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The biology of neoadjuvant chemotherapy for breast cancer

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Abstract

Neoadjuvant/pre-surgical medical therapy of breast cancer provides a unique opportunity to derive biological information related to tumour response. Large clinical trials of neoadjuvant chemotherapy have established that pathological complete remission is an independent predictor of improved disease-free survival. Clinical response has been found to parallel substantial reductions in the proliferation of breast cancer cells. Increased apoptosis also occurs, but it is not closely associated with response. Numerous biological markers such as p53, bcl-2, oestrogen receptor (ER) and HER2 have been assessed for their possible role in chemoresistance/response, but the data are not clear at this stage. Continuing work using cDNA microarrays may yield new, more reliable indices of likely response and an improved insight into biological processes related to chemotherapeutic response.

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Introduction

Biological rationale for neoadjuvant chemotherapy

Most breast cancer patients who receive chemotherapy do so immediately after surgery – that is, adjuvantly. However, chemotherapy may be given before surgery, as so-called neoadjuvant treatment. Synonymous terms include 'primary medical therapy' or 'induction chemotherapy'.

The use of neoadjuvant chemotherapy originates from the treatment of locally advanced and inoperable breast cancer. In the special case of inflammatory breast cancer, primary chemotherapy occupies a central role, with many centres favouring treatment with cytotoxic agents and radiotherapy, and avoiding surgery if at all possible.

Interest in neoadjuvant chemotherapy in part arose after animal studies conducted by Fisher *et al*. (1989*a, b*). In various animal models, they demonstrated that removal of the primary tumour resulted in an increase in the labelling index in residual tumour cells and an increase in circulating growth-stimulating factors (Fisher *et al*. 1989*a*). Administration of neoadjuvant chemotherapy, endocrine treatment or radiotherapy to these animals impaired the increase in cell growth observed in residual tumour cells in untreated animals (Fisher *et al*. 1989*b*). Furthermore, neoadjuvant treatment prevented the production of circulating factors that were capable of causing stimulation of growth of similar tumours in untreated recipients. On the basis of these results, it was hypothesised that the delivery of chemotherapy before surgical excision of tumour may improve patient outcome by preventing unfavourable kinetic responses.

Clinical rationale for neoadjuvant chemotherapy

Conventionally, neoadjuvant treatments are given to downstage tumours, with the aim of rendering them more amenable to conservative surgery. In the case of breast cancer, this translates to the ability to downstage tumours to a point at which mastectomy can be avoided whilst maintaining an equivalent rate of local control.

This review first considers the outcome of the major clinical studies and then discusses the molecular understanding of the mechanism of action of key cytotoxics of contemporary importance. Thereafter, biomarker studies are considered in relation to the prognostic and predictive information they provide, focusing on the insight into biological mechanisms that they provide.

Clinical experience with neoadjuvant chemotherapy

Randomised trials

Following the demonstration of the feasibility of neoadjuvant chemotherapy in phase II trials, several phase III trials have

been performed to compare mastectomy, local control and survival rates with those of conventional treatment in operable disease. Another objective of these trials was to assess whether the pathological response to chemotherapy could be correlated with outcome; although the opportunity fully to stage these patients pathologically at time of presentation is lost, the extent of residual viable disease after chemotherapy can be evaluated accurately.

The largest of these studies was the multi-centre National Surgical Adjuvant Breast and Bowel Project B-18 (NSABP B-18) trial (Fisher *et al*. 1997, 1998). Fifteen hundred women were allocated randomly to groups to receive either adjuvant or neoadjuvant chemotherapy with adriamycin and cyclophosphamide (AC). Thirty-six percent of the patients allocated to receive neoadjuvant chemotherapy achieved a complete clinical response (cCR) and 13% had no demonstrable disease on microscopic examination of the surgical sample (complete pathological response, pCR). After a follow up of 5 years, no differences have been observed between the two arms in terms of the incidence of regional or distant relapse. Patients demonstrating cCR were found to have a significantly improved disease-free survival (P= 0.0014), but not overall survival. In contrast, pCR was associated with improved disease-free survival (P=0.0001) and overall survival (P=0.06). This has led to pCR becoming a major endpoint in continuing trials, as a potential surrogate for long-term outcome.

The European Organization for Research and Treatment of Cancer recently reported on the results of a trial of very similar design (van Der Hage *et al*. 2001). Seven hundred patients were allocated randomly to groups to receive either four cycles of neoadjuvant or adjuvant 5-fluorouracil, epirubicin and cyclophosphamide (FEC) chemotherapy. No difference in overall or disease-free survival was seen between the two groups. cCR was seen in 6.6% of the neoadjuvant group, and pCR in only 3.7%. As in the NSABP study, pCR but not cCR was associated with an overall survival benefit, with the P value on the latter in this case reaching statistical significance ($P = 0.008$). In the neoadjuvant group, 23% of patients were downstaged to being suitable for breast-conserving surgery from previously requiring mastectomy. These patients did less well in terms of overall survival (hazards ratio 2.53; 95% confidence interval 1.02 to 6.25) compared with patients deemed to be suitable for breast-conserving surgery before chemotherapy although, interestingly, there was no effect on locoregional recurrence rates. The inferior survival of this group may be a reflection of the more aggressive biology of their tumours.

A smaller, single-centre study conducted in our unit yielded very similar results and is summarised along with the other randomised trials in Table 1 (Mauriac *et al*. 1991, 1999, Scholl *et al*. 1994, Semiglazov *et al*. 1994, Powles *et al*. 1995, Fisher *et al*. 1997, 1998, Makris *et al*. 1998*b*, Broet *et al*. 1999, van Der Hage *et al*. 2001).

The general consensus from these trials is that neoadjuvant chemotherapy results in a modest reduction in mastectomy rates, possibly accompanied by a small increase in the risk of local recurrence, especially in younger women. Importantly, for the future of presurgical studies, on the basis of the above observations it is possible to say that neoadjuvant chemotherapy is of no detriment to patients in circumstances when adjuvant chemotherapy would be indicated.

Newer regimens

The AC chemotherapy regimen remains the gold standard in neoadjuvant chemotherapy. However, several trials have reported high response rates for taxane-containing regimens

Table 1 Randomised clinical trials of neoadjuvant compared with adjuvant chemotherapy in operable breast cancer.

Study	Author	Clinical stage (all M0)	Year	No. of patients	Preop. arm treatment	Postop. arm treatment	DFS preop. (%)	DFS postop. (%)	*P*	OS preop. (%)	OS postop. (%)	*P*	Notes
NSABP-B18	Fisher *et al.* Fisher *et al.*	T1–3 N0–1	1997 1998	1523	AC	AC	67	67	NS	80	80	NS	
EORTC	Van Der Hage *et al.*	T1c–T4b N0–1	2001	698	FEC	FEC	65	70	NS	82	84	NS	
RMH	Powles *et al.* Makras *et al.*	T0–T4 N0–1	1995 1998	309	2M/3M + tam.	2M/3M + tam.	N/A	N/A	NS	N/A	N/A	NS	
Institute Bergonie	Mauriac *et al.* Mauriac *et al.*	T2–T3 N0–1	1991 1999	272	EVM × 3, MTV × 3	EVM × 3, MTV × 3 if high risk	50	50	NS	82	75	NS	Preop. arm: underwent RT or Sx
Institute Curie	Scholl *et al.* Broet *et al.*	T2–T3 N0–N1	1994 1999	414	CAF	(RT ± Sx) CAF	N/A	N/A	NS	65	60	NS	Sx if incomplete response to chemo/RT
St Petersburg	Semiglazov *et al.*	T1–2N2 T2N1 T3N0–1	1994	271	1–2 × TFM RT → Sx →MF × 4–5	RT→Sx→ TMF × 6	81	72	0.04	86	78	NS	

DFS, disease free survival; OS, overall survival; AC, adriamycin, cyclophosphamide; CAF, adriamycin, fluorouracil, cyclophosphamide; EVM, epirubicin, vincristine, methotrexate; FEC, 5-fluorouracil, epirubicin, cyclophosphamide; 2M/3M, mitoxantrone, methotrexate/mitoxantrone, methotrexate, mitomycin C; MTV, mitomycin, thiotepa, vindesine; TFM, thiotepa, fluorouracil, methotrexate; N/A, not available, RT, radiotherapy; Sx, surgery; tam., tamoxifen.
NS, p>0.05.

(Holmes *et al*. 1991, Moliterni *et al*. 1997), although phase III studies have failed to demonstrate an advantage over anthracyclines (Buzdar *et al*. 1999).

It is known that taxanes have activity in anthracycline-resistant disease, which prompted the design of the NSAPB B-27 study. This is a three-arm trial in which 2411 patients have been allocated randomly to (1) four cycles of neoadjuvant AC followed by surgery, (2) four cycles of AC followed by four cycles of docetaxel followed by surgery, and (3) four cycles of AC, followed by surgery and then adjuvant docetaxel. Preliminary results from the trial demonstrate that AC + docetaxel is associated with a statistically significant greater rate of pCR than AC alone (18.7% compared with 9.8%) and a statistically significant greater rate of pathologically negative nodes at time of surgery (58.1% compared with 50.7% (NSABP 2001). As yet, the impact of this on survival is unknown.

