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# Anthracycline Resistance in Breast Cancer: Clinical Applications of Current Knowledge

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## INTRODUCTION

THE STUDY of resistance to anthracyclines in breast cancer has two major goals: (1) to predict which patients will or will not respond to anthracyclines; and (2) to understand the basic underlying mechanisms of cellular resistance to anthracyclines. The interplay between these two perspectives is central to the development of new strategies for the improved use of anthracyclines in the treatment of breast cancer. In practical terms, the clinician wants to know: can he predict which patients will respond to anthracyclines; in anthracycline resistant patients, are there other antineoplastic agents that are either likely or very unlikely to induce clinical responses; and finally, are there specific ways to reduce anthracycline resistance and, therefore, make anthracyclines more efficacious.

The clinical definition of anthracycline resistance is a state in which anthracyclines do not cause tumour regression, or in the case of adjuvant therapy, does not delay tumour recurrence. Certain imprecisions in the clinical definition of anthracycline resistance must be recognised. First, issues of dose intensity [2] and therapeutic index must be recognised as having an impact on the percentage of patients demonstrating anthracycline resistance. High dose anthracycline programmes clearly cause more patients to respond than low dose programmes [3–6]. A second complicating factor in the use of clinical trial data is that most protocols use anthracyclines as part of multidrug regimens. Thus, it is often not clear that the development of resistance to a regimen is due to increased acquired resistance to the anthracycline component or due to changes in sensitiviy to other antineoplastic agents in the regimen. Another clinical scenario in which it is difficult to define the status of anthracycline resistance (and in particular relate it to underlying mechanisms of resistance) is when the tumour is clearly sensitive to therapy, but has a high mitotic rate which allows regrowth between treatment cycles. Here again, the clinical definition of anthracycline resistance is blurred.

Patients who have failed on anthracyclines and who then respond to a second anthracycline again illustrate that the clinical definition of anthracycline resistance does not define an absolute state. For example, patients who have failed on FEC (fluorouracil, epirubicin and cyclophosphamide) for metastatic breast cancer have then responded to FAC (fluorouracil, doxorubicin and cyclophosphamide) [7]. Also, studies have shown that patients given anthracyclines as adjuvant therapy have modestly decreased (<2-fold) response rate, but there is still a substantial possibility of secondary response to anthracyclines for metastatic recurrent disease [8]. We cannot infer from the published data whether these second responses to anthracyclines are due to dose intensification, loss of resistance over time or differing resistance mechanisms.

Despite the imprecision of terms resulting from the non-absolute nature of anthracycline resistance, it is, to some degree, worth defining the "drug resistance" that exists in previously treated patients participating in clinical trials so that this important predictive parameter can be utilised in comparing trial results. Here, primary resistance to anthracyclines generally refers to patients who have never responded to, and progressed on, an anthracycline-containing regimen. Secondary or acquired resistance to anthracyclines refers to the status of patients who may have responded, but most recently have progressed. These definitions often are not adhered to and the precise definition utilised for a resistant population should be given in clinical trial reports. For example, are patients who have developed recurrence within 6 months of an anthracycline-containing adjuvant regimen defined as belonging to a resistant population?

## UNDERLYING MECHANISMS OF ANTHRACYCLINE RESISTANCE

The majority of publications on anthracycline resistance centre on the multiple drug resistance (MDR) drug efflux mechanism [9], however there are five basic mechanisms proposed as playing important roles. These mechanisms include drug efflux pumps (chiefly MDR), topoisomerase II, glutathione metabolism, intracellular stress-related proteins and apoptotic mechanisms.

### *Drug efflux mechanisms*

The most widely studied resistance mechanism to anthracyclines is cellular drug efflux pumps. The work with multidrug resistant cell lines demonstrated that there was increased accumulation of therapeutic agents and crossresistance to multiple agents, including anthracyclines [10]. In subsequent work, the gene encoding the pump was sequenced and the structure was shown to be analogous to other recognised transport proteins [11]. This 170 kDa surface membrane (known as MDR1 or P-glycoprotein) was overexpressed in many multidrug resistant cell lines. It is now clear that MDR is not the only important efflux pump as overexpression of others, such as the multidrug resistance associated protein (MRP), have also been associated with drug resistance. That proteins other than MDR1 may be associated with anthracycline resistance, has been suggested by studies in which doxorubicin-resistant breast cancer cells *in vitro*

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expressed low levels of drug accumulation, normal topoisomerase II and MDR1, but overexpressed another drug efflux driving protein, MRP [12]. Other proteins involved in drug efflux have been only partially characterised to date, and have been shown to be overexpressed in some breast cancer tumour specimens [13, 14]. Drug efflux pumps are of particular interest as there are a number of agents, including commonly used drugs such as verapamil, quinine and cyclosporin, that can inferfere with their activity.

