# Biomarkers and Multiple Drug Resistance in Breast Cancer

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**Abstract:** Breast cancer, the most common form of cancer among women in North America and almost all of Europe, is a significant health problem in terms of both morbidity and mortality. It is estimated that each year this disease is diagnosed in over one million people worldwide and is the cause of more than 400,000 deaths. Although chemotherapy forms part of a successful treatment regime in many cases, as few as 50% patients may benefit from this, as a result of intrinsic or acquired multiple drug resistance (MDR). Through the use of *in vitro* cell culture models, a number of mechanisms of MDR have been identified; many, if not all, of which may contribute to breast cancer resistance in the clinical setting. This phenomenon is complicated by the likely multi-factorial nature of clinical resistance combined with the fact that, although apparently studied extensively in breast cancer, reported analyses have been performed using a range of analytical techniques; many on small sub-groups of patients, with different clinicopathological characteristics and receiving a range of therapeutic approaches. Larger defined studies, using standardised genomic and proteomics profiling approaches followed by functional genomics studies, are necessary in order to definitively establish the degree of complexity contributing to drug resistance and to identify novel therapeutic approaches - possibly involving chemotherapy, drug resistance modulators, and novel targeted therapies – to combat this disease.

**Keywords:** Breast cancer, biomarkers, chemotherapy, multiple drug resistance, molecular profiling, targeted therapy.

## INTRODUCTION

Breast cancer is a leading cause of cancer deaths in women all over the world [1], with approximately 12% of women directly affected by this disease. Although the median age for patients with breast cancer is 65 years [2], this disease may affect women of all ages. The incidence of breast cancer in women aged <20 years has been estimated to be 0.0001%; 0.0014% for ages 20-24years, 0.0081% for women between 25-29 years old, and 0.0248% for the 30-34 years age group [3]. Breast cancer, however, is not restricted to the female population – approximately 1% of all cases is diagnosed in men.

Ductal carcinoma is invasive and is the most common type of breast cancer. It originates in the milk ducts of the breast, but has developed the potential to metastasise to other parts of the body. Similarly, lobular carcinoma is invasive; this cancer begins in the milk-producing lobules. Inflammatory breast cancer is a rare type of advanced cancer. This form of breast cancer has poorest prognosis; it results from lymphatic vessels becoming blocked with tumour cells and, subsequently, becoming inflamed. Other forms of breast cancer include Paget's disease, comedocarcinoma, medullary carcinoma and colloidal carcinoma [4]. Although histology may influence treatment decisions, the stage of disease is usually considered to be more important. Poorly differentiated (high grade) tumours have a worse prognosis than well-differentiated (low grade) tumours. Inflammatory carcinoma has a poor prognosis, irrespective of stage. For patients with negative nodes, a group of "special tumour types" (typical medullary, mucinous, papillary, and pure tubular types) is associated with a better prognosis. For early disease without lymph node involvement (stage I), the 5 year survival rate is approximately 80% for invasive ductal carcinomas and 90-95% for invasive lobular, comedo-carcinomas, and colloid carcinomas. Unfortunately, breast cancers spread by lymphatic vessels and blood-borne metastases. The most common organs involved with symptomatic metastases are regional lymph nodes, skin, bone, liver, lung and brain. Indeed, axillary lymph node metastases are present in 55-70% of patients at the time of diagnosis when not detected by screening mammography [4]. Whereas in the US more than 90% of breast cancer diagnoses now occur during the early stage of this disease [5], in developing countries approximately 25-30% of cases have already advanced locally when first diagnosed [1].

## CURRENT THERAPIES

### Locoregional Treatment of Breast Cancer

Total mastectomy with axillary node dissection (modified radical mastectomy) was the standard surgical procedure for patients who choose surgery as their only local treatment. Alternatively, some centres have replaced lymph node dissection with "sentinel node" technique, which allows a more limited removal of lymph nodes (LN) for staging purposes. There is apparently no significant survival difference between total mastectomy with axillary node dissection and limited surgery (lumpectomy, tylectomy, total gross removal, or quadrantectomy) followed by definitive radiotherapy (*i.e.* generally given as a 4500-5000 cGy to the entire breast, followed by a boost to the area of the biopsy (1000-2000 cGy) [4]. New techniques of breast irradiation, including conformal radiotherapy (defined as a high precision technique, based on the tri-dimensional volumetric definition of the tumour and the anatomy of critical organs) and intensity-modulated radiotherapy, have

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1568-0096/06 $50.00+.00

been shown to reduce the post-irradiation problems of cardiac and lung irradiation associated with conventional methods [6].

### Systemic Therapy

At the time of initial treatment with surgery, most patients with invasive carcinoma of the breast have a systemic disease in which micrometastases have already occurred. Adjuvant systemic therapy (hormonal, monoclonal antibodies, chemotherapy; or a combination of these), given immediately after local treatment, is now a standard part of breast cancer management. Women at sufficient risk to warrant such therapy include nearly all those with positive axillary nodes and many with high-risk, node-negative, disease.

### Hormonal Therapy

Estrogen receptor (ER) and progesterone receptor (PgR) activities are the most predictive factors for response of primary and metastatic breast cancers to hormonal therapies. Endocrine therapy is, therefore, generally restricted to patients with hormone receptor-positive (or unknown) tumours, whose life is not in immediate danger from advanced cancer. Patients who develop relapse within 1 year of primary treatment usually respond poorly to endocrine treatment [4].

Tamixofen, an antiestrogen, is generally the first endocrine therapy used in patients with $ER^+$ or ER-unknown tumours. Recent results derived from cell line studies suggest that the use of vitamin D analogues (*e.g.* EB1089), used to inhibit growth of breast cancer cells, may act synergistically with anti-estrogens [7]. Unfortunately, tumours expressing high levels of ER co-activator AIB1 and Her-2/*neu* (see "*Monoclonal Antibodies*") often develop tamoxifen resistance [8]. Aromatase inhibitors (including anastrozole and letrozole) block the conversion of androgens to estrogens and are generally second-line endocrine agents. Such aromatase inhibitors, used in patients with large operable tumours as *pre*-operative therapy, enabling subsequent breast conservation surgery instead of mastectomy, have been proposed [9]. Megestrol acetate, a progestin, is an alternative second-line, or a third-line, choice of endocrine therapy. Fourth-line agents, including fluoxymesterone (an androgen) or diethylstilbestrol (an estrogen), may be used for those who initially respond but then become unresponsive to treatment with tamoxifen, an aromatase inhibitor, and megestrol acetate. (A comprehensive review of the use of hormonal therapy is beyond the scope of this document and has been dealt with in recent review articles *e.g.* [10-11]).

### Monoclonal Antibodies

The erbB receptor family, part of the receptor tyrosine kinase superfamily, has been shown to play an important role in both normal breast development and in the pathogenesis and progression of breast cancer. Receptor overexpression has also been shown to be a negative prognostic indicator, associated with tumour invasiveness and a lack of responsiveness to standard therapies, including both chemotherapy and hormonotherapy [12-13] and radioresistance in breast cancer [14]. Herceptin (trastuzumab), a humanised antibody to her-2/$neu^+$ which has been rationally developed based on tumour biology, is now utilised in the clinic for metastatic breast cancer treatment and results in an approximately 30% response rate for her-2/$neu^+$ patients. For less aggressive $ER^+$/her-2/$neu^+$ metastatic breast cancer, trastuzumab combined with endocrine therapy is a feasible approach; for aggressive her-2/$neu^+$ metastatic cancer, trasuzumab combined with chemotherapy is warranted [15]. Although phosphorylation of AKT and/or loss of nuclear expression of cyclin-dependent kinase inhibitor, p27, may be involved, resistance to erbB inhibitor therapy is not yet defined [16-18]. Other monoclonal antibodies currently in the development phase for use in breast cancer include cetuximab (erbitux), targeting EGFR (erbB-1), and pertuzumab (omnitarg), specific for her-2/$neu^+$ ligand-dependent signalling [19].