In another study that included some individuals with locally advanced disease, patients received neoadjuvant cyclophosphamide, doxorubicin, vincristine and prednisolone (CVAP). Non-responders after four cycles proceeded to four cycles of docetaxel and responders were allocated randomly to either four further cycles of CVAP chemotherapy or four cycles of docetaxel. An increased pCR rate was observed in those responders who switched to docetaxel, although docetaxel did not result in a reduction in the rate of axillary node involvement (Smith *et al*. 2002).

Neoadjuvant chemotherapy: mechanism of cytotoxic action

Background

Patients undergoing breast neoadjuvant chemotherapy provide an ideal research tool for the investigation of factors that may be used as indicators of outcome (prognostic factors) or indicators of chemoresponse (predictive factors). The breast is anatomically accessible and methods exist for obtaining serial biopsies atraumatically and in a manner that is acceptable to the patient. This provides an opportunity to observe the effects of chemotherapy on biological processes. As response to chemotherapy can also be prospectively assessed readily in this setting (an opportunity that never presents itself in the adjuvant setting), the relationship between the changes in the biological processes and the response can be determined. Because changes in proliferation or apoptosis are required for change in tumour growth, studies of these processes and their control pathways are of particular interest (Fig. 1, Fig. 2).

Mechanism of action of cytotoxic agents used in neoadjuvant chemotherapy treatment

The two classes of cytotoxic drugs that demonstrate most activity towards breast cancers are anthracyclines and taxanes. Experience over the years has shown that combination chemotherapy is more effective than single agents. The combination approach may succeed where single agents fail probably because, although individually the maximum tolerated doses of each drug may be insufficient to kill all malignant cells, in combination 100% kill may be achieved with tolerable toxicity, as long as the toxicity of the component drugs is non-overlapping. Furthermore, tumours represent a heterogeneous population of cell lineages that vary in terms of sensitivity (and resistance) to chemotherapy. A combination approach allows a broader range of coverage. Of the many chemotherapy regimens that have been used in the treatment of breast cancer, the most widely prescribed will be discussed briefly.

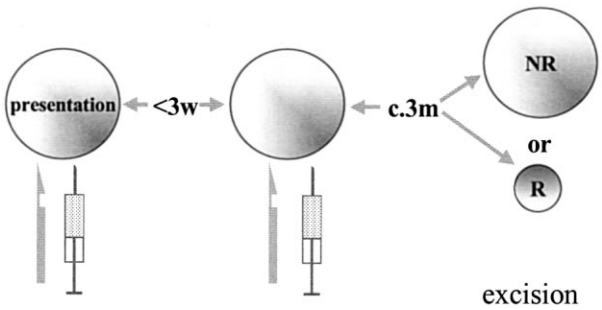

Figure 1 Primary medical (neoadjuvant) therapy sampling for biological assessment and response correlations. NR, non-responder; R, responder; 3w, 3 weeks; c.3m, approximately 3 months.

Anthracyclines

The most commonly used anthracycline, doxorubicin, is a natural compound produced by the *Streptomyces* species, whereas epirubicin, which is also widely used in the treatment of breast cancer, is synthetically produced. They act by several mechanisms, but most important is their interaction with the nuclear enzyme topoisomerase II (Smith & Soues 1994). In the resting state, DNA is tightly coiled into a compact secondary structure. Topoisomerases reduce DNA twisting and supercoiling, allowing selected regions of DNA to untangle and so engage in transcription, replication or repair processes.

Anthracyclines induce formation of covalent topoisomerase IIα–DNA complexes, which prevent the enzyme from completing the religation part of the ligation–religation reaction (Chen & Liu 1994). Anthracyclines are also DNA intercalators, causing single-stranded or double-stranded breaks. Furthermore, they can undergo reduction to produce free radicals that can cause oxidative damage to cellular proteins (Smith & Soues 1994).

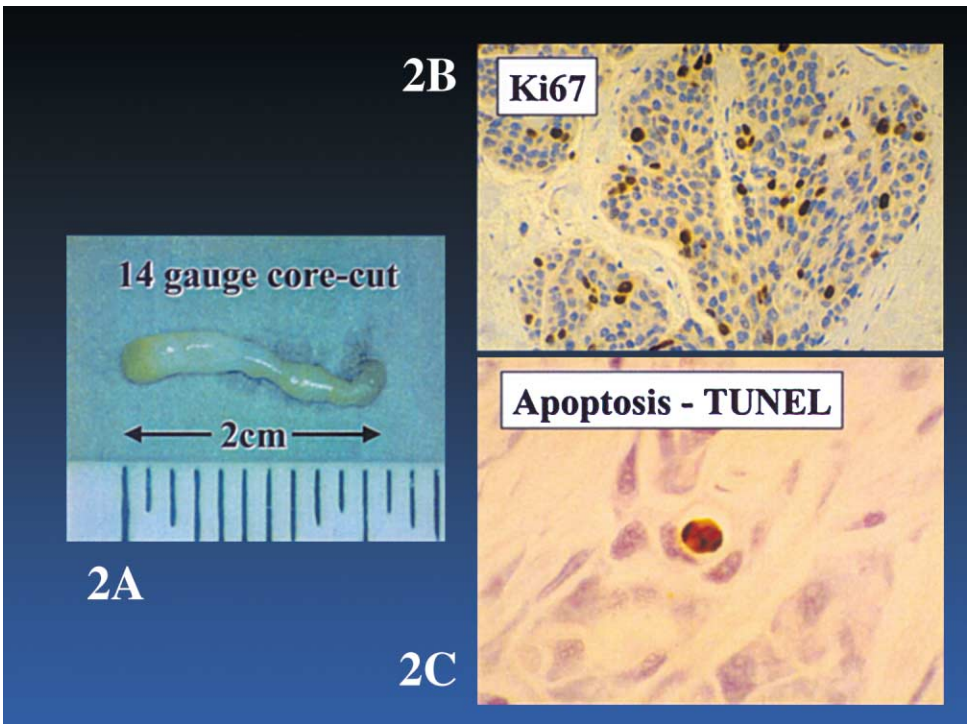

Figure 2 The assessment of proliferation or apoptosis during neoadjuvant therapy. (A) Core-cut specimen from breast cancer. (B) Ki67 staining of breast cancer specimen. (C) Result of TUNEL assay for apoptosis.

Taxanes

The first taxane, paclitaxel, was isolated from the bark of the Pacific Yew, *Taxus brevifolia*, in 1971. Since then, a similar compound, docetaxel, was isolated from more abundant species of yew and is now generated semi-synthetically.

Taxanes have been shown to bind to polymerised microtubules (Parness & Horwitz 1981), resulting in a shift in the equilibrium between tubulin dimers and microtubules, towards the latter. As a result microtubules are stabilised against depolymerisation, causing microtubule bundling in cells (Schiff & Horwitz 1980, Rowinsky *et al*. 1988). This prevents the microtubules from performing the functions necessary to facilitate mitosis, resulting in a sustained mitotic block (Jordan *et al*. 1993).

It has also been demonstrated that taxanes cause immune modulation via an increase in cytokines such as TNFα (Burkhart *et al*. 1994) and inhibition of angiogenesis (Sollott *et al*. 1995, Belotti *et al*. 1996).

Combination chemotherapy

An overview of breast cancer chemotherapy confirmed the superiority of combination chemotherapy over single agents (EBCTCG 1992). The chemotherapy regimen most rigorously assessed consists of cyclophosphamide, methotrexate and 5-fluorouracil. Cyclophosphamide, an alkylating agent, alkylates DNA, which leads to formation of crosslinks. Methotrexate is a folate antagonist. It acts by inhibiting the reduction of folate, which is required for the synthesis of a variety of essential coenzymes. In particular, it causes inhibition of thymidylate synthetase, so inhibiting DNA and RNA synthesis. 5-Fluorouracil, a fluoropyrimidine, acts by inactivating thymidylate synthetase, so blocking DNA synthesis (Dutcher *et al*. 2000).

Response and resistance to chemotherapy

General

Defective apoptosis and, to a degree, inappropriate proliferative activity, underpin the process of tumourigenesis (Johnstone *et al*. 2002). It is important to emphasise that, despite variations in individual mechanism of action, all the above agents bring about cancer cell death by activation of the apoptotic cascade as the principal mechanism of chemotherapy-induced cancer cell death (Hickman *et al*. 1992). As will be discussed, evidence indicates that defective apoptosis is probably the basis of chemotherapeutic resistance in many

cases. Study of the changes in the components of the apoptotic and proliferative pathways in breast cancers during chemotherapy may yield information about the molecular pathways associated with chemotherapy response and resistance.

