors. These include a better appreciation of the biology and behaviour of tumours and the development of efficient and more targeted non-surgical modalities of therapy with the aid of immunohistochemical and molecular markers. The transformation over time of the management

of breast carcinoma from the super radical mastectomy of Wangensteen and the radical mastectomy of Halsted to the modified radical mastectomy and breast conservation surgery with sentinel node sampling exemplifies this shift away from mutilating to more acceptable yet equally effective forms of surgery. This has been matched by the increased use of targeted systemic hormonal and immunotherapy in the treatment of breast carcinoma.

As such, it is of importance that surgeons are aware of the new therapeutic strategies available and the basis for their use in the management of breast cancer.

### Implications of genetics, epigenetics, cell signaling pathways in targeted treatment of breast cancer

The knowledge of genes, gene products, cell signaling pathway receptors and other molecules associated with breast cancer has provided us with a better understanding of the disease. This has created a new window to develop targeted treatment strategies, predict therapeutic responses, and prognostication.

Breast cancer is a heterogeneous disease. Tumours with different biological features have different clinical outcomes and responses to therapy. Although epidemiological evidence supports certain risk factors (such as age, obesity, alcohol intake, lifetime oestrogen exposure such as early menarche, late menopause, nulliparity) a family history and a personal history of breast cancer remain the strongest risk factors for the disease. The interaction of inherited and environmental factors leads to progressive accumulation of genetic and epigenetic changes in breast cancer cells. Although most breast cancers are due to lifetime noninherited mutations, germline mutations transmitted down the families comprise nearly 20% of all cases and have a distinctive pathogenesis related to the gene involved [6].

Although the genes responsible for most familial breast cancers have not been identified, half of these cancers are caused by germ-line mutations in tumour suppressor genes (TSGs). Of these, BRCA1 and BRCA2 are the most well known and comprise 5 - 10% of all breast cancers [7]. There are a number of other gene mutations which could be responsible for breast cancer such as p53, PTEN, ATM, CHEK2, BRIP1, NBS1, RAD50, MSH2 and MLH [8]. The estimated lifetime risk of developing breast cancer for women with BRCA1 and BRCA2 mutations is 40% -

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85%. These women have an increased risk of ovarian cancer as well as contralateral disease that may be as great as 5% per year [9]. Male carriers of BRCA2 mutations are also at increased risk of breast cancer. Mutations in these TSGs lead to defects in DNA repair mechanisms and are associated with genetic predisposition to breast cancer. Poly ADP-ribose polymerase 1 inhibitors (PARP inhibitors) dramatically reduce repair of DNA strand breaks in BRCA deficient tumours, resulting in increased tumour sensitivity to DNA damaging agents such as cisplatin [10]. As a result, PARP inhibitors are expected to be highly specific for cancer cells and yet non-toxic for healthy tissues in BRCA associated breast cancer.

In addition to the inherited mutations described above, sporadic breast cancers exhibit mechanisms referred to as epigenetic mechanisms, where there are no changes in DNA nucleotide sequences, but aberrations in processes such as DNA methylation and histone deacetylation. These lead to alterations of gene expression such as inactivation of several DNA repair genes including BRCA1, ATM, CHK2, and P53. The end result is a cancer with a biological behaviour similar to the phenotypes produced by inherited mutant carriers such as BRCA1 [11]. These abnormalities are potentially reversible by inhibitors of DNA methylation and histone deacetylation and may be useful in treating relevant subsets of breast cancer patients [12].

There are multiple cell signalling pathways implicated in breast cancer biology. The role of oestrogen in the pathogenesis of breast cancer is well known and the most documented. It has a marked proliferative effect on normal mammary epithelium through the activation of the oestrogen (ER) a nuclear hormone receptor. ER is over-expressed in nearly 70% of breast cancers and it is the most used biological target in breast cancer treatment. Oestrogen exerts its genomic action of ER by transcriptional regulation of oestrogen responsive growth promoting genes. Circulating oestrogen action can be inhibited by selective oestrogen receptor modulators (SERMs) such as Tamoxifen which blocks breast related receptors while preserving oestrogen action on bones and the endometrium. An alternative therapeutic approach is reducing the circulating levels of oestrogen by oophorectomy or the use of gonadotropin releasing hormone agonists (GNRH) such as Gosereline in premenopausal females. In post menopausal women who lack ovarian oestrogen, aromatase inhibitors are used to block the enzyme aromatase which produces peripheral oestrogen from androgens. The hormone receptor status ie. oestrogen receptor (ER) and progesterone receptor (PR) positivity is a good prognostic feature and oestrogen blockade has been shown to be an effective and minimally toxic targeted strategy in these patients.