## CHEMOTHERAPY

Breast cancer is moderately sensitive to cytotoxic drugs [20] and so chemotherapy plays an important role in the management of this disease [21-22]. Adjuvant chemotherapy in early breast cancer decreases the chances of recurrence and death by 24% and 15% annually, respectively [23]. *Neo*-adjuvant/induction chemotherapy (*i.e.* treatment given before the primary treatment) was first reported in the 1970s and initially utilised to convert unresectable tumour to smaller tumours, making them more amenable to local control with either surgery or radiotherapy, in addition to indicating chemosensitivity of the tumour [24]. This is an attractive treatment option for patients with locally advanced breast cancer [25]. This form of therapy can reduce tumour size in 80-90% of breast cancer patients [26], with pathological complete response rates approaching 20% [27]. Randomised trials have shown that *neo*-adjuvant chemotherapy is equally effective, but not superior, to *post*-operative chemotherapy in breast cancer *i.e.* it does not significantly increase disease/relapse-free survival (DFS) or overall survival (OS) when compared to adjuvant chemotherapy [28-29]. For those with metastatic disease, chemotherapy results in response rates of 25-55% [30] and improves the quality and duration of life but, with rare exception, does not result in a cure [20].

Many cytotoxic agents used singly are effective in achieving partial response in 20-35% of cases. Remission commonly lasts 4-6 months [4]. As combination therapy, the antimetabolites 5-fluorouracil and methotrexate in combination with the alkylating agent cyclophosphamide (CMF) have conventionally been used as breast cancer treatment [21], with a response rate of approximately 60% achieved for a median duration of 1 year or more [4].

Anthracycline-based regimes (including doxorubicin or epirubicin) are widely used as first-line chemotherapy for primary and metastatic breast cancers. Results from meta-analysis of 5 studies including a total of 1088 cases indicated that anthracycline-containing regimes confer a survival benefit over CMF regimes [31]. Conventional first-line regimes include doxorubicin with cyclophosphamide (AC); epirubicin with cyclophosphamide (EC); fluorouracil

with doxorubicin and cyclophosphamide (FAC); and fluorouracil with epirubicin and cyclophosphamide (FEC) [2]. For patients previously treated with adjuvant CMF chemotherapy, anthracycline-based regimes are frequently favoured as first-line chemotherapy for metastatic disease. Anthracycline-taxane combinations have been reported to result in excess of 50% response rates, with complete remission in approximately 15% of cases [32]. In some cases (*albeit* few; 3.1%) long-term remissions (> 5 years) have occurred [33]. Results from recent clinical trials, however, support the use of nonanthracycline-containing regimes as the first option for treating metastatic breast cancer. Analysis of 93 node-positive patients treated with different combinations of doxorubicin, docetaxel and CMF, indicated that, in terms of overall survival (OS) and disease-free survival (DFS), docetaxel-based regimes were at least as successful as standard anthracycline-based adjuvant therapy [34]. Docetaxel combined with cisplatin as first-line chemotherapy in metastatic breast cancer resulted in a 60% response rate, while taxanes with carboplatin, docetaxel with gemcitabine, or docetaxel with capecitabine, have all been proposed as feasible combinations [35]. Benefits from taxanes, particularly docetaxel, have also been seen in the *neo*-adjuvant setting.

Following failure of first-line chemotherapy, a significant response may be obtained with second-line anticancer drugs [36]. The choice of second-line chemotherapy is generally determined by the first-line regime used, with the taxanes (paclitaxel and docetaxel) commonly considered to be the most effective options; particularly if anthracycline-based regimes have previously been used [2]. Indeed, in a phase II study of 50 patients treated with docetaxel (blocks cells in G2/M phase) plus gemcitabine (blocks cells in G1/S phase) following docetaxel failure, 46% of patients responded (3 complete responses, 20 partial responses) suggesting an *in vivo* synergism between these two drugs and supporting advancement to a randomised trial [37]. Interestingly, partial response to paclitaxel in 31.8% (14/44) of metastatic breast cancer patients who were un-responsive to docetaxel has also been reported [38].

Results from clinical trials indicate that capecitabine (xeloda), a rationally designed cytotoxic drug designed to generate 5-fluorouracil preferentially in tumour cells by exploiting their higher thymidine phosphorylase (TP) enzyme activity compared to that in normal cells, is effective and has a favourable safety profile in the chemotherapeutic treatment of metastatic breast cancer [18]. Results from phase I [39] and phase II [40-41] clinical trials combining capecitabine with docetaxel and epirubicin have indicated an acceptable safety profile and some anti-tumour activity, warranting more extensive trials in advanced breast cancer. The ability of certain cytotoxic drugs, such as the taxanes and cyclophosphamide [42], as well as iressa [43], to up-regulate the activity of TP indicates the potential for synergistic activity, if used in combination.

Other agents commonly used in breast cancer include vinca alkaloids, anthraquinones (mitoxantrone), epipodophyllotoxins (etoposide) [44] and bisphosphonates (which are increasingly used to treat the hypercalcaemia associated with malignant disease). Pamidronate, in particular, may be useful in postponing "skeletal events" including fracture and pain in patients with breast cancer metastatic to bone [4].

To overcome problems associated with cell developing drug resistance (see "*Drug Resistance*") and to increase the efficacy of chemotherapeutic regimes, oncologists have investigated ways of "fine-tuning" chemotherapy doses and schedule, including high-dose (based on hypothesis that escalating the dose will overcome drug resistance and improve outcome) [20, 45-47] and dose-dense (delivering standard dose, but with shorter intervals between cycles) [48] approaches. Whereas high-dose regimes have, in general, shown no significant improvement over standard regimes [47], dose-dense adjuvant chemotherapy with doxorubicin apparently improves clinical outcome [48].

## DRUG RESISTANCE

Chemotherapy resistance, whether inherent or acquired, is a major problem in the management of breast cancer. Patients refractory to chemotherapy exhibit resistance to multiple cytotoxic agents of differing structures and, often, differing functions. This clinical resistance, comparable to the experimental phenomenon termed multiple drug resistance (MDR), is likely to be multifactorial and heterogenous [49-51], with many molecular mechanisms potentially contributing to the drug resistance phenotype [21]. Mechanisms (see Fig. **1** for some examples) include reduction in the intracellular accumulation of anticancer drugs, by both increasing drug efflux and/or decreasing drug uptake; drug sequestration; alterations in drug targets (*e.g.* topoisomerases) or activation of de-toxifying systems (including glutathione/glutathione-S-transferases; cytochrome P450s); increased repair of drug-induced DNA damage; disruption in cell signalling; alterations in factors involved in cell cycle control; and inhibition of apoptosis [52]. Although drug resistance is conferred to a large number of clinically and pharmacologically unrelated compounds *via* some cellular mechanisms, other mechanisms (*e.g.* topoisomerase II alterations) are more target-specific, resulting in resistance to a limited number of agents (see "*Other Mechanisms of Drug Resistance in Breast Cancer*"). However, it should be considered that cancer cells may display a range of these resistance mechanisms at any given time, often resulting in multiple drug resistant cells. Many breast cancer cell lines, which are sensitive, inherently resistant, or have acquired drug resistance, have been developed and evaluated as useful *in vitro* models of this phenomenon. An extensive review of these cell lines is beyond the space limitations of this paper. However, a number of examples of these models is summarised in Table **1**. Many of the cellular mechanisms described here are apparently simultaneously involved in clinical drug resistance.