There are very few studies that have set out to study the biology of neoadjuvant chemotherapy in breast cancer, and those that exist are limited in the number of time points. However, numerous studies have tried to identify markers or changes in markers that are associated with response or resistance to treatment, with the goal of introducing them as predictive markers. Areas of key importance are:

- What changes occur to underpin the regression of tumours?
- What biological processes are required to facilitate the changes and which, if deficient, may lead to resistance?
- Are there identifiable features in the cells that remain at the end of chemotherapy, which allow their survival?

Predictive markers in the setting of neoadjuvant chemotherapy have been assessed in terms of correlation with clinical or pathological response, on the basis that clinical and pathological responses to treatment are surrogates of disease-free and overall survival respectively (Fisher *et al.* 1998, van Der Hage *et al.* 2001). It should be noted therefore that, for full validation, biological markers require that a direct correlation with patient outcome be demonstrated.

Changes in proliferation and apoptosis caused by chemotherapy

Chemotherapy, radiotherapy and, in part, hormonal treatments act by inducing apoptosis (Hickman 1992, Ellis *et al.* 1997*b*, Verheij & Bartelink 2000). Apoptosis, or programmed cell death, is an active process controlled by several regulators that initiate or inhibit activity (Hengartner 2000). It is characterised morphologically at the cellular level by membrane blebbing, cell shrinkage, protein fragmentation, chromatin condensation and DNA degradation. There then follows a rapid engulfment of the remainder by neighbouring phagocytes. In normal development and growth, apoptosis is a tightly regulated mechanism that can potentially lead to multiple pathology, including carcinogenesis (Reed 1999), if dysregulation occurs. The balance between proliferation and apoptosis is crucial in determining the overall growth or regression of the tumour (Reed 1999, Tamm *et al.* 2001). Given that changes in apoptosis and proliferation are ultimately involved in the response process, these parameters of cell growth are suitable candidates for the study of predictors of response. The pathways leading to apoptosis and proliferation are inextricably linked at various points, for example by the tumour suppressor gene, *p53* (Eastman & Rigas 1999).

Detection and quantification of apoptosis in breast cancer

The 'gold standard' for detection and quantification of apoptotic cell death *in situ* has been morphological assessment with either electron microscopy or light microscopy (Kerr *et al.* 1994). However, this is often difficult and time consuming, even for trained histopathologists, and therefore a variety of methods have been developed for the identification of these cells. The most widely used techniques are *in situ* end labelling (Wijsman *et al.* 1993) and terminal deoxyribonucleotidyl tranferase-mediated dUTP nick end labelling (TUNEL) assay (Gavrieli *et al.* 1992) (Fig. 2). Both these methods involve incorporation of labelled nucleotides at the free 3′ OH end of DNA, including the 3′ ends produced by endonuclease action during apoptosis. Cell morphology can be examined simultaneously with these techniques, and infrequent events are more readily identified. Other methods of detecting apoptosis include characterisation of the membrane changes that occur during apoptosis (annexin V) or detection of release of enzymes (tissue transglutaminases). It must be recognised that most of these methods provide only a snapshot of a dynamic process, and that apoptotic bodies are removed rapidly by surrounding macrophages as they arise.

Detection and quantification of proliferation

A variety of methods have been used to evaluate proliferation in breast carcinomas, some of which depend on the cell cycle phase that they measure. Mitotic index, a component of the histopathological tumour grading system, measures the proportion of the cells in the mitosis phase (M) of the cell cycle only. Detection of cells synthesising DNA (i.e. in S-phase) can be evaluated using the thymidine labelling index (Silvestrini *et al.* 1985) and labelling with bromodeoxyuridine, a halogenated analogue of thymidine (Thor *et al.* 1999). However, these materials require fresh material of sufficient quantity. Another method detects the percentage of cells in S-phase. The S-phase fraction, measured by DNA flow cytometry, uses fresh, frozen or paraffin-embedded tissue. Immunohistochemical methods utilising the Ki67 monoclonal antibody to determine cell proliferation are widely accepted. The Ki67 antibody reacts with a nuclear antigen that is expressed in G1, S, G2 and mitosis, but not in G0 (resting phase) (Gerdes *et al.* 1984), but its use is restricted to frozen tissue (Fig. 2). MIB-1 is a murine monoclonal antibody against recombinant parts of the Ki67 antigen, and can be used in formalin-fixed, paraffin-embedded tissue sections. A good correlation between Ki67 antibody and MIB-1

measurement has been reported (Querzoli *et al.* 1996) ($r = 0.73$, $P < 0.001$).

Apoptosis and proliferation as prognostic/predictive markers

Apoptosis and proliferation markers have been studied in breast cancer extensively, as both prognostic and predictive markers. In untreated breast cancer, apoptosis correlates strongly with proliferation (Lipponen *et al.* 1994, Berardo *et al.* 1998, Ellis *et al.* 1998, van Slooten *et al.* 1998, Zhang *et al.* 1998). Thus the cell population of highly proliferative tumours appears to also be lost at a high rate. However, the evidence for apoptosis as an independent prognostic marker appears to be largely inconclusive (Lipponen *et al.* 1994, Berardo *et al.* 1998, Zhang *et al.* 1998). High apoptosis is associated with tumours of high grade, HER2 expression and p53 overexpression, and is inversely associated with Bcl-2 and ER (Lipponen *et al.* 1994, Hori *et al.* 1997, Rochaix *et al.* 1999, Liu *et al.* 2001). Therefore a poor outcome for such tumours is expected. Despite comprehensive large studies (n>700) examining the relationship between apoptosis and disease-free and overall survival, apoptosis failed to emerge as an independent prognostic marker in patients with node-negative or node-positive breast cancer (Liu *et al.* 2001). In contrast, tumours of high proliferation as detected by Ki67 or MIB-1 have largely been associated with decreased disease-free and overall survival (Pinder *et al.* 1995, Brown *et al.* 1996, Railo *et al.* 1997, Wintzer *et al.* 1991). Interestingly, this is despite the widely reported positive relationship between level of cell proliferation and response to chemotherapy in the neoadjuvant setting, supporting pre-clinical observations of high proliferation and chemosensitivity (Bonetti *et al.* 1996, Chevillard *et al.* 1996, MacGrogan *et al.* 1996).

Biological studies during neoadjuvant treatment have revealed that a measurable increase in apoptosis occurs in breast tumours within 24 h after the start of chemotherapy (Ellis *et al.* 1997*c*, Archer *et al.* 1999). This was also associated with a concomitant decrease in proliferation (Archer *et al.* 1999). A small pilot study by Chang *et al.* (1999) using serial fine needle aspirations (FNAs) and flow cytometry demonstrated a significant relationship between early changes in apoptosis (24–48 h) and eventual clinical response (Chang *et al.* 1999). A later, more extensive study using more rugged methodology failed to confirm the relationship between early changes in apoptosis and proliferation with response (C D Archer, personal communication) (Parton *et al.* 2001). Decreases in proliferation are relatively modest after 24 h, but by 21 days are more extensive, and have also been shown to correlate with a favourable response (Makris *et al.* 1998*a*, Chang *et al.* 1999). Thus it appears that timing of subsequent biopsy or FNA is pivotal in demonstrating a relationship between significant changes as a result of treatment-associated cell death and clinical response; this in turn may vary between tumours and mode of action of chemotherapy. Nonetheless, the relationship between reduced Ki67 or increased apoptosis is not close at any time point examined. In addition to variable kinetics between tumours, this may result from the need for a greater change in these parameters to lead to regression in a fast-growing tumour than is required in a slowly progressing tumour. This becomes clear from examination of Fig. 3, in which tumour regression/progression are seen in MCF-7 xenografts during oestrogen withdrawal or tamoxifen treatment. Parallel measurements of Ki67 and the apoptotic index showed that twofold reductions or threefold increases, respectively, were associated with only stable disease in this fast-growing tumour. Lesser changes would be associated with continued progression but, in a slowly growing tumour, could result in regression. Although these data pertain to endocrine treatment, similar principles should hold for chemotherapy.

In residual tissue at the cessation of chemotherapy, both apoptosis and proliferation are significantly reduced (Ellis *et al.* 1998); thus, in resistant cell populations at the end of treatment, the balance between apoptosis and proliferation is maintained. This raises the question of whether the decrease in proliferation is the result of downregulation in the entire cell population by a triggered 'switching off' of proliferative regulators by treatment, or reflects a selection of residual, less proliferative cells that are intrinsically less sensitive to chemotherapy and have been preserved throughout treatment. The latter concept is in keeping with the finding that the residual cell population has greater concentrations of detectable Bcl-2 protein, an inhibitory apoptosis regulator (Ellis *et al.* 1998). However, it is simplistic to consider that Bcl-2 may be the sole factor related to this reduced proliferation, as Bcl-2 is a member of an extensive family of regulators of apoptosis. Honkoop *et al.* (1998) were able to demonstrate that a high proliferative index in residual tumour was associated with decreased survival. Interestingly, this may be reflected in the finding by Vakkala *et al.* (1999) that increased proliferation and reduced apoptosis in the recurrent breast tumours in comparison with the primary lesion also predicted for a worse prognosis.

