Available online at www.sciencedirect.com
ScienceDirect
Biochimica et Biophysica Acta 1785 (2008) 96 – 132
Biochimica et Biophysica Acta
BBA
www.elsevier.com/locate/bbacan

Review

Taxanes, microtubules and chemoresistant breast cancer

Barbara T. McGrogan [a,b], Breege Gilmartin [a,b], Desmond N. Carney [c], Amanda McCann [a,b,*]

[a] *UCD School of Medicine and Medical Science (SMMS), University College Dublin, UCD, Belfield, Dublin 4, Ireland*
[b] *Conway Institute of Biomolecular and Biomedical Research, University College Dublin, UCD, Belfield, Dublin 4, Ireland*
[c] *Mater Misericordiae Hospital, Eccles Street, Dublin 7, Ireland*

Received 28 June 2007; received in revised form 23 October 2007; accepted 28 October 2007
Available online 12 November 2007

Abstract

The taxanes, paclitaxel and docetaxel are microtubule-stabilizing agents that function primarily by interfering with spindle microtubule dynamics causing cell cycle arrest and apoptosis. However, the mechanisms underlying their action have yet to be fully elucidated. These agents have become widely recognized as active chemotherapeutic agents in the treatment of metastatic breast cancer and early-stage breast cancer with benefits gained in terms of overall survival (OS) and disease-free survival (DFS). However, even with response to taxane treatment the time to progression (TTP) is relatively short, prolonging life for a matter of months, with studies showing that patients treated with taxanes eventually relapse. This review focuses on chemoresistance to taxane treatment particularly in relation to the spindle assembly checkpoint (SAC) and dysfunctional regulation of apoptotic signaling. Since spindle microtubules are the primary drug targets for taxanes, important SAC proteins such as MAD2, BUBR1, Synuclein-gamma and Aurora A have emerged as potentially important predictive markers of taxane resistance, as have specific checkpoint proteins such as BRCA1. Moreover, overexpression of the drug efflux pump MDR-1/P-gp, altered expression of microtubule-associated proteins (MAPs) including tau, stathmin and MAP4 may help to identify those patients who are most at risk of recurrence and those patients most likely to benefit from taxane treatment.

Keywords: Breast cancer; Taxanes; Microtubules; Chemoresistance; Spindle assembly checkpoint (SAC); Tubulin; BRCAl; Mitotic assembly deficient protein 2 (MAD2)

Contents

* Corresponding author. Conway Institute of Biomolecular and Biomedical Research, University College Dublin, UCD, Belfield, Dublin 4, Ireland. Tel.: +353 1 7166742; fax: +353 1 7166888.
E-mail address: amanda.mccann@ucd.ie (A. McCann).

0304-419X/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbcan.2007.10.004

1. Introduction

Cellular chemotherapeutic resistance is a major factor involved in poor response and reduced overall survival (OS) in patients with metastatic breast cancer (MBC) [1]. One class of drugs commonly used in the treatment of breast cancer is the microtubule-stabilizing agents (MSAs) also know as microtubule inhibitors (MIs), namely paclitaxel and docetaxel [2]. The commercially available taxanes, paclitaxel (Taxol®) and docetaxel (Taxotere®), have become widely recognized as extremely active chemotherapeutic agents in the treatment of breast cancer [3], with a response rate of between 25 and 69% observed when used as first-line treatment [4].

MI agents, such as the taxanes (microtubule polymerizing agents) and vinca alkaloids (microtubule depolymerizing agents) form part of an increasing number of cytotoxic drugs currently available for the treatment of breast cancer. In the early 1970′s treatment regimes for breast cancer were limited to the use of the alkylating agent, cyclophosphamide in combination with methotrexate and 5-fluorouracil (CMF), with a median survival rate of 14 months [5]. This regime was superseded in the 1980′s with the use of the anthracyclines, doxorubicin and epirubicin, which have been widely used as single first-line treatment or in a combinatorial regime with other chemotherapeutic agents [6]. By the 1990′s the taxanes, paclitaxel and docetaxel were introduced in combination with anthracyclines and alkylating agents, thereby improving overall survival (OS) and disease-free survival (DFS) in metastatic breast cancer (MBC) patients [7,8]. Moreover, the taxanes have been incorporated into the management of early-stage breast cancer and are now routinely used in combination with anthracyclines and trastuzumab (Herceptin®) [5,8]. However, resistance to taxanes is common and there is an increasing need to try and identify those patients who will respond to treatment.