### Drug Transport Proteins

One of the mechanisms of drug resistance that may be clinically active in breast cancer patients is the prevention of intracellular drug accumulation by the expression of transporter proteins that pump drugs out of cells before they can reach their site(s) of action. In addition, expression of these transporters on sub-cellular compartments may result

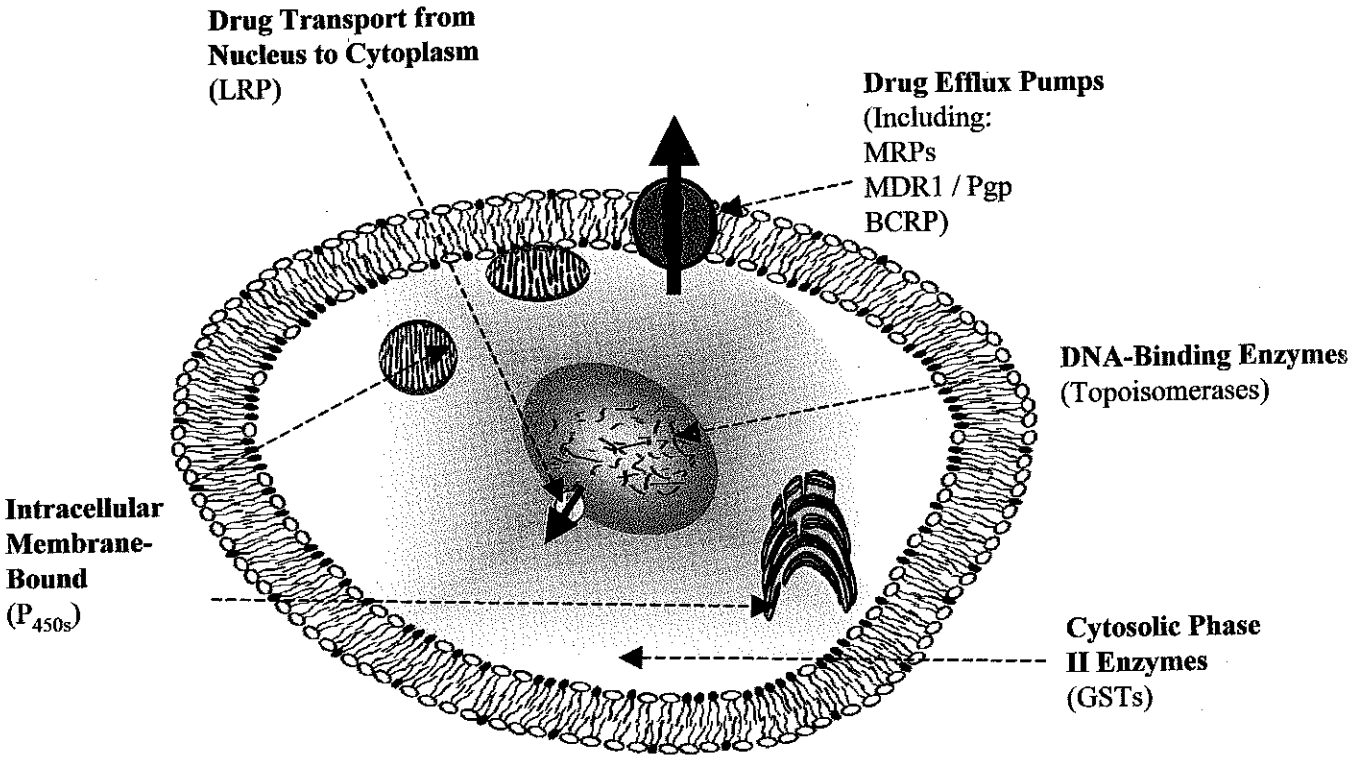


**Fig. 1.** Examples of drug resistance mechanisms within the cell.

in drug sequestration within the cell [71], but remote from its target site of action [72]. Several of these proteins belong to the ATP-binding cassette (ABC) transmembrane protein superfamily that utilises energy from ATP hydrolysis to translocate substrates across cell membranes [21, 52].

Based on sequence homology, 48 different ABC transporters (grouped into 7 sub-families ranging from A-G) have been defined in the human genome (see http://www.gene.ucl.ac.uk/nomenclature/genefamily/abc.html and http://www.med.rug.nl/mdl/humanabc.htm; [73-76]). ABC family members involved in MDR include MDR1/P-glycoprotein (ABCB1); MRP family members, MRP1 (ABCC1), MRP2 (ABCC2), MRP3 (ABCC3), MRP4 (ABCC4), MRP5 (ABCC5), MRP6 (ABCC6), and breast cancer resistant protein/BCRP (ABCG2). The lung resistance protein, LRP (major vault protein/MVP), is not an ABC transporter, but it is frequently expressed at high levels in drug-resistant cell lines and tumour specimens (see Table **2**). However, its role in drug resistance remains uncertain.

### MDR1/P-Glycoprotein

The multiple drug resistance 1 (MDR1) gene encodes P-glycoprotein, a 170 kDa plasma membrane protein consisting of 12 transmembrane domains and 2 ATP-binding domains [87-88]. Expression of MDR1/Pgp mRNA and protein have been observed in many normal cell types, including those of the gastrointestinal tract, liver, kidney, brain, testes, ovaries, and adrenal glands. Although the physiological role of MDR1/Pgp has not yet been clearly defined, it may include de-toxification and excretion of xenobiotics, as well as hormone transport [52]. Anthracyclines [77], epipodophyllotoxins, vinca alkaloids, geldanamycin [76] and taxanes [78] are amongst the drugs to which MDR1/Pgp confers resistance. Compounds that are, apparently, not transported by MDR1/Pgp include methotrexate, 5-fluorouracil, camptothecins, and hydroxyurea [76].

In breast cancer, the role of MDR1/Pgp gene expression has been extensively investigated, with contradictory results ranging from 0% to 100% expression in tumour cells [21, 77] and conflicting reports as to its prognostic/predictive relevance. A reason for the broad range of conflicting results may be the different methods of analysis employed – including immunohistochemistry, with different antibodies; *in situ* hybridization; Northern blotting; RT-PCR; and Western blotting. Collating results from twelve RT-PCR breast cancer studies performed between 1996 and 2001, an overall detection rate of *mdr1* mRNA in 63% (334/531 cases) was reported [21]. Pooled results from Northern blot studies resulted in an overall 28% *mdr1* mRNA positivity. While immunohistochemical detection of MDR1/Pgp protein in untreated breast cancer ranged from 0% [89] to 100% [90], pooled results from twenty-nine studies reported between 1989-2001 indicated an approximately 40% (744/1840 cases) positivity; with 42% and 44% positivity found in locally advanced and primary operable breast cancer, respectively. No significant difference was observed between expression of MDR1/Pgp protein (by immunohistochemistry) in primary lesions from non-metastatic (74.3%) and metastatic (72.7%) cases [21, 91-94]. In our study of 177 invasive breast carcinoma cases, we detected MDR1/Pgp expression in approximately 66% of cases [95].

**Table 1. Examples of MDR Associated Genes Expressed in Human Breast Cancer Cell Lines**

| Cell Line | Drug Resistance Mechanism | Drug Resistance/Sensitivity | References |
|---|---|---|---|
| **Endogenous Expression of MDR-associated genes** | | | |
| T47D | $MRP1^-$ | drug sensitive cells | [53] |
| MCF-7 | $MRP1^-$ | | |
| | *BCRP protein* | | |
| BT20 | + | ND | [54] |
| MCF-7 | + | ND | |
| CAMA1 | +/- | ND | |
| HBL100 | +/- | ND | |
| 1.6.2.6. | +/- | ND | |
| MPL13E | - | ND | |
| SKBR-3 | - | ND | |
| T47D | - | ND | |
| ZR75-1 | - | ND | |
| | *MDR1/Pgp protein* | *5-FU, cytoxin, dox, paclitaxel, vincristine (R)* | [55] |
| MCF-7 | +++ | +++ | |
| BT-20 | + | +++ | |
| BT474 | ++ | ++ | |
| SKRB-3 | ++++ | ++ | |
| MDA-MB-453 | +++ | + | |
| MDA-MB-231/c1.9 | $transglutaminase^-$ | doxorubicin (S) | [56] |
| MDA-MB-231/c1.16 | $transglutaminase^+$ | doxorubicin (R) | |
| **DRUG SELECTED CELL LINES** | | | |
| MCF-7/VP | $MRP1^+$ | VP-16 (R) | [57] |
| | *mdr1* & $MDR1/Pgp^-$ | VM-16 (R) | [58] |
| | | doxorubicin (R) | |
| | | mitoxanthrone (R) | |
| | | vincristine (R) | |
| | | genistein (S) | |
| | | camptothecin (S) | |
| | | melphalan (S) | |
| | | chlorambucil (S) | |
| MCF-7/Adr | $MDR1/Pgp^+$ | doxorubicin (R) | [59] |
| MCF-7/Adr-20 | defects in caspases, no change in MDR1/Pgp or MRP1 | doxorubicin (R) | [60] |
| MDA-MB-435S-F/Taxol-10p4p | ↑ MDR1/Pgp & MRP1 | paclitaxel (R) | [61] |
| | | vincristine (R) | |
| | | docetaxel (R) | |
| | | doxorubicin (S) | |

(Table 1)contd.....