The biological basis of drug resistance in breast cancers

General

In the large neoadjuvant studies discussed above, the majority of patients haboured residual viable tumour cells at time of surgery after completing chemotherapy (Fisher *et al.* 1997, van Der Hage *et al.* 2001). Innate or acquired chemoresistance is therefore a common occurrence in the setting of neoadjuvant breast cancer treatment. Disabling of apoptosis is a central event in tumour development, and most

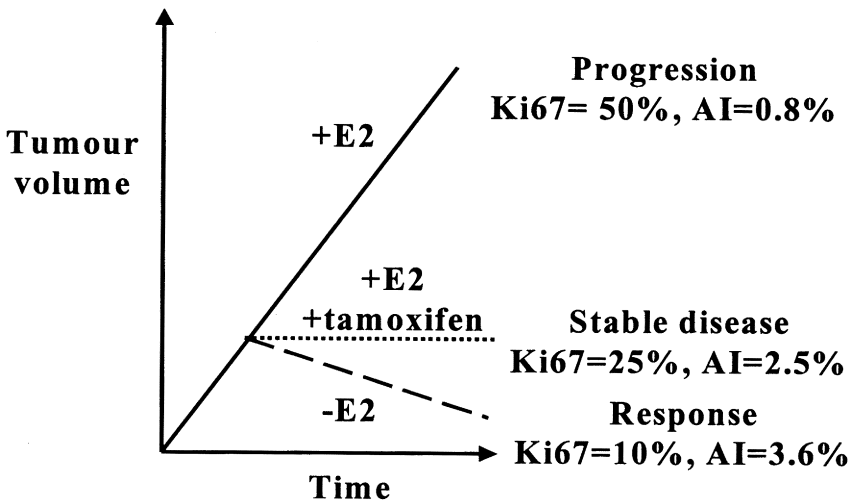

Figure 3 Clinical response and relationship with apoptotic and proliferative features of tumours. Data are plotted as straight lines for illustrative purposes. Original data relate to MCF-7 xenografts in nude mice (Johnston *et al.* 1999). AI, apoptotic index; E2, oestradiol.

chemotherapeutic drugs require functioning apoptotic pathways to induce cell death (Johnstone *et al*. 2002). This provides a possible explanation of why drug-naive tumours can be resistant. Drug resistance can also be acquired, as is demonstrated through the common clinical observation that chemotherapy resistance is even more evident in the setting of second- or third-line treatment. Cytotoxic drugs can be mutagenic and, in the context of cells that have defective apoptotic pathways, it is possible that such induced mutations might occur, resulting in resistance to other drugs. The fact that chemically unrelated drugs can kill tumours by activating a common pathway means that single mutations may lead to resistance to a range of drugs (see below).

'Classical drug resistance'

A significant component of anthracycline resistance is enhanced efflux of drug via the P-glycoprotein pump, which is encoded by multidrug-resistant (*MDR*) genes (Kartner *et al*. 1985). At present, the best-described mechanism of resistance to tubulin-binding agents is also the *MDR* phenotype (Ling 1992). Evidence is now emerging that the P-glycoprotein pump can cause drug resistance much further down the apoptotic pathway, by inhibition of caspases (Johnstone *et al*. 2000), downstream effectors of apoptosis.

It has been recognised for some years that glutathione upregulation represents another common mechanism of resistance. This antioxidant is believed to give rise to resistance by providing protection against free-radical damage (Lutzky *et al.* 1989). Investigation of these possible pathways of resistance in neoadjuvant studies has not been reported.

Molecular defects

A panel of biological markers including regulators such as p53, Bcl-2 family proteins, caspases and DNA fragmentation factor have been described as having a role in apoptosis. The assessment of these in cell lines and in clinical samples, particularly in the neoadjuvant setting, has helped build a picture of their contribution to the biology of chemoresistance.

Apoptotic pathways

p53

DNA damage and other cellular insults are detected by Chk2 and the product of the ataxia telangectasia mutated gene (ATM), resulting in activation of *p53* (Khanna & Jackson 2001). This triggers transcriptional activation of Bax and other pro-apoptotic Bcl-2 family members and repression of Bcl-2 and other anti-apoptotic genes. Loss of *p53* disrupts apoptosis and accelerates tumour development in transgenic mice (Attardi & Jacks 1999). There is evidence from *in vitro* (Lowe *et al*. 1993) and animal studies (Lowe *et al*. 1994) that defective *p53* is associated with resistance to chemotherapy. Furthermore, loss of *p53* function correlates with multidrug resistance in many tumour types (Wallace-Brodeur & Lowe 1999).

p53 muations are common in breast malignancies (Andersen *et al*. 1993) and are associated with a poor prognosis (Overgaard *et al*. 2000). Mutant *p53* is characterised by a long half-life, leading to nuclear accumulation. Thus immunohistochemical staining of *p53* is commonly taken to indicate the presence of mutant *p53*. However, not all *p53* mutations cause increased staining (Aas *et al*. 1996). Several

neoadjuvant studies have failed to detect a predictive value to *p53* staining with regards to chemoresponsiveness in breast cancers (MacGrogan *et al*. 1996, Niskanen *et al*. 1997, Bonetti *et al*. 1998, Rozan *et al*. 1998). Work by Geisler *et al*. (2001) has demonstrated that specific *p53* mutations are associated with resistance to doxoubicin in the neoadjuvant setting. A number of these mutations were not associated with enhanced staining for *p53*, which would explain why immunohistochemical studies have been inconclusive. Assessment of *p21* (*WAF1*/*cyp1*) staining as a downstream marker of *p53* activity improved the relationship between *p53* staining and mutational status, but was not an absolute predictor of functional *p53* (Ellis *et al*. 1997*c*). The same group of investigators also demonstrated a strong correlation between *p53* mutation and HER2 expression.

In another study, *p53* staining and mutations were studied in relation to the response of 67 breast tumours to neoadjuvant 5-fluorouracil epirubicin cyclosphate (FEC) or docetaxel chemotherapy. In the FEC group, treatment failure was associated with *p53* mutation and absence of apoptotic cells after treatment. For paclitaxel treatment, in which the role of *p53*-mediated cell death is less clear (Lanni *et al*. 1997), a response was supported by deficient *p53* and normal *p53* was associated with resistance (Kandioler-Eckersberger *et al*. 2000).

Bcl-2 family

Bcl-2 is one of a family of cytoplasmic proteins that are regulated by *p53* and phosphorylation by tyrosine kinases. Bcl-2 and Bcl-XL inhibit apoptosis, whereas Bax, Bag-1 and Bad promote it. Bcl-2 is overexpressed in approximately 80% of breast tumours (Krajewski *et al*. 1999) and correlates with low proliferative indices, high oestrogen receptor (ER) content, absence of p53 and HER2 staining, and good outcome (van Slooten *et al*. 1996). Bcl-2 overexpression is classically associated with follicular lymphomas and appears to be responsible for the chemoresistance exhibited by these tumours (Reed 1997). The situation in breast cancer is less clear-cut. In the randomised study conducted by van Slooten *et al*. (1996) referred to above, Bcl-2 had no predictive value for a single cycle of preoperative FEC chemotherapy (van Slooten *et al*. 1996). Other non-randomised neoadjuvant studies have also failed to show any predictive value of Bcl-2 in terms of chemoresponse (Frassoldati *et al*. 1997, Collecchi *et al*. 1998, Ellis *et al*. 1998). However, Bonetti *et al*. (1998) found that, in a series of women with advanced breast cancer, Bcl-2 negativity was associated with increased response rate to neoadjuvant chemotherapy; however, their numbers of patients were very small. As mentioned previously, the finding that residual tumour at cessation of chemotherapy has increased Bcl-2 concentrations relative to pre-chemotherapy specimens accords with the concept of a role of Bcl-2 expression in tumour resistance (Collecchi *et al*. 1998, Ellis *et al*. 1998).

Overexpression of Bcl-X_L, a related member of the Bcl-2 family, promotes chemotherapy resistance of mammary tumours in a syngeneic mouse model (Liu *et al*. 1999), but clinical data are currently lacking. In contrast, reduced staining for Bax, a proapoptotic regulator, is associated with poor breast cancer prognosis in the setting of metastatic disease (Krajewski *et al*. 1995). Furthermore, in this and a further metastatic study (Sjostrom *et al*. 1998), response to combination chemotherapy was significantly worse in tumours with reduced Bax.

Other defects in apoptosis involved in drug resistance

Functional mutations in many p53 upstream regulators and downstream effectors occur in human tumours. A*paf-1* is downstream to the Bcl-2 family, and required for activation of caspase-9 activation. Loss of A*paf-1* expression in human leukaemia and ovarian cell lines is associated with a decrease in drug-induced apoptosis, which can be re-established after transfection with *Apaf-1* (Jia *et al*. 2001).