In addition to its genomic action oestrogen also has a non-

genomic action with a much more rapid onset due to activation of membrane bound and/or cytosolic ERs. This non-nuclear ER action leads to the phosphorylation and activation of epidermal growth factor receptors (EGFRs) and some components of the phosphatidylinositol - 3 kinase (PI3-K) pathway [13]. This cross talk between ER and growth factor receptors is bidirectional and human epidermal growth factor receptor 2 (HER2) can increase ER signalling to the extent of the tumour being unresponsive to anti-oestrogen therapy. This is the mechanism by which some patients with both ER and HER2 positivity develop resistance to oestrogen blockade.

Well recognised growth factor receptor pathways include epidermal growth factor receptors - EGFR (HER 1, ErbB1) and human epidermal growth factor receptor - HER (EGFR2 or ErbB2). Amplification and over expression of HER2 (found in 20-30 % of breast cancers) is clearly associated with accelerated cell growth and proliferation as well as an increased risk of disease recurrence with shortened overall patient survival [14]. HER2 acts by deregulation of the G1/S phase of cell cycle control via cyclins and also interaction with important 2nd messengers such as kinases [15]. HER2 amplification is a marker of therapeutic response to monoclonal anti-HER2 antibody, trastuzumab (Herceptin). HER2 receptors also form heterodimers with other EGFRs. Therefore, inhibiting tyrosine phosphorylation and the tyrosine kinase signaling pathway by using a dual-tyrosine kinase inhibitor (TKI) such as Lapatinib, provides a useful therapeutic option in trastuzumab refractory patients with metastases [16]. HER2 status is commonly tested by immunohistochemistry of the tumour tissue, but in equivocal HER2 status, fluorescence in situ hybridisation (FISH) should be employed to confirm HER2 status prior to starting anti-HER treatment. Of late, reverse-transcriptase polymerase chain reaction RT-PCR also has been used to assess HER2 status.

Insulin like growth factor 1 (IGF-1) also known as somatomedin C initiates intracellular signalling primarily through its IGF-1 (IGF-1R) tyrosine kinase receptor present in many cell types in the body. IGF-1 is one of the most potent natural activators of the AKT signalling pathway, a stimulator of cell growth and proliferation, and a potent inhibitor of programmed cell death leading to anti-apoptosis. IGF-1 and other tyrosine kinase growth factor receptors signal through multiple pathways. A key pathway is phosphatidylinositol 3 kinase (PI3-K) and its downstream partner, mammalian target of rapamycin (mTOR) which has a pro-growth effect. mTOR inhibitors such as rapamycin analogues are currently under trial as anti-cancer agents. Elevated IGF-1 levels have been implicated in breast cancer. Monoclonal antibodies and other

IGF-1R inhibitors are under study as therapeutic agents for breast cancer [17].

Breast cancer cells have been shown to express vascular endothelial growth factor receptors VEGFRs which are another group of tyrosine kinase receptors with the ability to induce angiogenesis. Bevacizumab a monoclonal antibody and sunitinib a TKI have anti-VEGFR activity and are under therapeutic trials [18].

### Therapeutic implications of molecular subtyping

Molecular subtyping of breast cancer is the subject of much research but is yet to influence standard clinical management protocols. Currently prognosis and treatment decisions are guided by tumour stage, grade, hormone receptor status and HER2 status. Molecular subtyping is a step beyond this and future therapeutic decisions and options in breast carcinoma are likely to be guided by this.

Human mammary glands contain two distinct subtypes of epithelial cells, basal (myoepithelial) and luminal, which can be recognised by cytokeratin expression. Most breast cancers are of the luminal type but less commonly basal types which represent more aggressive tumours occur [19]. Using cDNA microarrays followed by immune histochemistry (IHC) breast cancer can be divided into four major molecular subtypes: luminal A, Luminal B, triple negative/basal-like, HER2 type [20]. Other less common molecular subtypes include normal breast-like, apocrine molecular type. Breast cancers that do not fall into any of these subtypes are often listed as unclassified.