A number of investigators have examined the expression of the *MDR1* gene (P-glycoprotein) in breast cancer tumour specimens [15], studying the frequency of *MDR* expression in primary tumours, whether it allowed prediction of response to anthracyclines, and whether modulators could increase anthracycline efficacy. *MDR1* gene expression is one of several markers suggested to have a role in the prediction of breast cancer response to systemic treatment [16]. Investigators have found quite a different incidence of *MDR* expression in primary breast cancers. This may be, in part, due to the different methodologies for measuring *MDR*. Some studies using tissue homogenates of primary tumours and measuring mRNA [17], DNA or protein expression [18] have reported no detectable levels and no gene amplification in primary tumour specimens. This would suggest that examination for *MDR* in primary tumours will not allow prediction of which patients should recieve anthracycline-based adjuvant therapy, or, which patients would respond to anthracycline therapy at relapse. This work conflicts with other studies. In a Glasgow study, 25/49 primary breast tumours exhibited detectable levels of mRNA for *MDR*. The presence of mRNA for *MDR* in the tumour specimens predicted responsiveness to doxorubicin in soft agar cloning assays *in vitro* [19].

In a French study, the expression of the mRNA for *MDR* was measured in a prospective series of 30 patients treated with primary chemotherapy and who were sequentially biopsied. The patients in this study had stage II or III breast cancer and were included in a randomised protocol receiving either 4 cycles of 5-fluorouracil, doxorubicin and cyclophosphamide (FAC) or 5-fluorouracil, thiotepa and cyclophosphamide (FTC) [20]. The results showed that levels of *MDR* gene expression (mRNA) increased after the first cycle of chemotherapy in 32% of patients, and that this increase in gene overexpression is more often observed in patients treated with FAC than FTC (42% versus 11%, respectively). An Italian study reported the presence of high levels of *MDR* expression detected by immunohistochemistry in 23/30 patients with locally advanced breast cancer, and found that overexpression correlated with poor response to therapy [21]. Some studies, incorporating serial biopsies during chemotherapy, have shown evidence that expression may increase during therapy [22, 23]. However, it cannot simply be inferred that overexpression of *MDR* is due to selection of resistant clones in the presence of an anthracycline. A similar phenomenon of increased *MDR* expression has been seen in stage IV breast cancer patients undergiong repeated biopsies during endocrine therapy [24, 25].

Studies of drugs which interfere with the *MDR* efflux pump give conflicting data regarding the utility of this strategy. In studies with small numbers of patients at the University of Arizona, in women with cyclophosphamide, vincristine, doxorubicin, dexamethasone (CVAD)-resistant breast cancer [26, 27], 5/27 (19%) achieved partial (PR) or complete remission (CR) with the addition of verapamil and quinine. These findings indicate that resistance can be clinically reversed, and supports further development and testing of agents that interfere with MDR. The use of amiodarone or quinidine, together with either vinblastine or doxorubicin, has been studied in 18 women with advanced breast cancer [28]. These women had been evaluated for *MDR* expression, detected by immunohistochemistry, and it was detected in 9 patients. *MDR* expression did not appear predictive of response to therapy in this small study, although there was a suggestion that patients who were *MDR* positive had longer durations of response. Other small studies have similarly reported the addition of a MDR modulator to salvage patients. For example, a CR has been reported after the addition of verapamil in a breast cancer patient progressing on continuous infusion of vinblastine [29].

Not all studies using modulators of MDR have found evidence of enhanced therapeutic effect. In a randomised placebo controlled prospective trial [1] with 233 patients treated with epirubicin (100 mg/m$^2$ every 3 weeks) for locally advanced or metastatic breast cancer, those patients treated with quinidine had no increased level of response (57% versus 54% in the placebo group), and no increase in overall survival. Serum and tissue levels of the quinidine appeared adequate for an observable modulation of the MDR-related drug efflux [30].

An interesting but often unexplored aspect of MDR modulator studies is whether the modulators affect the pharmokinetics of the chemotherapeutic agent. Efflux pumps may play an important role in drug metabolism. For example, cyclosporin modulates MDR action, but it also dramatically slows daunorubicin and doxorubicin metabolism, dramatically increasing drug toxicity [31, 32]. It is, therefore, possible that some apparent modulators of MDR may induce responses, not by affecting intracellular concentrations of anthracycline though interference with the MDR in neoplastic cells, but by indirect effects on systemic metabolism of the anthracycline.