Altered function of upstream regulators (ATM and Chk2) and downstream effectors and regulators (PTEN, heat-shock proteins, inhibitor of apoptosis proteins (IAPs), caspase-8) occurs in tumours and, in many cases, correlates with drug resistance (Johnstone *et al*. 2002).

Although this information is likely to be important to our understanding of the probable underlying mechanisms of drug resistance in breast cancer, assessment of these defects in neoadjuvant studies of the type described above is still awaited, to assess their clinical significance.

Proliferative pathways

Steroid receptor status

There are data that suggest that steroid receptor negativity predicts for chemosensitivity. In one recent study, tumours negative for ER or progesterone receptor demonstrated clinical response and pCR rates superior to that of neoadjuvant chemotherapy (various regimens) superior to that of ER positive tumours (Colleoni *et al*. 2000). This is also supported by adjuvant/perioperative data from the same centre (Colleoni *et al*. 2001). Several other studies have demonstrated a statistically significant greater response rate to neoadjuvant chemotherapy in steroid receptor-negative patients (Bonadonna *et al*. 1990, MacGrogan *et al*. 1996, Daidone *et al*. 1999).

The underlying mechanism by which lack of ER sensitises cells to apoptosis by chemotherapy is not fully established, but *in vitro* studies suggest that ER signalling can increase levels of Bcl-2 and induce anthracycline resistance (Teixeira *et al*. 1995).

HER2 (c-*erb*B-2, c-*neu*)

HER2 is a transmembrane receptor similar to epidermal growth factor receptor that is overexpressed in about 25% of

breast tumours (Slamon *et al*. 1989) and associated with poor outcome (Menard *et al*. 2001). Activation of HER2 induces activation of *ras*, leading to a phosphorylation cascade ultimately resulting in cell proliferation.

Despite some earlier positive studies, recently reported results suggest that stable transfection of normal human mammary epithelial cells with multiple copies of HER2 does not result in changes in sensitivity to a wide variety of chemotherapy agents (Orr *et al*. 2000).

Clinical data suggest that HER2 overexpression may affect drug sensitivity. Most data have been derived from adjuvant clinical trials in which the outcome of HER2-positive and HER2-negative patients (as assessed by immunohistchemistry) receiving adjuvant chemotherapy has been compared with HER2-positive and HER2-negative patients receiving no treatment or treatment with drugs at lower doses. The majority of studies suggest that HER2-negative, but not HER2-positive, patients derive benefit from chemotherapy based on alkylating agents (Allred *et al*. 1992, Gusterson *et al*. 1992, Miles *et al*. 1999), and other adjuvant studies have shown that overexpression of HER2 is associated with relative sensitivity to anthracycline regimens (Paik *et al*. 1998, Thor *et al*. 1998). These data have not been corroborated by results from studies assessing the predictive value of HER2 status in the metastatic setting (Revillion *et al*. 1996, Niskanen *et al*. 1997).

The optimal setting for assessing the response rates to chemotherapeutic agents is the neoadjuvant, for reasons given above. A recent study by Petit *et al*. (2001) demonstrated that HER2 overexpressers responded better to FEC100 than to FEC50, where the number denotes the dose of epirubicin (in mg m^{-2}), whereas HER2-negative patients responded equally to both regimens. Furthermore, HER2-positive patients responded better to FEC100 than did HER2-negative patients (P=0.07). A study published by Geisler *et al*. (2001) found that HER2 overexpression predicted resistance to a low-dose, weekly doxorubicin schedule, and a study by Willsher *et al*. (1998) similarly found a correlation between HER2 expression and resistance to neoadjuvant mitoxantrone, methotrexate and mitomycin C. In contrast, some studies of this kind have failed to show a predictive value of this marker (MacGrogan *et al*. 1996, Rozan *et al*. 1998, Vargas-Roig *et al*. 1999, Vincent-Salomon *et al*. 2000). Thus, at present, the data in neoadjuvant studies are conflicting with respect to HER2 status and response to anthracyclines.

To date, the studies correlating HER2 status and response to taxanes have been small and inconclusive, as reviewed recently (Yamauchi *et al*. 2001).

Directions for research

Resistance to chemotherapy remains a major challenge in the treatment of breast cancers. Some tumours do respond to chemotherapy and patients benefit from it. However, as yet there is no reliable means of predicting chemotherapy responsiveness. Even in those patients who respond, pCR is rare, indicating a sub-population of chemoresistant cells. Most drugs in common usage require an intact apoptotic mechanism. Cancers, however, often arise or at least persist as a consequence of abnormal functioning of this pathway.

Serial biopsies obtained from breast cancers during neoadjuvant chemotherapy provide a valuable opportunity to observe the pathways involved in cell death over time. Cells remaining in poorly responsive tumours at time of surgery are *de facto* resistant to treatment and are the cells responsible for treatment failure. Their molecular characterisation may provide an improved understanding of the mechanisms of chemoresistance and lead to new, targeted treatment strategies, possibly in conjunction with current treatments. The data described above provide only a first glimpse of the biology of chemotherapy in primary tumours, but reveal the potential for further studies of this type to provide additional valuable information. Comparisons of other components of the apoptotic and proliferative pathways in responding and non-responding cells may provide further mechanistic information and provide the basis for more rational treatments and better drug development.

The new technologies of comparative genomic hybridisation, real-time polymerase chain reaction, expression microarray and proteomics will be useful in performing these comparisons. The feasibility of these approaches has been demonstrated (Assersohn *et al*. 2002, Sotiriou *et al*. 2002), and data from such studies are eagerly awaited.

References

Aas T, Borresen AL, Geisler S, Smith-Sorensen B, Johnsen H, Varhaug JE, Akslen LA & Lonning PE 1996 Specific P53 mutations are associated with de novo resistance to doxorubicin in breast cancer patients. *Nature Medicine* **2** 811–814.

Allred DC, Clark GM, Tandon AK, Molina R, Tormey DC, Osborne CK, Gilchrist KW, Mansour EG, Abeloff M & Eudey L 1992 HER-2/neu in node-negative breast cancer: prognostic significance of overexpression influenced by the presence of in situ carcinoma. *Journal of Clinical Oncology* **10** 599–605.

Andersen TI, Holm R, Nesland JM, Heimdal KR, Ottestad L & Borresen AL 1993 Prognostic significance of TP53 alterations in breast carcinoma. *British Journal of Cancer* **68** 540–548.

Archer CD, Salter J, Hills M, Dowsett MD & Smith IE 1999 Induction of apoptosis and reduction in proliferation 24 hours after chemotherapy in early breast cancer. *Procedings of the American Society of Clinical Oncologists* **18** 81A.

Assersohn L, Gangi L, Zhao Y, Dowsett M, Simon R, Powles TJ & Liu ET 2002 The feasibility of using fine needle aspiration from primary breast cancers for cDNA microarray analyses. *Clinical Cancer Research* **8** 794–801.

Attardi LD & Jacks T 1999 The role of p53 in tumour suppression: lessons from mouse models. *Cell Molecular Life Sciences* **55** 48–63.

Belotti D, Vergani V, Drudis T, Borsotti P, Pitelli MR, Viale G, Giavazzi R & Taraboletti G 1996 The microtubule-affecting drug paclitaxel has antiangiogenic activity. *Clinical Cancer Research* **2** 1843–1849.

Berardo MD, Elledge RM, de Moor C, Clark GM, Osborne CK & Allred DC 1998 bcl-2 and apoptosis in lymph node positive breast carcinoma. *Cancer* **82** 1296–1302.

Bonadonna G, Veronesi U, Brambilla C, Ferrari L, Luini A, Greco M, Bartoli C, Coopmans de Yoldi G, Zucali R & Rilke F 1990 Primary chemotherapy to avoid mastectomy in tumors with diameters of three centimeters or more. *Journal of the National Cancer Institute* **82** 1539–1545.

Bonetti A, Zaninelli M, Rodella S, Molino A, Sperotto L, Piubello Q, Bonetti F, Nortilli R, Turazza M & Cetto GL 1996 Tumor proliferative activity and response to first-line chemotherapy in advanced breast carcinoma. *Breast Cancer Research and Treatment* **38** 289–297.

Bonetti A, Zaninelli M, Leone R, Cetto GL, Pelosi G, Biolo S, Menghi A, Manfrin E, Bonetti F & Piubello Q 1998 bcl-2 but not p53 expression is associated with resistance to chemotherapy in advanced breast cancer. *Clinical Cancer Research* **4** 2331–2336.

Broet P, Scholl SM, de la Rochefordiere A, Fourquet A, Moreau T, De Rycke Y, Asselain B & Pouillart P 1999 Short and long-term effects on survival in breast cancer patients treated by primary chemotherapy: an updated analysis of a randomized trial. *Breast Cancer Research and Treatment* **58** 151–156.