Though molecular and genetic information is needed to accurately determine subtypes, characteristics such as hormone receptor status, HER2/neu status and proliferation rate (eg: Ki67) can also assist in defining the four major subtypes. Tumour behaviour and therapeutic response of each subtype is related to these known characteristics. The most common profile for each subtype has been identified, though all within each subtype may not share all these features. Luminal A: ER+ ve and/or PR+ve, HER2- ve and low Ki67 and a prevalence of 42-59% ; Luminal B : ER+ ve and/or PR+ve, HER2+ ve (or HER2- ve with high Ki67) and a prevalence of 6-19% ; Triple negative/basal-like : ER-ve, PR-ve, HER2-ve, cytokeratin 5/6 + and/or HER1+ ve and a prevalence of 14-20%; HER type : ER-ve, PR-ve, HER2+ ve and a prevalence of 7-12% [21,22].

Luminal A tumours generally have a low or moderate tumour grade, a 15% p53 mutation rate (poor prognostic sign) and have the best prognosis, with relatively higher survival rates and low recurrence rates. Women with luminal B tumours also have high survival rates, but compared to luminal A tumours are often diagnosed at a younger age and have factors that lead to a poorer prognosis [21].

Following loco-regional treatment luminal tumours can be controlled with oestrogen blockade but especially in type B patients who have poorer prognostic features such as young age, lymphovascular invasion, nodal involvement, high Ki67 or HER 2 positivity, chemotherapy may be indicated. Trastuzumab (Herceptin) will have a place in HER2 +ve type B cases.

Most triple negative tumours are basal-like and most basal-like tumours are triple negative, but the overlap of these two categories (which account for 14-20% of all breast cancers) is not 100%. These tumours tend to occur more often in younger women, and most BRCA1 breast cancers and many BRCA2 breast cancers have both these features. Many of these tumours contain p53 mutations and are often aggressive with a poorer prognosis compared to ER+ luminal subtypes [22]. Triple negative/basal-like tumours are usually treated with a combination of surgery, radiation therapy and chemotherapy. These tumours cannot be treated with hormone therapies or trastuzumab because they are hormone receptor-negative and HER2 negative. The genes linked to basal-like tumours are not well understood at this time and thus, targeted therapies do not yet exist. However, potential targets for future therapies include the EGF receptor, aB-crystallin and cyclin E [23]. Clinical trials studying treatment options for triple negative/basal-like tumours are underway.

About 7-12% of breast cancers are of the HER2 subtype (HER+ve, ER-ve, PR-ve). Many HER2+ tumours contain p53 mutations [22]. HER2+ ve tumours have a poor prognosis and are prone to early and frequent recurrences and metastases [24]. Women with HER2+ tumours appear to be diagnosed at a younger age than those with other subtypes and are currently offered trastuzumab (Herceptin) in prophylactic settings as well as for treatment of metastases.

Normal breast like sub-type is a less common molecular subtype of tumour. About 6-10% of all breast cancers fall into this category [22]. These tumours are most often small and tend to have a good prognosis. Normal breast-like tumours are more common among post-menopausal than pre-menopausal women [25]. Some researchers question though whether these tumours are a distinct molecular subtype.

### Using gene expression profiling to fine tune treatment

Gene expression profiling is a tool that allows researchers to study thousands of genes at a given time. Studying which genes are active (expressed) and which are inactive in different types of tumour cells may help researchers to develop more targeted treatments. This tool may enable researchers to compare gene expression in treated versus untreated tumour cells to understand the effects of treat-

ment on tumour tissue. The genetic profiles of tumours could also help predict which cancers may be more aggressive and more likely to recur. Tumours with gene profiles showing a high risk of recurrence may be more likely to respond to chemotherapy than tumours with low risk gene profiles. Oncotype Dx is a multigene expression assay which helps to differentiate the subset of patients initially assessed to have a good prognosis (ER +, early stage breast cancers) but who later go on to develop metastases. This test uses real-time RT-PCR to check for a panel of 21 genes and gives a likelihood of recurrence score and has been approved by US food and drug administration (FDA) to identify these patients as candidates for initial adjuvant chemotherapy, prior to starting long term hormone therapy [26].

Mammaprint is a 70 gene expression profiling tool under study to assess its validity as a predictor of lymph node-negative breast cancer (both ER +ve and -ve) [27].