| Cell Line | Drug Resistance Mechanism | Drug Resistance/Sensitivity | References |
|---|---|---|---|
| | | carboplatin (S) | |
| | | VP-16 (S) | |
| | | 5-FU (S) | |
| MDA-MB-435S-F/Adr-10p10p | ↑ MDR1/Pgp & MRP1 | doxorubicin (R) | |
| | | VP-16 (R) | |
| | | docetaxel (R) | |
| | | paclitaxel (S) | |
| | | carboplatin (S) | |
| | | 5-FU (S) | |
| MCF-7/dox | no ↑ *mdr1* or *mrp1* | doxorubicin (R) | [62] |
| | no ↑ MDR1/Pgp | VP-16 (R) | |
| | no change in GSH | vincristine (R) | |
| | no change in GSH peroxidase | cisplatin (R) | |
| | no change in GSH reductase | mitomycin C (R) | |
| | no change in GSH transferase | | |
| MCF-7/MX | novel MTX-pump suggested | methotrexate (R) | [63] |
| | | Topotecan (R) | |
| | | VP-16 (R) | |
| MCF-7/AdrVp | no ↑ MDR1/Pgp | doxorubicin (R) | [64] |
| | no change in GST | melphalan (R) | |
| | ↓ topoisomerase II | vinblastine (S) | |
| | ↑ novel 95 kDa protein | | |
| MCF-7/AdrVp3000 | ↑ *abca4, abca5, abca7, abcb3, abcb10,* | mitoxanthrone (R) | [206] |
| | ↑ *abcc2, abcc3, abcc5, abcc8, abcg2/bcrp;* | doxorubicin (R) | |
| | ↓ *abca3, abca10, abca12, abcb4, abcc4, abcc11;* no change in *mdr1* or *mrp1* | daunorubicin (R) | |
| MCF-7-VP17* | ↓ topoisomerase II mRNA & protein | VP-16 (R) | [65] |
| ZR-75B-VP13* | ↑ MRP1 | | |
| MDA-MB-231-VP7* | | | |
| [* VP-16 selectants] | | | |
| MCF-7/C4 [camptothecin] | ↑ DNA repair | camptothecin (R) | [66] |
| | | cisplatin (R) | |
| | | VP-16 (S) | |
| MCF-7/TPT300 [topotecan] | no MDR1/Pgp | topotecan (R) | [67] |
| | no MRP1 | camptothecin (R) | |
| | ↑ BCRP | mitoxanthrone (R) | |
| | ↓ topoisomerase I | | |

(Table 1)contd.....

| Cell Line | Drug Resistance Mechanism | Drug Resistance/Sensitivity | References |
|---|---|---|---|
| CaLc18/AMSA | ↓ topoisomerase II activity | ASMA (R) | [68] |
| | ↑ topoisomerase I activity | | |
| | ↑ *GST* mRNA | | |
| TRANSFECTED CELL LINES | | | |
| MDA-MB-435 p185c-erbB2 | c-erbB2 | paclitaxel (R) | [69] |
| MDA-MB-435 E1A | her2/*neu* repression | sensitises to paclitaxel | [70] |

Adr = Dox = adriamycin = doxorubicin; MX = methotrexate; AMSA = 4'-(9-acridinylamino)methanesulphon-m-anisidide; ND = not described; Vp = verapamil; VP = VP-16/etoposide; ↑= increased levels; ↓ = decreased levels; $^{+}$ = expressing; $^{-}$ = deficient

Evidence for the induction of MDR1/Pgp protein expression by exposure to MDR1 substrate anticancer drugs is the fact that MDR1/Pgp protein was detected (by immunohistochemistry) in 52% of cases *post* treatment, compared to 41% of untreated breast cancer cases [21]. Significant (p=0.0033) induction of *mdr1* mRNA (detected by RT-PCR) was also reported by Lizard-Nacol *et al.* [96] in a study of 75 patients receiving primary chemotherapy. In agreement with this, pooled data from a range of studies indicated that the use of such chemotherapy in the *neo-*

**Table 2. Drug Efflux Pumps Associated Genes Expressed in Breast Cancer**

| Gene | GenBank Accession No. | Alternative Names | Substrates | References |
|---|---|---|---|---|
| MDR1/Pgp | AF016535 | ABCB1 | anthracyclines | [76] |
| | | | epipodophyllotoxins | [77] |
| | | | vinca alkaloids | [78] |
| | | | geldanamycin | |
| | | | taxanes | |
| MRP1 | L05628 | ABCC1 | methotrexate | [79] |
| | | | anthracyclines | |
| | | | epipodophyllotoxins | |
| | | | vinca alkaloid - vincristine | |
| | | | (taxanes – poor substrates) | |
| BCRP | AF098951 | ABCG2 / MXR | methotrexate | [52] |
| | | | mitoxanthrone | [67] |
| | | | topotecan | [80] |
| | | | irinotecan | [81] |
| | | | flavopiridol | [82] |
| | | | campothecin-derived topoisomerase I inhibitors | |
| | | | indolocarbazole topoisomerase I inhibitors | |
| | | | imatinib mesylate (gleevec) | |
| | | | anthracyclines (depends on mutation at codon 482) | |
| LRP | X79882 | Major vault protein | platinum agents | [83] |
| | | | alkylating agents | |
| | | | doxorubicin | [84] |
| ATP7B | U03464 | P-type ATPase | cisplatin | [85] |
| | | | carboplatin | [86] |
| | | | oxiliplatin | |

adjuvant setting resulted in induction of MDR1 gene expression *i.e. mdr1* mRNA expression was induced from 50% to 73% of cases, with MDR1/Pgp protein expression increased from 43% of cases to 64% [21]. In a study of *pre-* and *post-neo*adjuvantly treated locally advanced breast carcinomas, MDR1/Pgp protein expression was reported to increase from 55% to 100% of cases, regardless of whether the chemotherapy was anthracycline-based, taxane-based, or CMF [97]. Furthermore, results from a study of 359 freshly resected breast carcinoma specimens indicated that, compared with the MDR1/Pgp protein-negative tumours, a significant increase in doxorubicin and taxol resistance occurred in MDR1/Pgp expressing tumours, regardless of prior treatment [98]. Faneyte *et al.* [99], however, reported a study of both breast cancer cell lines and primary specimens where only very low levels of *mdr1* mRNA, and no MDR1/Pgp protein, was detected in the cell lines, with MDR1/Pgp protein undetectable in 88% tumour specimens from anthracycline-treated patients. Similarly, *mdr1* mRNA expression (by RT-PCR) was described in approximately 40% of breast cancer specimens, with no MDR1/Pgp protein detected (by Western blot or immunohistochemistry) in breast carcinoma cells; expression was only detected in interstitial mononuclear cell types [89].

MDR1/Pgp protein expression has generally been found not to correlate with many clinicopathological characteristics of breast cancer patients. In an analysis of 94 specimens obtained from mastectomy without *pre*-operative chemotherapy, no correlation was found between MDR1/Pgp protein expression (found in 37.2% of cases) and hormonal receptor status, menopausal status, tumour size, or axillary node involvement [100]. Likewise, in a study of 63 primary breast cancers, no significant association was found to exist between MDR1/Pgp protein expression and tumour size, lymph node status, ER or PgR status [101]. In locally advanced breast carcinomas (80 cases studies), MDR1/Pgp protein expression was found to be more frequently detected in lobular carcinomas compared to ductal carcinomas, and in patients with positive lymph nodes, compared to those with negative nodes; it was independent of all other clinical parameters evaluated [97]. In our recent study of 177 invasive breast carcinomas where MDR-1/Pgp protein was found to be expressed in approximately 66% of cases, although there was a trend towards its expression being associated with higher grade tumours (grade III) (*albeit* statistically insignificant; log rank $p$ value = 0.085), there was no correlation with any of the other clinicopathological features studied, including age at diagnosis, tumour type, tumour size, ER status and lymph node status [95]. This is in agreement with other studies indicating clinicopathological features not to be significantly associated with expression of MDR1/Pgp protein [102-104].