Brown RW, Allred CD, Clark GM, Osborne CK & Hilsenbeck SG 1996 Prognostic value of Ki-67 compared to S-phase fraction in axillary node-negative breast cancer. *Clinical Cancer Research* **2** 585–592.

Burkhart CA, Berman JW, Swindell CS & Horwitz SB 1994 Relationship between the structure of taxol and other taxanes on induction of tumor necrosis factor-alpha gene expression and cytotoxicity. *Cancer Research* **54** 5779–5782.

Buzdar AU, Singletary SE, Theriault RL, Booser DJ, Valero V, Ibrahim N, Smith TL, Asmar L, Frye D, Manuel N *et al.* Fluorouracil, doxorubicin, and cyclophosphamide as neoadjuvant therapy in patients with operable breast cancer. *Journal of Clinical Oncology* **17** 3412–3417.

Chang J, Powles TJ, Allred DC, Ashley SE, Clark GM, Makris A, Assersohn L, Gregory RK, Osborne CK & Dowsett M 1999 Biologic markers as predictors of clinical outcome from systemic therapy for primary operable breast cancer. *Journal of Clinical Oncology* **17** 3058–3063.

Chen AY & Liu LF 1994 DNA topoisomerases: essential enzymes and lethal targets. *Annual Review of Pharmacological Toxicology* **34** 191–218.

Chevillard S, Pouillart P, Beldjord C, Asselain B, Beuzeboc P, Magdelenat H & Vielh P 1996 Sequential assessment of multidrug resistance phenotype and measurement of S-phase fraction as predictive markers of breast cancer response to neoadjuvant chemotherapy. *Cancer* **77** 292–300.

Collecchi P, Baldini E, Giannessi P, Naccarato AG, Passoni A, Gardin G, Roncella M, Evangelista G, Bevilacqua G & Conte PF 1998 Primary chemotherapy in locally advanced breast cancer (LABC): effects on tumour proliferative activity, bcl-2 expression and the relationship between tumour regression and biological markers. *European Journal of Cancer* **34** 1701–1704.

Colleoni M, Minchella I, Mazzarol G, Nole F, Peruzzotti G, Rocca A, Viale G, Orlando L, Ferretti G, Curigliano G *et al.* 2000 Response to primary chemotherapy in breast cancer patients with tumors not expressing estrogen and progesterone receptors. *Annals of Oncology* **11** 1057–1059.

Colleoni M, Gelber S, Coates AS, Castiglione-Gertsch M, Gelber RD, Price K, Rudenstam CM, Lindtner J, Collins J, Thurlimann B *et al.* 2001 Influence of endocrine-related factors on response to perioperative chemotherapy for patients with node-negative breast cancer. *Journal of Clinical Oncology* **19** 4141–4149.

Daidone MG, Veneroni S, Benini E, Tomasic G, Coradini D, Mastore M, Brambilla C, Ferrari L & Silvestrini R 1999 Biological markers as indicators of response to primary and adjuvant chemotherapy in breast cancer. *International Journal of Cancer* **84** 580–586.

van Der Hage JA, van De Velde CJ, Julien JP, Tubiana-Hulin M, Vandervelden C & Duchateau L 2001 Preoperative chemotherapy in primary operable breast cancer: results from the European Organization for Research and Treatment of Cancer trial 10902. *Journal of Clinical Oncology* **19** 4224–4237.

Dutcher JP, Novik Y, O'Boyle K, Marcoullis G, Secco C & Wiernik PH 2000 20th-century advances in drug therapy in oncology – Part. II. *Journal of Clinical Pharmacology* **40** 1079–1092.

Eastman A & Rigas JR 1999 Modulation of apoptosis signaling pathways and cell cycle regulation. *Seminars in Oncology* **26** 7–16; discussion 41–12.

EBCTCG 1992 Systematic treatment of early breast cancer by hormonal, cytotoxic or immune therapy. *Lancet* **339** 1–15.

Ellis PA, Lonning PE, Borresen-Dale A, Aas T, Geisler S, Akslen LA, Salter I, Smith IE & Dowsett M 1997*a* Absence of p21 expression is associated with abnormal p53 in human breast carcinomas. *British Journal of Cancer* **76** 480–485.

Ellis PA, Saccani-Jotti G, Clarke R, Johnston SR, Anderson E, Howell A, A'Hern R, Salter J, Detre S, Nicholson R *et al.* 1997*b* Induction of apoptosis by tamoxifen and ICI 182780 in primary breast cancer. *International Journal of Cancer* **72** 608–613.

Ellis PA, Smith IE, McCarthy K, Detre S, Salter J & Dowsett M 1997*c* Preoperative chemotherapy induces apoptosis in early breast cancer. *Lancet* **349** 849.

Ellis PA, Smith IE, Detre S, Burton SA, Salter J, A'Hern R, Walsh G, Johnston SR & Dowsett M 1998 Reduced apoptosis and proliferation and increased Bcl-2 in residual breast cancer following preoperative chemotherapy. *Breast Cancer Research and Treatment* **48** 107–116.

Fisher B, Gunduz N, Coyle J, Rudock C & Saffer E 1989*a* Presence of a growth-stimulating factor in serum following primary tumor removal in mice. *Cancer Research* **49** 1996–2001.

Fisher B, Saffer E, Rudock C, Coyle J & Gunduz N 1989*b* Effect of local or systemic treatment prior to primary tumor removal on the production and response to a serum growth-stimulating factor in mice. *Cancer Research* **49** 2002–2004.

Fisher B, Brown A, Mamounas E, Wieand S, Robidoux A, Margolese RG, Cruz AB Jr, Fisher ER, Wickerham DL, Wolmark N *et al.* 1997 Effect of preoperative chemotherapy on local-regional disease in women with operable breast cancer: findings from National Surgical Adjuvant Breast and Bowel Project B-18. *Journal of Clinical Oncology* **15** 2483–2493.

Fisher B, Bryant J, Wolmark N, Mamounas E, Brown A, Fisher ER, Wickerham DL, Begovic M, DeCillis A, Robidoux A *et al.* 1998 Effect of preoperative chemotherapy on the outcome of women with operable breast cancer. *Journal of Clinical Oncology* **16** 2672–2685.

Frassoldati A, Adami F, Banzi C, Criscuolo M, Piccinini L & Silingardi V 1997 Changes of biological features in breast cancer cells determined by primary chemotherapy. *Breast Cancer Research and Treatment* **44** 185–192.

Gavrieli Y, Sherman Y & Ben-Sasson SA 1992 Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. *Journal of Cell Biology* **119** 493–501.

Geisler S, Lonning PE, Aas T, Johnsen H, Fluge O, Haugen DF, Lillehaug JR, Akslen LA & Borresen-Dale AL 2001 Influence of TP53 gene alterations and c-erbB-2 expression on the response to treatment with doxorubicin in locally advanced breast cancer. *Cancer Research* **61** 2505–2512.

Gerdes J, Lemke H, Baisch H, Wacker HH, Schwab U & Stein H 1984 Cell cycle analysis of a cell proliferation-associated human nuclear antigen defined by the monoclonal antibody Ki-67. *Journal of Immunology* **133** 1710–1715.

Gusterson BA, Gelber RD, Goldhirsch A, Price KN, Save-Soderborgh J, Anbazhagan R, Styles J, Rudenstam CM, Golouh R, Reed R *et al.* 1992 Prognostic importance of c-erbB-2 expression in breast cancer. International (Ludwig) Breast Cancer Study Group. *Journal of Clinical Oncology* **10** 1049–1056.

Hengartner MO 2000 The biochemistry of apoptosis. *Nature* **407** 770–776.

Hickman JA 1992 Apoptosis induced by anticancer drugs. *Cancer Metastasis Reviews* **11** 121–139.

Hickman JA, Beere HM, Wood AC, Waters CM & Parmar R 1992 Mechanisms of cytotoxicity caused by antitumour drugs. *Toxicology Letters* **64–65** 553–561.

Holmes FA, Walters RS, Theriault RL, Forman AD, Newton LK, Raber MN, Buzdar AU, Frye DK & Hortobagyi GN 1991 Phase II trial of taxol, an active drug in the treatment of metastatic breast cancer. *Journal of the National Cancer Institute* **83** 1797–1805.

Honkoop AH, van Diest PJ, de Jong JS, Linn SC, Giaccone G, Hoekman K, Wagstaff J & Pinedo HM 1998 Prognostic role of clinical, pathological and biological characteristics in patients with locally advanced breast cancer. *British Journal of Cancer* **77** 621–626.

Hori M, Nogami T, Itabashi M, Yoshimi F, Ono H & Koizumi S 1997 Expression of Bcl-2 in human breast cancer: correlation between hormone receptor status, p53 protein accumulation and DNA strand breaks associated with apoptosis. *Pathology International* **47** 757–762.

Jia L, Srinivasula SM, Liu FT, Newland AC, Fernandes-Alnemri T, Alnemri ES & Kelsey SM 2001 Apaf-1 protein deficiency confers resistance to cytochrome c-dependent apoptosis in human leukemic cells. *Blood* **98** 414–421.