### Personalising chemotherapy schedules

Chemotherapy sensitivity and resistance assays (CSRAs) are a relatively new wave of tests done for many types of cancers. The extreme drug resistance (EDR) assay is an advanced laboratory test for cancer, also known as a chemotherapy drug resistance test. These tests are performed by growing a fresh samples of the cancer cells in the presence of different cytotoxic drugs in the laboratory. If the cells grow in the presence of a very high (extreme) dosage of a cytotoxic agent, studies have shown that the cancer is unlikely to show a clinical response in vivo. The percentage of viable cells is given as a resistance score ranging from low to high. In the chemosensitivity assay the test design is similar to the above, but the percentage of cells that has been destroyed is taken as the test result. These assays are still considered experimental, and would be offered only in clinical trial settings [28].

It is also important to gauge the benefit of chemotherapy in relation to the patient and the tumour. One tool that allows for such refinement is 'Adjuvant' an online programme that quantifies the benefit of adjuvant treatment [29]. This integrates tumour size and bio marker information, patient age, health status, and the relative benefits of chemotherapy as measured in clinical trials. The report is given in a bar graph format and helps patients and physicians to make rational choices regarding chemotherapy.

### What is new in radiotherapy?

The two main drawbacks of radiation therapy are the frequency and length of the treatment. Treatment is usually given daily, five days a week, for five to seven weeks. New techniques aim at shortening the course of treatment, while maintaining efficacy and minimising morbidity. These

techniques are under trial and results will decide whether these therapies become part of standard care.

Accelerated, hypofractionated whole-breast irradiation resembles standard radiation therapy apart from using a slightly higher radiation dose per session (hypofractionation) leading to a shorter course (accelerated therapy). The few randomised clinical trials that have been done show promising results with similar long-term (10 year) side effects [30].

Accelerated partial breast irradiation delivers radiation only to the area around the tumour bed. This reduces the number of treatment sessions (twice a day for one week). Radiation can be delivered by brachytherapy, three-dimensional conformal external beam or by intraoperative radiation therapy. Brachytherapy uses targeted radiation therapy placed inside the tumour bed in the form of implanted radiation 'seeds' (interstitial radiation therapy) or a single small balloon device (intracavitary radiation therapy, MammoSite) that is used to deliver the radiation. Three-dimensional (3D) conformal external beam radiation therapy uses standard external beam radiation to target only the tumour bed. Intra-operative radiation therapy delivers a single dose of radiation to the tumour bed during breast surgery (lumpectomy) which is of a higher dose than in a standard radiation session. All these three techniques though apparently effective are still under trial [3].

### Circulating tumour cells

Many studies have shown circulating tumour cell levels can help predict survival time for people diagnosed with metastatic breast cancer. The more circulating tumour cells in the blood, the more advanced metastatic breast cancer is likely to be. Having more of these cells may also predict a lack of response to treatment. At this time, circulating tumour cell testing is not routinely done in clinical practice. It is still not known how much more information these tests provide over standard tests and tumour markers in guiding the treatment of metastatic breast cancer. However, a large clinical trial is studying how best to use circulating tumour cell test results to improve chemotherapy choices for metastatic breast cancer. Circulating tumour cells may also help predict disease-free survival times in women with early breast cancer, but study findings are mixed [32].

### Developing an effective breast cancer vaccine

Harnessing the immune response in treating breast cancer would potentially offer a less toxic, more targeted approach to eradicating residual disease. Breast cancer vaccines are being developed to effectively train cytotoxic T cells to recognise and kill transformed cells while spar-

ing normal ones. However, achieving this goal has been problematic due to the ability of established cancers to suppress and evade the immune response. These cancer vaccines are likely to work better in a minimal residual disease state. The presently available cancer vaccine strategies, including dendritic cell-based, tumour-associated antigen peptide-based and whole cell-based therapies have various pros and cons and to date, no one approach has proven to be superior. Combining a properly optimised cancer vaccine with novel immunomodulating agents that overcome tumour-related immune-suppression in a well-designed clinical trial offers the best hope for developing an effective breast cancer vaccine strategy [33].

### Conclusion

The objective of this review is to motivate surgeons to think beyond surgery. This will allow them to customise surgical procedures that conform to a multimodality treatment plan. Some of the therapies mentioned are still under evaluation and may fall out of favour. Controlled clinical trials are attempting to address these issues. However, surgery still has a definite curative value as our knowledge of systemic targeted adjuvant therapies continues to advance.

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