The clinical significance of MDR1 gene expression in breast cancer remains controversial with regard to any prognostic or predictive role. Although MDR1/Pgp expression has been associated with poor patient outcome in both primary and advanced cancers, reports have been largely conflicting [104]. An extensive meta-analysis of such earlier studies reported MDR1/Pgp protein to be expressed in approximately 41% of breast tumours, with expression associated with poor response to treatment [104]. Similarly, MDR1/Pgp protein expression (37.2%; 35/94 cases) was reported to be significantly (p=0.0433) associated with shortened DFS in chemotherapy-naïve breast tumours, when compared to those that were MDR1/Pgp negative [100]. Furthermore, recent studies have concluded that *mdr1* mRNA expression in primary breast tumours is inversely correlated with the efficacy of first-line chemotherapy and that high *mdr1* (and lung resistance related protein (*lrp*)) gene expression is significantly associated with poor progression-free survival; although no correlation was found to exist between expression of these genes and *post*-relapse overall survival [105]. In contrast to this, in a study of breast carcinomas *pre-* (43 cases) and *post-* (38 cases) doxorubicin treatment, *mdr1* mRNA expression was found not to correlate with age at diagnosis, TNM catagories, ER or PgR status, or DFS (similar findings were observed on analysis of *mrp1*, *lrp*, and *bcrp* mRNAs in these specimens [106]). Similarly, overexpression of MDR1/Pgp protein in locally advanced breast cancer (48 cases analysed) following *neo*-adjuvant chemotherapy (cyclophosphamide + doxorubicin + 5-fluorouracil (CAF); taxotere + doxorubicin; or CMF) did not significantly correlate with response to chemotherapy [107].

In invasive breast tumour analysis, in agreement with results of analysis of 63 such cases where MDR1/Pgp expression was not found to have potential as a prognostic marker [101], our study of 177 invasive breast carcinomas failed to show any significant association between MDR-1/Pgp protein expression at diagnosis (when analysing expression as present/absent and <25% tumour cells positive *versus* ≥25% positivity). Furthermore, when patients were sub-stratified according to chemotherapy status, lymph node status, tumour histological grade and size, no significant associations were found to exist between MDR-1/Pgp expression and either DFS or OS, in any of the sub-groups studied [95].

As previously mentioned, however, MDR1/Pgp is not restricted to cancerous cells of the breast, but has also been detected in normal breast tissue, at both the mRNA and protein levels. In a study of 40 locally advanced breast cancers, higher levels of *mdr1* mRNA were found in cancerous compared to normal tissue prior to *neo*-adjuvant chemotherapy; however, this difference was no longer significant following treatment, mainly due to induced levels of *mdr1* mRNA in benign tissue [108]. At the protein level, a broad range of results has been reported on analysis of normal breast tissue. MDR1/Pgp protein expression has been reported to be weak in normal tissue and confined to breast epithelial cells, but absent from stroma [109]; expressed in 88% (21/24 cases) of normal/benign breast tissues, but restricted to the luminal surface of ductal epithelium [110]; found in 67% of normal tissue sections from regions adjacent to locally advanced breast cancer [111]; and absent from all (5/5) normal breast specimens [112]. It may be noteworthy that all of these studies, except the latter immunoblot analysis, involved immunohistochemical techniques.

At the mRNA level, in studies of 75 locally advanced breast cancers, significantly induced levels of *mdr1* (detected by RT-PCR) were detected in both tumour and normal specimens, following primary chemotherapy [96]. Similarly, analysis of 40 locally advanced breast cancer patients showed a significant induction of *mdr1* mRNA following primary

treatment with the anthracycline-based regime, CAF, with greater levels of induction in normal compared to tumour cells [108]. This suggests that MDR1 gene expression in response to chemotherapy may be a general event, occurring in both normal and tumour cell types.

**MRP1**

The MRP (ABCC) subfamily is comprised of nine members that transport structurally diverse lipophilic anions and hydrophilic peptides [113] and function as drug efflux pumps [76, 114]. MRP1, the first described member of the MRP1 family, was identified when using differential hybridisation to analyse gene transcripts overexpressed in doxorubicin-resistant lung cancer cell lines lacking MDR1/Pgp overexpression [79]. The MRP1 gene encodes a 190 kDa trans-membrane protein associated with both cell membrane and intracellular membrane expression [52]. Its activity on intracellular vesicles has been shown to be sufficient to confer a drug resistance phenotype, *in vitro* [72]. MRP1 apparently has an overlapping, but not identical, substrate specificity to MDR1/Pgp. MRP1 confers resistance to anthracyclines, epipodophyllotoxins, methotrexate, and the vinca alkaloid vincristine (but, apparently, confers no/only low level resistance to vinblastine [115-117]). In contrast to MDR1/Pgp, taxanes are poor substrates for MRP1 and its overexpression in cell lines has been shown not to confer resistance to cisplatin and mitoxanthrone [118].

MRP1 is expressed by the majority of untreated breast tumours, with results from studies of MRP1 expression in breast cancer patients apparently proving less conflicting than those involving MDR1/Pgp. Using immunohistochemical analysis of breast tumours, MRP1 protein has been reported to be expressed in 10% to 100% of cases, with 80% (16/20) positivity in operable cases and 10% (2/10 cases) in locally advanced [119] breast carcinomas; 34% (88/259) [120] and 61.5% (107/174) positivity have been reported in invasive breast carcinomas [95]; 34.8% (8/23) in recurrent cancers; 100% (19/19) in a study of mixed breast cancer tumour [121], and 80% in a study of 100 primary tumour with no reported metastases [122]. At the mRNA level (analysing by RT-PCR), *mrp1* expression has been detected in the majority of breast cancers studied. In primary tumours (described in most cases as "primary operable"), *mrp1* mRNA was detected in 70.4% (19/27) [123] to 100% *i.e.* 74/74 [124], 85/85 [125] and 43/43 [106] of cases. In our study of 106 invasive breast carcinomas, we detected *mrp1* gene transcripts in 72.8% of specimens [126].

In primary breast cancers, MRP1 expression has been associated with an overall poorer prognosis [120, 122, 127]; this association has been observed in patients with small tumours and in node negative patients. Furthermore, MRP1 expression was found to be predictive of overall survival in patients who received adjuvant CMF [120]. MRP1 expression has also been shown to be an important predictor of poor prognosis in patients with recurrent breast cancer [128]. In our recent study of 177 invasive breast carcinomas [95], MRP1 protein was found to be expressed in approximately 61% of tumours analysed; confirming previous observations that MRP1 protein is detectable in a large proportion of breast cancers. Membranous and granular MRP1 positivity was observed in the majority of tumours studied [95]; again, in agreement with previous analyses [129]. MRP1 protein expression did not correlate with established clinical or pathologic characteristics namely, ER status of patients, LN status, histological sub-type, histological grade, tumour size or subsequent treatment with adjuvant chemotherapy. In agreement with our findings, others have reported that expression of this protein at diagnosis is independent of node status, menopausal status, histological sub-type and age of patients [127-128]. Although some reports indicate MRP1 protein expression to be associated with intermediate histological tumour grade and larger tumour size, other studies suggest that MRP1 expression is independent of these parameters [120, 127]. Increased MRP1 expression has, however, been shown to be associated with primary tumours which have distant metastases and in lymph node metastases compared to primary tumours [130].