Johnston SR, Boeddinghaus IM, Riddler S, Haynes BP, Hardcastle IR, Rowlands M, Grimshaw R, Jarman M & Dowsett M 1999 Idoxifene antagonizes estradiol-dependent MCF-7 breast cancer xenograft growth through sustained induction of apoptosis. *Cancer Research* **59** 3646–3651.

Johnstone RW, Ruefli AA & Smyth MJ 2000 Multiple physiological functions for multidrug transporter P-glycoprotein? *Trends in Biochemical Sciences* **25** 1–6.

Johnstone RW, Ruefli AA & Lowe SW 2002 Apoptosis. A Link between cancer genetics and chemotherapy. *Cell* **108** 153–164.

Jordan MA, Toso RJ, Thrower D & Wilson L 1993 Mechanism of mitotic block and inhibition of cell proliferation by taxol at low concentrations. *Procedings of the National Academy of Sciences of the USA* **90** 9552–9556.

Kandioler-Eckersberger D, Ludwig C, Rudas M, Kappel S, Janschek E, Wenzel C, Schlagbauer-Wadl H, Mittlbock M, Gnant M, Steger G *et al.* 2000 TP53 mutation and p53 overexpression for prediction of response to neoadjuvant treatment in breast cancer patients. *Clinical Cancer Research* **6** 50–56.

Kartner N, Evernden-Porelle D, Bradley G & Ling V 1985 Detection of P-glycoprotein in multidrug-resistant cell lines by monoclonal antibodies. *Nature* **316** 820–823.

Kerr JF, Winterford CM & Harmon BV 1994 Apoptosis. Its significance in cancer and cancer therapy. *Cancer* **73** 2013–2026.

Khanna KK & Jackson SP 2001 DNA double-strand breaks: signaling, repair and the cancer connection. *Nature Genetics* **27** 247–254.

Krajewski S, Blomqvist C, Franssila K, Krajewska M, Wasenius VM, Niskanen E, Nordling S & Reed JC 1995 Reduced expression of proapoptotic gene BAX is associated with poor response rates to combination chemotherapy and shorter survival in women with metastatic breast adenocarcinoma. *Cancer Research* **55** 4471–4478.

Krajewski S, Krajewska M, Turner BC, Pratt C, Howard B, Zapata JM, Frenkel V, Robertson S, Ionov Y, Yamamoto H *et al.* 1999 Prognostic significance of apoptosis regulators in breast cancer. *Endocrine-Related Cancer* **6** 29–40.

Lanni JS, Lowe SW, Licitra EJ, Liu JO & Jacks T 1997 p53-independent apoptosis induced by paclitaxel through an indirect mechanism. *PNAS* **94** 9679–9683.

Ling V 1992 Charles F Kettering Prize: P-glycoprotein and resistance to anticancer drugs. *Cancer* **69** 2603–2609.

Lipponen P, Aaltomaa S, Kosma VM & Syrjanen K 1994 Apoptosis in breast cancer as related to histopathological characteristics and prognosis. *European Journal of Cancer* **30A** 2068–2073.

Liu R, Page C, Beidler DR, Wicha MS & Nunez G 1999 Overexpression of Bcl-x(L) promotes chemotherapy resistance of mammary tumors in a syngeneic mouse model. *American Journal of Pathology* **155** 1861–1867.

Liu S, Edgerton SM, Moore DH, 2nd & Thor AD 2001 Measures of cell turnover (proliferation and apoptosis) and their association with survival in breast cancer. *Clinical Cancer Research* **7** 1716–1723.

Lowe SW, Ruley HE, Jacks T & Housman DE 1993 p53-dependent apoptosis modulates the cytotoxicity of anticancer agents. *Cell* **74** 957–967.

Lowe SW, Bodis S, McClatchey A, Remington L, Ruley HE, Fisher DE, Housman DE & Jacks T 1994 p53 status and the efficacy of cancer therapy in vivo. *Science* **266** 807–810.

Lutzky J, Astor MB, Taub RN, Baker MA, Bhalla K, Gervasoni JE Jr, Rosado M, Stewart V, Krishna S & Hindenburg AA 1989 Role of glutathione and dependent enzymes in anthracycline-resistant HL60/AR cells. *Cancer Research* **49** 4120–4125.

MacGrogan G, Mauriac L, Durand M, Bonichon F, Trojani M, de Mascarel I & Coindre JM 1996 Primary chemotherapy in breast invasive carcinoma: predictive value of the immunohistochemical detection of hormonal receptors, p53, c-erbB-2, MiB1, pS2 and GST pi. *British Journal of Cancer* **74** 1458–1465.

Makris A, Powles TJ, Allred DC, Ashley S, Ormerod MG, Titley JC & Dowsett M 1998*a* Changes in hormone receptors and proliferation markers in tamoxifen treated breast cancer patients

and the relationship with response. *Breast Cancer Research and Treatment* **48** 11–20.

Makris A, Powles TJ, Ashley SE, Chang J, Hickish T, Tidy VA, Nash AG & Ford HT 1998*b* A reduction in the requirements for mastectomy in a randomized trial of neoadjuvant chemoendocrine therapy in primary breast cancer. *Annals of Oncology* **9** 1179–1184.

Mauriac L, Durand M, Avril A & Dilhuydy JM 1991 Effects of primary chemotherapy in conservative treatment of breast cancer patients with operable tumors larger than 3 cm. Results of a randomized trial in a single centre. *Annals of Oncology* **2** 347–354.

Mauriac L, MacGrogan G, Avril A, Durand M, Floquet A, Debled M, Dilhuydy JM & Bonichon F 1999 Neoadjuvant chemotherapy for operable breast carcinoma larger than 3 cm: a unicentre randomized trial with a 124-month median follow-up. Institut Bergonie Bordeaux Groupe Sein (IBBGS). *Annals of Oncology* **10** 47–52.

Menard S, Fortis S, Castiglioni F, Agresti R & Balsari A 2001 HER2 as a prognostic factor in breast cancer. *Oncology* **61** 67–72.

Miles DW, Harris WH, Gillett CE, Smith P & Barnes DM 1999 Effect of c-erbB(2) and estrogen receptor status on survival of women with primary breast cancer treated with adjuvant cyclophosphamide/methotrexate/fluorouracil. *International Journal of Cancer* **84** 354–359.

Moliterni A, Tarenzi E, Capri G, Terenziani M, Bertuzzi A, Grasselli G, Agresti R, Piotti P, Greco M, Salvadori B *et al.* 1997 Pilot study of primary chemotherapy with doxorubicin plus paclitaxel in women with locally advanced or operable breast cancer. *Seminars in Oncology* **24** S17-10–S17-14.

Niskanen E, Blomqvist C, Franssila K, Hietanen P & Wasenius VM 1997 Predictive value of c-erbB-2, p53, cathepsin-D and histology of the primary tumour in metastatic breast cancer. *British Journal of Cancer* **76** 917–922.

NSABP 2001 The effect of primary tumour response of adding sequential taxotere to adriamycin and cyclophosphamide: preliminary results from NSABP Protocol B-27. *Breast Cancer Research and Treatment* **69** 210.

Orr MS, O'Connor PM & Kohn KW 2000 Effects of c-erbB2 overexpression on the drug sensitivities of normal human mammary epithelial cells. *Journal of the National Cancer Institute* **92** 987–994.

Overgaard J, Yilmaz M, Guldberg P, Hansen LL & Alsner J 2000 TP53 mutation is an independent prognostic marker for poor outcome in both node-negative and node-positive breast cancer. *Acta Oncologica* **39** 327–333.

Paik S, Bryant J, Park C, Fisher B, Tan-Chiu E, Hyams D, Fisher ER, Lippman ME, Wickerham DL & Wolmark N 1998 erbB-2 and response to doxorubicin in patients with axillary lymph node-positive, hormone receptor-negative breast cancer. *Journal of the National Cancer Institute* **90** 1361–1370.

Parness J & Horwitz SB 1981 Taxol binds to polymerized tubulin in vitro. *Journal of Cell Biology* **91** 479–487.

Parton MM, Archer C, Ashley S, Smith IE & Dowsett M 2001 Ki67/apoptotic index, a growth index for prediction of response to neoadjuvant chemotherapy. *Breast Cancer Research and Treatment* **69** 245.

Petit T, Borel C, Ghnassia JP, Rodier JF, Escande A, Mors R & Haegele P 2001 Chemotherapy response of breast cancer depends on HER-2 status and anthracycline dose intensity in the neoadjuvant setting. *Clinical Cancer Research* **7** 1577–1581.

Pinder SE, Wencyk P, Sibbering DM, Bell JA, Elston CW, Nicholson R, Robertson JF, Blamey RW & Ellis IO 1995 Assessment of the new proliferation marker MIB1 in breast carcinoma using image analysis: associations with other prognostic factors and survival. *British Journal of Cancer* **71** 146–149.