*Topoisomerase II*

Topoisomerase II is the major mechanistic target of anthracyclines [33], and responsible for strand breaks crucial to DNA replication. Anthracyclines result in stabilisation of a topoisomerase II–DNA intermediate in this process. Cells expressing high levels of topoisomerase II are more susceptible to generation of these intermediates (and interference with cell division), while cells expressing low levels of topoisomerase II are relatively resistant [34].

Topoisomerase II is the major target for anthracyclines, although to date relatively few clinical studies correlating levels of its expression and resistance have been done in breast cancer. Topoisomerase II levels detected by immunohistochemistry have suggested that it may be a marker for cell proliferation as it is highly correlated with Ki67, and inversely correlated with steroid hormone receptors [35]. Genetic analyses show that, in some cases of primary breast cancer, topoisomerase II is clearly amplified [36, 37] and often co-amplified with *c-erbB-2* which shares a similar chromosome location (17q21-22).

Only one published study correlated topoisomerase levels with response to doxorubicin in breast cancer patients. This study, using dot blots to detect mRNA levels of topoisomerase II, found that in 15 patients, 6 had high topoisomerase levels and in these patients there were five objective responses. There were no responses in the 9 patients with low topoisomerase levels ($P<0.01$). In the same small study, *MDR1* and *GST-pi* expression did not correlate with a clinical response to doxorubicin [38].

*Glutathione-related proteins*

Glutathione-related mechanisms mediate a variety of normal detoxification reactions in cells, and may represent potential drug resistance mechanisms in neoplastic cells. Because many anthracyclines have free radical intermediates, which induce damage to DNA, part of the cytotoxicity of anthracyclines is due to free radical-induced mechanisms, which would be reduced by glutathione-dependent protective mechanisms. Increased expression of glutathione transferase has been documented in some anthracycline-resistant breast cancer cell lines [39] and also in some primary breast tumours [40]. Loading cells with glutathione peroxidase has also demonstrated increased resistance to doxorubicin, suggesting the possible importance of this mechanism [41]. Clearly, however, this mechanism is not universal in anthracycline-resistant cell lines [42]. No clinical studies of breast cancer patients have been done to demonstrate an association between anthracycline resistance and overexpression in these systems.

*Stress-related proteins (heat shock proteins)*

Studies in breast cancer cell lines have shown that the induction of heat shock proteins is associated with induction of resistance to doxorubicin. This resistance was associated, not with induction of MDR protein, but with a several-fold increase in the levels of heat shock proteins [43]. Studies have provided data, albeit conflicting, on the prognostic significance of heat shock protein expression of primary breast cancers prior to treatment, but have not addressed whether they predict resistance to anthracycline-based adjuvant therapy [44, 45]. The demonstration that isoquinolinesulphonamides interfere with heat shock protein induction suggests a means by which heat shock proteins might be modulated [46].

*Apoptosis and other mechanisms*

A decrease in the ability of cells, particularly aberrant cells, to undergo programmed cell death — apoptosis — may be one of the mechanisms which explains the growth of breast tumours. Thus, relative resistance to a chemotherapeutic agent might, in part, be conferred by a change that protects heavily damaged cells from undergoing apoptosis. It has been suggested that aberrant expression of *TP53* may be implicated. This hypothesis suggests that escalated cell division may decrease the opportunity for cells to correct aberrations before they pass through "checkpoints", thus rendering them more susceptible and thereby more susceptible to chemotherapeutic agents that damage DNA, due to an apoptotic surveillance mechanism. This hypothesis provides a framework for predicting how the expression of some commonly obtained prognostic factors might correlate with anthracycline senstivity. This topic warrants further clinical investigation. The trial results of one co-operative group suggest a higher level of anthracycline sensitivity in patients with high *c-erbB-2* expression or S-phase [47], and relative resistance in patients with low *c-erbB-2* or S-phase. This provocative result, as yet unreplicated, suggests that some proteins not directly affected by or interacting with anthracyclines may be important in modulating their effects, and might be used to predict anthracycline sensitivity.

## SUMMARY

Anthracyclines are highly effective antineoplastic agents for the treatment of breast cancer. Nevertheless, essentially all breast cancer patients have tumours which are intrinsically resistant or which develop resistance during the course of therapy. Clinical trails provide indirect information on the nature of anthracycline resistance and work in the basic sciences has demonstrated molecular mechanisms which play a role. Initial clinical attempts to expolit and translate these mechanisms to predict, and interfere with, anthracycline resistance have met with mixed success, and have not yet led to accepted clinical applications.

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