Although, in general, there appears to be a correlation between MRP1 protein expression and poor outcome for breast cancer patients, a direct causal role for MRP1 in clinical drug resistance remains to be determined [21]. In a study of 100 primary breast cancer patients, the absence of MRP1 protein was found to be significantly associated with increased DFS and OS, compared to those with $MRP1^+$ tumours [122]. Although in our study of invasive breast carcinomas we found no association between MRP1 expression at diagnosis (presence/absence and <25% MRP1 expression *versus* ≥25% MRP1 expression) and either DFS or OS, following sub-stratification of cases for more detailed analyses (*i.e.* patients who received chemotherapy *versus* patients who did not receive chemotherapy; patients with grade I *versus* II *versus* III tumours; LN positive patients *versus* LN negative patients; patients with tumours <2cm *versus* >2cm), a significant correlation was observed between MRP1 protein expression in ≥25% tumour cells and both DFS (log rank $p$ value = 0.0181) or OS (log rank $p$ value = 0.0171) for patients with high grade tumours (grade III) who received CMF chemotherapy, regardless of lymph node status. When this sub-group was subjected to multivariate analysis, MRP1 protein expression in <25% of tumour cells at diagnosis was identified as an independent favourable prognostic factor for both DFS (log rank $p$ value = 0.008) and OS (log rank $p$ value = 0.008) [95]. The fact that expression of MRP1 protein in these invasive carcinomas only showed prognostic value in chemotherapy (CMF +/- doxorubicin or paclitaxel) treated patients with grade III tumours and failed to show similar prognostic relevance in any of the subgroups of patients who did not receive chemotherapy, suggests that MRP1 protein expression may have predictive value in some CMF treated patients. Furthermore, the relevance of MRP1 in patients treated with CMF has more recently been confirmed in a study of 516 premenopausal, hormone receptor-positive breast cancer patients with stage I and II disease, where MRP1 expression was found to be an independent predictor of shorter RFS and OS [131]. These observations are in agreement with a previous report of patients treated with a regime of FAC or FEC, where *mrp1* (and *bcrp*) mRNA expression correlated with progression-free survival [105]; this effect was not seen in cyclophosphamide, methotrexate and 5-fluorouracil treated patients [105]. Similarly, in a study of 27 cases, an

increased risk of relapse within 10 years of receiving chemotherapy (mainly mitomycin C) was observed where primary tumours expressed high levels of *mrp1* mRNA [123]. However, we [126], like others [106, 125], found no correlation between *mrp1* mRNA expression and outcome for patients with invasive breast carcinomas.

Information on possible induction of MRP1 gene expression by chemotherapy is very limited. Results from a small study of MRP1 protein expression in locally advanced breast cancers *pre-* and *post- neo*adjuvant anthracycline-based chemotherapy showed increased expression (*i.e.* in 56% (9/16) of cases *post*-therapy compared to 20% (2/10) of cases *pre*-therapy), suggesting an inducing effect of treatment [119]. In a more recent study of patients with locally advanced breast carcinomas, MRP1 protein expression was found to be significantly ($p<0.001$) increased from 62% of cases prior to chemotherapy to 88% after treatment, regardless of whether patients received anthracycline-based, taxane-based, or CMF chemotherapy. *Pre*-chemotherapy MRP1 protein expression was more frequently ($p=0.02$) observed in patients with distant metastases than in those without and was associated with shorter DFS ($p=0.02$). From this study it was concluded that although response to chemotherapy was not associated with *pre-* or *post*-chemotherapy expression of MRP1, time to disease progression may be [97].

As for MDR1/Pgp, MRP1 gene expression is not restricted to cancer cells and is frequently detected – at both the mRNA and protein levels – in normal breast tissue. In a study of normal tissue adjacent to 55 breast tumours, *mrp1* mRNA was detected in all cases [121]. Similarly, Ito *et al.* [123] reported *mrp1* mRNA in normal breast tissue, but at levels that were significantly lower than in the associated tumours. In a study of 6 normal breast specimens, MRP1 protein was found in 50% of cases (3/6), in epithelial cells; but not in stroma [119].

Recently, studies have addressed the possible contribution to breast cancer clinical resistance of some of the newly described members of the MRP family such as MRP-2 [132] and MRP-8 [133]. Although other MRPs [134], in addition to MRP1, are frequently expressed in malignant disease, their possible function(s) and role(s) in clinical drug resistance have yet to be fully elucidated. A small study (30 cases) of MRP1, MRP2, and MRP3 gene expression in untreated and *post-neo*adjuvant anthracycline-based tumours indicated mRNA expression for all 3 genes in all specimens studied, with no significant increase in levels *post*-chemotherapy. MRP1, -2, and -3 proteins were undetected [135]. Recent studies involving the analysis of MRP-7 overexpression in HEK293 cells indicate that, unlike other MRPs, MRP-7 may confer resistance to taxanes [136]. As taxanes are often considered as first- or second- line therapy in breast cancer, the involvement of MRP-7 in this disease warrants investigation. Furthermore, more extensive studies are now required to determine the relevance – if any - of expression of all members of the MRP family to clinical outcome for cancer patients.

**Breast Cancer Resistance Protein**

Breast cancer resistance protein (BCRP; also known as mitoxanthrone resistance gene (MXR); ABC transporter in placenta (ABC-P)), initially cloned by Doyle *et al.* in 1998 [137], is a 72 kDa half-transporter consisting of only six transmembrane domain with one ATP-binding domain. BCRP apparently functions as a homodimer at the cell membrane [52, 138-140]. The MDR phenotype conferred by BCRP is overlapping with, but distinct from, that due to MDR1/Pgp *i.e.* BCRP transports a number of anticancer drugs including mitoxanthrone, methotrexate, topotecan, irinotecan, camptothecin-derived and indolocarbazole topoisomerase I inhibitors, flavopiridol, quinazoline ErbB1 inhibitors and its transportation of anthracyclines depends on a mutation at codon 482 [52, 67, 80-81, 141-142]. Imatinib mesylate (ST1571; gleevec), a potent tyrosine kinase inhibitor, has recently been reported to be a substrate for BCRP [82]. BCRP expression has been observed in a number of normal tissue types, including the placenta [143], brain [144], colon and bile canaliculi [145-146], suggesting that its physiological role may involve protection from potentially harmful xenobiotics. BCRP expression in stem cells is proposed to be associated with the maintenance of the undifferentiated stem cell phenotype [147]. Although described as "breast cancer resistance protein", the definitive role of BCRP in clinical drug resistance in breast cancer is still unclear [54, 148].

**Lung Resistance Protein**

Lung resistance protein (LRP) (also known as major vault protein (MVP)), a 110 kDa protein, is not an ABC transporter, but its expression is frequently detected at high levels in drug resistant cell lines and tumour specimens. LRP is involved in drug transportation from nucleus to cytoplasm [149-150]. Its expression has been associated with resistance to platinum, alkylating agents [83] and doxorubicin [84]. In a study of 99 primary breast carcinomas, LRP protein (analysed by immunohistochemistry) was undetected in 12% of cases; it was expressed at low levels in 20%, intermediate levels in 47%, and high levels in 21% of cases. This expression was not significantly associated with clinicopathological characteristics evaluated, including age at diagnosis, tumour type, size, or grade, ER status, PgR status or lymph node status, and showed no correlation with outcome for patients in terms of either DFS or OS [151].

Analysis of 48 locally advanced breast cancers following *neo*adjuvant CAF chemotherapy (except 2 cases where CMF or taxotere + doxorubicin were used) indicated no correlation between LRP protein overexpression *post*-therapy and response, whether "response" was described as complete response, partial response, no response, or disease progression [107]. Similarly, a study of 80 locally advanced breast carcinomas indicated LRP protein expression not to be significantly associated with any clinical parameters evaluated [97]. Conflicting results exist with regards to the induction of LRP by chemotherapeutic agents. Analysis of 13 paired specimens indicated no increased expression of LRP protein after *neo*-adjuvant chemotherapy [119], while a study of locally advanced breast carcinomas *pre-* and *post*-chemotherapy (80 and 68 cases, respectively) showed significant ($p<0.001$) increases (from 65% to 97% cases) in LRP protein expression after *neo*adjuvant chemotherapy, regardless of the drug regime (CMF, anthracycline- or taxane-based therapy) used [97].

### Co-Expression of Transport Pumps in Breast Cancer

Following comparative genomic hybridisation (CGH) analysis of 4 breast tumours *post-neo*adjuvant chemotherapy (including 3X CMF; 3X ED; 4X FEC; and 6X FEC), Fazeny-Dorner *et al.* [25] reported that 3 patients showed involvement of genomic regions containing MDR1, MRP1 and BCRP.

Although some breast cancer studies have failed to find a correlation between *mdr1* and *mrp1* mRNA expression [125] and between MDR1/Pgp and MRP1 protein expression (*e.g.* Filipits *et al.* [127]), in other cases, a significant relationship between expression of these efflux pumps has been reported. In 1998, Mechetner *et al.* [98] reported that *pre-* chemotherapy MDR1/Pgp and *pre-* and *post-* MRP1 expression predicted tumour recurrence and patient death. In our study of invasive breast carcinomas, a highly significant association was shown between expression of MDR1/Pgp and MRP1 proteins at diagnosis (log rank $p$ value <0.0001) [95]. Furthermore, at the mRNA level, in a study of *mdr1*, *mrp1*, *lrp*, and *bcrp*, while *bcrp* and *lrp* expression did not correlate with that of other genes, expression of *mdr1* and *mrp1* were associated [106].