Powles TJ, Hickish TF, Makris A, Ashley SE, O'Brien ME, Tidy VA, Casey S, Nash AG, Sacks N, Cosgrove D *et al.* 1995 Randomized trial of chemoendocrine therapy started before or after surgery for treatment of primary breast cancer. *Journal of Clinical Oncology* **13** 547–552.

Querzoli P, Albonico G, Ferretti S, Rinaldi R, Magri E, Indelli M & Nenci I 1996 MIB-1 proliferative activity in invasive breast cancer measured by image analysis. *Journal of Clinical Pathology* **49** 926–930.

Railo M, Lundin J, Haglund C, von Smitten K, von Boguslawsky K & Nordling S 1997 Ki-67, p53, Er-receptors, ploidy and S-phase as prognostic factors in T1 node negative breast cancer. *Acta Oncologica* **36** 369–374.

Reed JC 1997 Bcl-2 family proteins: regulators of apoptosis and chemoresistance in hematologic malignancies. *Seminars in Hematology* **34** 9–19.

Reed JC 1999 Dysregulation of apoptosis in cancer. *Journal of Clinical Oncology* **17** 2941–2953.

Revillion F, Hebbar M, Bonneterre J & Peyrat JP 1996 Plasma c-erbB2 concentrations in relation to chemotherapy in breast cancer patients. *European Journal of Cancer* **32A** 231–234.

Rochaix P, Krajewski S, Reed JC, Bonnet F, Voigt JJ & Brousset P 1999 In vivo patterns of Bcl-2 family protein expression in breast carcinomas in relation to apoptosis. *Journal of Pathology* **187** 410–415.

Rowinsky EK, Donehower RC, Jones RJ & Tucker RW 1988 Microtubule changes and cytotoxicity in leukemic cell lines treated with taxol. *Cancer Research* **48** 4093–4100.

Rozan S, Vincent-Salomon A, Zafrani B, Validire P, De Cremoux P, Bernoux A, Nieruchalski M, Fourquet A, Clough K, Dieras V *et al.* 1998 No significant predictive value of c-erbB-2 or p53 expression regarding sensitivity to primary chemotherapy or radiotherapy in breast cancer. *International Journal of Cancer* **79** 27–33.

Schiff PB & Horwitz SB 1980 Taxol stabilizes microtubules in mouse fibroblast cells. *PNAS* **77** 1561–1565.

Scholl SM, Fourquet A, Asselain B, Pierga JY, Vilcoq JR, Durand JC, Dorval T, Palangie T, Jouve M, Beuzeboc P *et al.* 1994 Neoadjuvant versus adjuvant chemotherapy in premenopausal patients with tumours considered too large for breast conserving surgery: preliminary results of a randomised trial: S6. *European Journal of Cancer* **30A** 645–652.

Semiglazov VF, Topuzov EE, Bavli JL, Moiseyenko VM, Ivanova OA, Seleznev IK, Orlov AA, Barash NY, Golubeva OM & Chepic OF 1994 Primary (neoadjuvant) chemotherapy and radiotherapy compared with primary radiotherapy alone in stage IIb-IIIa breast cancer. *Annals of Oncology* **5** 591–595.

Silvestrini R, Daidone MG & Gasparini G 1985 Cell kinetics as a prognostic marker in node-negative breast cancer. *Cancer* **56** 1982–1987.

Sjostrom J, Krajewski S, Franssila K, Niskanen E, Wasenius VM, Nordling S, Reed JC & Blomqvist C 1998 A multivariate analysis of tumour biological factors predicting response to cytotoxic treatment in advanced breast cancer. *British Journal of Cancer* **78** 812–815.

Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong SG, Keith DE, Levin WJ, Stuart SG, Udove J, Ullrich A *et al.* 1989 Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. *Science* **244** 707–712.

van Slooten HJ, Clahsen PC, van Dierendonck JH, Duval C, Pallud C, Mandard AM, Delobelle-Deroide A, van de Velde CJ & van de Vijver MJ 1996 Expression of Bcl-2 in node-negative breast cancer is associated with various prognostic factors, but does not predict response to one course of perioperative chemotherapy. *British Journal of Cancer* **74** 78–85.

van Slooten HJ, van de Vijver MJ, van de Velde CJ & van Dierendonck JH 1998 Loss of Bcl-2 in invasive breast cancer is associated with high rates of cell death, but also with increased proliferative activity. *British Journal of Cancer* **77** 789–796.

Smith PJ & Soues S 1994 Multilevel therapeutic targeting by topoisomerase inhibitors. *British Journal of Cancer (Supplement)* **23** S47–S51.

Smith IC, Heys SD, Hutcheon AW, Miller ID, Payne S, Gilbert FJ, Ah-See AK, Eremin O, Walker LG, Sarkar TK *et al.* 2002 Neoadjuvant chemotherapy in breast cancer: significantly enhanced response with docetaxel. *Journal of Clinical Oncology* **20** 1456–1466.

Sollott SJ, Cheng L, Pauly RR, Jenkins GM, Monticone RE, Kuzuya M, Froehlich JP, Crow MT, Lakatta EG, Rowinsky EK *et al.* 1995 Taxol inhibits neointimal smooth muscle cell accumulation after angioplasty in the rat. *Journal of Clinical Investigation* **95** 1869–1876.

Sotiriou C, Powles TJ, Dowsett M, Jazaeri AA, Feldman AL, Assersohn L, Gadisetti C, Libutti SK & Liu ET 2002 Gene expression profiles derived from fine needle aspiration correlate with response to systemic chemotherapy in breast cancer. *Breast Cancer Research* **4** R3.

Tamm I, Schriever F & Dorken B 2001 Apoptosis: implications of basic research for clinical oncology. *Lancet Oncology* **2** 33–42.

Teixeira C, Reed JC & Pratt MA 1995 Estrogen promotes chemotherapeutic drug resistance by a mechanism involving Bcl-2 proto-oncogene expression in human breast cancer cells. *Cancer Research* **55** 3902–3907.

Thor AD, Berry DA, Budman DR, Muss HB, Kute T, Henderson IC, Barcos M, Cirrincione C, Edgerton S, Allred C, Norton L & Liu ET 1998 erbB-2, p53, and efficacy of adjuvant therapy in lymph node-positive breast cancer. *Journal of the National Cancer Institute* **90** 1346–1360.

Thor AD, Liu S, Moore DH, 2nd & Edgerton SM 1999 Comparison of mitotic index, in vitro bromodeoxyuridine labeling, and MIB-1 assays to quantitate proliferation in breast cancer. *Journal of Clinical Oncology* **17** 470–477.

Vakkala M, Lahteenmaki K, Raunio H, Paakko P & Soini Y 1999 Apoptosis during breast carcinoma progression. *Clinical Cancer Research* **5** 319–324.

Vargas-Roig LM, Gago FE, Tello O, Martin de Civetta MT & Ciocca DR 1999 c-erbB-2 (HER-2/neu) protein and drug resistance in breast cancer patients treated with induction chemotherapy. *International Journal of Cancer* **84** 129–134.

Verheij M & Bartelink H 2000 Radiation-induced apoptosis. *Cell Tissue Research* **301** 133–142.

Vincent-Salomon A, Carton M, Freneaux P, Palangie T, Beuzeboc P, Mouret E, de Cremoux P, Coue O, Zafrani B, Nicolas A *et al.* 2000 ERBB2 overexpression in breast carcinomas: no positive correlation with complete pathological response to preoperative high-dose anthracycline-based chemotherapy. *European Journal of Cancer* **36** 586–591.

Wallace-Brodeur RR & Lowe SW 1999 Clinical implications of p53 mutations. *Cell Molecular Life Sciences* **55** 64–75.

Wijsman JH, Jonker RR, Keijzer R, van de Velde CJ, Cornelisse CJ & van Dierendonck JH 1993 A new method to detect apoptosis in paraffin sections: in situ end-labeling of fragmented DNA. *Journal of Histochemistry and Cytochemistry* **41** 7–12.

Willsher PC, Pinder SE, Gee JM, Ellis IO, Chan SY, Nicholson RI, Blamey RW & Robertson JF 1998 C-erbB2 expression predicts response to preoperative chemotherapy for locally advanced breast cancer. *Anticancer Research* **18** 3695–3698.

Wintzer HO, Zipfel I, Schulte-Monting J, Hellerich U & von Kleist S 1991 Ki-67 immunostaining in human breast tumors and its relationship to prognosis. *Cancer* **67** 421–428.

Yamauchi H, Stearns V & Hayes DF 2001 When is a tumor marker ready for prime time? A case study of c-erbB-2 as a predictive factor in breast cancer. *Journal of Clinical Oncology* **19** 2334–2356.

Zhang GJ, Kimijima I, Abe R, Watanabe T, Kanno M, Hara K & Tsuchiya A 1998 Apoptotic index correlates to bcl-2 and p53 protein expression, histological grade and prognosis in invasive breast cancers. *Anticancer Research* **18** 1989–1998.