### ATP7B

Copper-transporting P-type adenosine triphosphatase (ATP7B) protein, a transporter involved in tumour cell uptake of cisplatin, carboplatin, and oxiplatin *in vitro* [85-86], was found to be expressed in 22% (9/41) of untreated primary breast tumours. Although present in adjacent normal tissue, ATP7B levels were upregulated in tumour cells, with significantly (p=0.012) higher levels of expression in poorly differentiated breast carcinomas, compared to moderately- or well-differentiated carcinomas [152]. However, analysis of ATP7B in breast cancer is limited and so further evaluation is necessary to determine if its expression (or lack of expression) is clinically relevant in resistance to chemotherapy.

### Other Mechanisms of Drug Resistance in Breast Cancer

As previously mentioned, in addition to drug efflux pumps, other mechanisms of drug resistance have been described in breast cancer.

#### *Topoisomerase IIα*

Topoisomerase IIα, a nuclear DNA-binding enzyme that combines nucleas, helicase, and ligase activity and so modifies the topological state of DNA, is a specific target for several chemotherapeutic agents, including anthracyclines. Both amplification and protein overexpression of topoisomerase IIα have been associated with chemosensitivity to anthracycline-based therapy [153-155]. Recent analysis of topoisomerase IIα expression in breast cancer (50 cases) reported a significant (p<0.05) association between $ER^+$ and topoisomerase IIα-expression. In this study, expression of topoisomerase IIα in higher percentages of tumour cells indicated increased probability of disease recurrence, suggesting a prognostic value for topoisomerase IIα [156]. However, as with the analyses of efflux pumps, conflicting reports have emerged on the predictive potential of topoisomerase IIα. Results from a phase III clinical trial in advanced breast cancer patients, comparing single agents (*i.e.* doxorubicin with docetaxel), indicate topoisomerase IIα protein expression to confer a higher probability of response to doxorubicin only [157]. In an immunohistochemical analysis of 41 primary breast cancers, where patients received *neo*-adjuvant anthracycline-based chemotherapy (FAC or FEC), topoisomerase IIα overexpression (lost after chemotherapy) was significantly (p=0.03) associated with clinical response [158]. Similarly, an analysis of 36 patients with progressive metastatic breast cancer, previously treated with an anthracycline and now receiving cisplatin and etoposide phosphate, topoisomerase IIα (but not topoisomerase IIβ) indicated protein levels to be significantly (p<0.001) higher in responding patients, compared to those with stable or progressive disease [159]. In contrast, a study of 199 patients with operable breast cancer and treated with *neo*-adjuvant FEC, showed neither overexpression or amplification of topoisomerase IIα to be predictive of response [160].

A direct role/association for her-2/*neu* amplification, in parallel with topoisomerase IIα amplification, in breast cancer drug resistance has yet to be established. The topoisomerase IIα gene is located adjacent to her-2/*neu* oncogene on chromosome 17q12-q21 and it is described as either amplified or deleted in almost 90% of her-2/*neu* amplified breast tumours [161-162]. A number of recent studies have, therefore, co-investigated topoisomerase IIα and her-2/*neu* in breast cancer, to establish if they may have a co-operative relevance. Topoisomerase IIα amplification in breast cancer has been described as often, but not exclusively, accompanied by her-2/*neu* gene amplification [163]; conversely, lack of topoisomerase IIα amplification in the absence of her-2/*neu* gene amplification has been reported [164]. Further conflicting studies report amplification of her-2/*neu* and topoisomerase IIα associated with increased response to *pre*-operative doxorubicin [153-154]; amplification of topoisomerase IIα, but not her-2/*neu*, correlating with response to anthracyclines [165]; while in other cases, amplification of neither her-2/*neu* or topoisomerase IIα was predictive of response to FEC [160].

#### *Glutatione-S-Transferases*

Glutatione-S-transferases (GSTs) are phase II enzymes involved in detoxification and cell protection. In humans, 5 major classes of GSTs have been identified, including α, μ, π, δ, and θ [166]. Both GST π and μ classes are expressed in breast cancer tissue [167]. GSTs have been implicated in resistance to doxorubicin, melphalan, cisplatin, chlorombucil, and other alkylating agents [168] but, although GSTs may inactivate chemotherapeutic agents by conjugating to glutathione, their involvement in *in vivo* resistance to chemotherapy in unclear [169-175]. A study of 42 primary breast patients was recently reported where GST π expression in the tumour was associated with poor response to FAM [176]. Although analysis of 2048 cases of breast cancer and 1969 controls indicated that single GST polymorphisms do not confer a risk of breast cancer [166], studies of GST polymorphisms in 1034 patients with invasive breast cancer showed no relevance to any of the GST μ or GST θ genotypes, but found a significant association between GST π polymorphisms and clinical outcome in patients who had received chemotherapy [177].

GST-dependent drug resistance may be of a limited relevance *in vivo*. Further studies are necessary to investigate this.

### *Cytochrome $P_{450}$ (CYP450)*

CYP450 is a super-family of heme-containing mono-oxygenases that is involved in the synthesis and metabolism of a wide range of endogenous and exogenous compounds [178]. Fifty-seven human CYP genes have been sequenced and have been grouped into 18 families and 43 sub-families, according to sequence homology. The families involved in drug metabolism include CYP1, CYP2 and CYP3. Almost all anti-cancer drugs are chemically modified by one or more member(s) of this large family of enzymes. Potentially, therefore, the expression of certain key metabolic enzymes in tumours could be a significant determinant of the sensitivity of these cells to the effects of cytotoxic agents.

Chemotherapeutic drugs are susceptible to different cytochrome P450 metabolic enzymes; however, in the majority of cases, a limited number of enzymes are thought to be particularly important for the conversion of the active cytotoxic species. Members of the cytochrome P4503A (CYP3A) family metabolise the vast majority of xenobiotics; with one isoform, CYP3A4, being responsible for the metabolism of approximately 50 % of all known drugs. CYP3A4 is a major metabolising enzyme for docetaxel, doxorubicin and cyclophosphamide, so its presence in a tumour may contribute significantly to drug sensitivity/resistance. Limited information available to date suggests that CYP3A induction may be frequent in primary breast tumours, where there is also an association with lower proliferation rates [179-180]. Evidence of a role for CYP3A4 expression in breast cancer has been further suggested by a study of normal (5 specimens) and tumour (7 specimens) breast tissue specimens indicating the presence of two CYP3A4 variants *i.e.* one predominantly associated with tumour tissue and the other associated with normal tissue [181]. Furthermore, analysis of CYP3A4 in (38 cases) breast biopsies suggest that intratumoral *cyp3A4* mRNA levels may be a predictor of response to docetaxel, but not to cyclophosphamide + epirubicin [182]. However, most studies reported have been performed on small numbers of cases with no definitive reports on correlations with prognostic/predictive variables.

While reduced levels of P450 reductase expression are associated with resistance to mitomycin C in breast cancer [168], the relevance – if any – of other P450s, including CYP19 (encoding for cytochrome P450 aromatase [183]; inhibitors of which have shown substantial activity in primary and advanced breast cancer [184-186]); CYP1B1 (detected in many tumour types including breast [187]); and CYP2E1 (expression described as being significantly higher in tumours compared to normal breast tissue in some [188], but not in other [189], studies) have yet to be determined.

### Mis-Match Repair, Transglutaminase, Glucosyl-ceramide, p27, Annexin-1, PTEN, BRCA1 and 2, NFkB

Other mechanisms of resistance to chemotherapy in breast cancer include mis-match repair deficiency. Loss of DNA mis-match repair has been reported to result in resistance to cisplatin, alkylating agents, and the topoisomerase II poison doxorubicin, but apparently this does not result in resistance to taxanes [190]. Loss of mis-match repair protein (MLH1) expression after *neo*-adjuvant chemotherapy in node-positive breast cancer (29 cases studied *pre-* and *post-* therapy) has been described as an independent predictive factor for poor DFS [191]. This mechanism has also been associated with resistance to adjuvant CMF in a study of 71 sporadic invasive ductal carcinomas of the breast [192]. Furthermore, results from *in vitro* cell models and a limited number of clinical studies of breast cancer indicate overexpression of tissue transglutaminase [56], glucosylceramide synthase [193-194] and annexin-1 [195], as well as reduced levels of p27 tumour suppressor gene expression [196-197] and loss of phosphatase and tensin homologue deleted on chromosome 10 (PTEN) tumour suppressor gene expression [198-199] to be associated with chemo-resistance in breast cancer. Recent studies have associated deficiencies in hENT1 (human equilibrative nucleoside transporter 1 [200]) expression with gemcitabine resistance and have proposed that this mechanism may be associated with poor clinical response to capecitabine in breast cancer [201].

Although mutations in BRCA1 and BRCA2 are present in only 5-10% of all breast cancers, compared to the general population carriers of these mutations have a higher risk of developing breast cancer, with increased risk of aggressive contralateral cancer [202]. Results from a study of 125 patients, however, indicate that although BRCA positive patients more frequently have negative prognostic factors, their overall prognosis is generally equal or better than those with wild-type BRCA [203]. Studies evaluating an association between BRCA1 and BRCA2 mutations and/or expression and drug resistance are very limited. However, real-time PCR analysis of *brca1* and *brca2* mRNAs in 25 patients with locally advanced (n=13) or locally recurrent (n=12) tumours indicates a significant association between *brca2* mRNA levels and response to docetaxel *i.e. brca2* mRNA levels of responders were significantly lower than those for non-responders. In this study, no such association was found for *brca1* mRNA expression [204]. However, increased expression of *brca1* mRNA has been associated with favourable response to anthracycline (epirubicin)-containing chemotherapy [205]. Recently the activity of NFkB has been shown to prognostically sub-divide $ER^+$ primary breast cancers, with increased p50 subunit DNA binding activity apparently more clinically relevant than increased p65 activity [206]. A definitive relationship between NFkB and drug resistance has yet to be determined.

## TARGETED THERAPIES

It is becoming increasingly likely that future therapies will be determined not only by patients characteristics, but also by the molecular biology of the individual's tumour [207-209]. More extensive molecular characterisation, using modern techniques to obtain information on the genomic profile of breast tumours will enable more specific, individualized, treatment. The use of such systems, including DNA microarrays [131,210-211] and proteomics [212], in addition to exploiting methods identified *in vitro* for cell re-sensitising to chemotherapy (*e.g.* through the use of proteasome inhibitors [213], specific taxane-based MDR reversal reagents (TRAs) [214], and SiRNA/RNAi [215-

216]), will allow further development of customised therapies directed toward the particular molecular defects in a given cancer [217].

Recent advances in molecular biology have already led to a new era of anticancer drugs targeting specific genetic defects/genes involved in cell proliferation, apoptosis, and angiogenesis in malignant cells. These include the use of imatinib mesylate (ST1571; gleevec) for bcr/abl-positive chronic myelogenous leukaemia; trastuzumab (herceptin), an antibody-based targeted therapy for her-2/*neu* overexpressing metastatic breast cancer; cetuximab (erbitux), for EGFR overexpressing metastatic colorectal cancer and approved for phase III development for breast cancer [19]; rituximab (rituxan), a monoclonal antibody to CD20 used in non-Hodgkin's lymphoma; gefitinib (ZD1839; iressa) a tyrosine kinase inhibitor of EGFR used in NSCLC and shown to inhibit BCRP-mediated drug resistance *in vitro* and *in vivo* [218]; as well as the proteasome inhibitor bortezomib (valcade) and the anti-angiogenesis agent bevacizumab (avastin)) are the cause of cautious optimism for the future [219-220]. To date, enhanced therapeutic efficacy with these molecular targeting agents over traditional chemotherapy has been shown in patients with advanced or recurrent disease. Several vaccines against the HER family of proteins have also been developed and are currently being investigated as therapies for breast cancer [19, 221-222]. Further extensive studies, aimed at establishing appropriate combinations of targeted therapies (and possibly vaccines) with anticancer drugs appropriate for specific patient sub-groups, are now needed.

## CONCLUSION

Chemotherapy is an essential component of the current treatment regimes for breast cancer. Unfortunately, the efficacy of this treatment is often limited by either inherent or acquired resistance to cytotoxic drugs, whether used singularly or in combination. Since MDR1/Pgp was reported almost 30 years ago, through analysis of relevant cell lines as *in vitro* models and studies of clinical specimens, our understanding of this multifactorial phenomenon has greatly increased. However, as a consequence of the complex nature of drug resistance and the range of analytical techniques used to assess the relevance of limited numbers of gene products, many reports to date have produced conflicting results; leaving us with as many questions as answers.

The recent development of advanced technologies for high-throughput genomic (expression microarrays) and proteomic profile analyses of breast tumours in comparison to normal tissue, and of drug -resistant compared to -sensitive cells, should allow us to clarify discrepancies observed in previous studies, as well as enabling novel markers and pathways involved in drug resistance to be identified and investigated, simultaneously. The relevance of mRNA/microarray analysis in breast cancer has been highlighted by Van't Veer *et al.* [210] who performed expression microarray analysis of 78 sporadic lymph node-negative tumours (<5 cm in diam.) in women under 55 yrs. old. Approximately 50% of the cases had developed metastasis at 5 years. Very few of the patients had received systemic treatment. Expression analysis of approx. 25,000 gene transcripts was performed, from which a list of 70 discriminatory genes was identified whose expression patterns in primary tumours associated with a group of cases that did not result in metastasis, despite no systemic therapy. In a more extensive (295 cases) follow-up study, including lymph node-positive as well as -negative tumours (again where tumours were <5 cm; age ≤52 yrs.), with longer follow-up information, Van de Vijver *et al.* [223] reported their previously predicted gene expression profile to also be a strong predictor of distant metastases development in lymph node-positive disease.

These exciting microarray studies [210, 223] have been heralded a break-through and a relevant way to analyse and evaluate breast biopsies – to the extent that they have formed the basis of a clinical trial entitled MINDACT (Microarray for Node Negative Disease may Avoid Chemotherapy), using the first microarray-based diagnostic, MammaPrint® (Agendia BV)). This phase III clinical trial is supported by €7m EU funds (contributing approx. 1/3 of the overall funds), is planned to begin in late 2005 in approximately 39 institutions in 21 countries and it is proposed to involve approximately 5,000 women over a 3 year period, to specifically establish if the 70 gene signature identified can predict response to chemotherapy and so help to reduce the numbers (estimated at 12-20%) of patients who are "over-treated" with chemotherapy. More extensive studies, including different cohort of patients and including non-cancer breast specimens as controls, are now necessary. It is anticipated that our increased understanding of the molecular profile of different breast cancers, compared to normal tissue, and how these profiles relate to response to different kinds of therapies, will allow us to translate this knowledge to selection of best chemotherapeutic and targeted therapy combinations for individual breast cancer patients.

## ACKNOWLEDGEMENTS

We wish to acknowledge support from the Albert College Fellowship, Dublin City University; the Faculty of Science and Health's Targeted Research Initiatives Fund, Dublin City University; and Ireland's Higher Educational Authority Program for Research in Third Level Institutes (PRTLI) Cycle 3.

## ABBREVIATIONS

ABC = ATP-binding cassette

AC = Doxorubicin with cyclophosphamide

Adr = Dox = adriamycin = doxorubicin

CAF = Cyclophosphamide and doxorubicin and 5-fluorouracil

CGH = Comparative genomic hybridisation

CMF = Cyclophosphamide with methotrexate and 5-fluorouracil

DFS = Disease-free survival

EC = Epirubicin with cyclophosphamide

ED = Epirubicin and docetaxel

ER = Estrogen receptor

FAC = 5-Fluorouracil with doxorubicin and cyclophosphamide

FAM = 5-Fluorouracil with doxorubicin and mitomycin C

FEC = 5-Fluorouracil with epirubicin and cyclophosphamide

LN = Lymph node

MDR = Multiple drug resistance

MVP = Major vault protein

MX = Methotrexate

ND = Not described

nX = Cycle number

OS = Overall survival

PgR = Progesterone receptor

RFS = Relapse-free survival

Vp = Verapamil

TP = Thymidine phosphorylase

VP = VP-16/etoposide

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**Received: July 20, 2005** **Revised: February 1, 2006** **Accepted: February 3, 2006**