2. Microtubule inhibitor (MI) chemotherapy — the taxanes and vinca alkaloids

Paclitaxel was originally isolated from the bark of the Pacific yew tree, *Taxus brevifolia* in 1971 [9]. The chemical structure is illustrated in Fig. 1 [10]. It was initially approved by the United States (US) Food and Drug administration (FDA) for use in advanced ovarian cancer in 1992 [11] and subsequently endorsed for the treatment of metastatic breast cancer (MBC) in 1994 [11]. Since paclitaxel was originally isolated from a natural source having a limited supply, it is now derived semi-synthetically from the inactive taxane precursor,

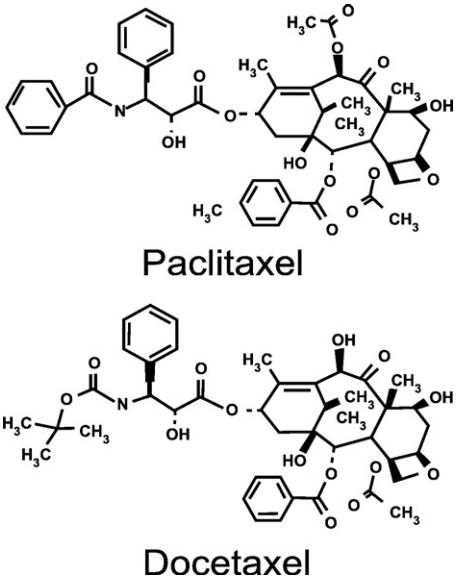


Fig. 1. Chemical structure of paclitaxel and docetaxel. (Figure printed with permission Fitzpatrick and Wheeler [10]).

10-deacetylbaccatin III, found in the needles of the European yew tree, *Taxus baccata* [11].

As paclitaxel is a highly hydrophobic compound it is administered in solution with alcohol and purified Cremophor ® EL (polyoxyethylated castor oil) to aid delivery [12]. This solvent can cause severe hypersensitivity reactions, thus premedication with dexamethasone is recommended, where clinically indicated as individual patient factors may preclude treatment with steroids [12]. Following intravenous (IV) infusion, paclitaxel demonstrates nonlinear pharmacokinetics, is metabolized in the liver and is excreted predominantly in bile [13]. The elimination T $^1/_2$ is 15–50 h [13]. Recent development of ABI-007, an albumin bound paclitaxel particle, helps to avoid the hypersensitivity induced by Cremophor ® EL [12]. In one phase III trial comparing ABI-007 to standard paclitaxel treatment, 454 patients with MBC were randomized to receive either 260 mg/m^2 of ABI-007 or standard paclitaxel 175 mg/m^2 every 3 weeks for 6 cycles [14]. Patients treated with ABI-007 vs. standard paclitaxel had significantly higher response rates 33% vs. 19% ($p=0.001$) respectively and longer time to disease progression 23% vs. 16.9%, hazard ratio [HR]=0.75 ($p=0.006$). Moreover, the incidence of grade 4 neutropenia was significantly lower for the ABI-007 arm compared with the standard paclitaxel arm 9% vs. 22% ($p<0.001$) respectively [14]. This positive outcome is further highlighted by the recent US FDA approval of ABI-007 for use in the treatment of MBC after either failure of combination chemotherapy, or relapse within 6 months of adjuvant chemotherapy [15]. Common side-effects of paclitaxel include alopecia, myelosuppression, gastrointestinal symptoms and febrile neutropenia [13]. Peripheral neuropathy is particularly associated with paclitaxel and increases with cumulative dose [13]. When paclitaxel is administered in a dose-dense setting, that is, as a weekly or two-weekly regime, it is more effective in terms of response rates compared to conventional three-weekly scheduling. Paclitaxel given neoadjuvantly to a cohort of 258 node-positive and node-negative breast cancer patients, resulted in a significantly higher pathological complete response (pCR) rate in those patients who received weekly treatments ($n=127$) vs. three-weekly treatment regimes ($n=131$) 28% vs. 15.7% ($p=0.02$) [16]. Furthermore, the Cancer and Leukemia Group B (CALGB) 9840 trial also found that weekly administration of paclitaxel was superior to a three-weekly regime in terms of time to progression (TTP) and overall response rate (ORR) in the metastatic setting [17].

Docetaxel, a second generation taxane, is derived semi-synthetically by the esterification of a side chain to 10-deacetylbaccatin III (the inactive taxane precursor) [18]. The chemical structure of docetaxel is illustrated in Fig. 1 [10]. It is administered in solution with polysorbate 80 and ethanol, a vehicle associated with significantly less hypersensitivity reactions than Cremophor ® EL [13]. Docetaxel can be given as part of a weekly or standard three-weekly regime [19,20], although weekly docetaxel is poorly tolerated clinically and is therefore not used routinely [20]. Docetaxel differs from paclitaxel with its linear pharmacokinetics and elimination T $^1/_2$ of 1 h but shares the same common side-effects with paclitaxel [13]. In addition, oedema and fluid accumulation, including pleural effusions and ascites, are also commonly seen with docetaxel and can be dose-limiting [13]. With regard to docetaxel treatment, the three-weekly regime is associated with increased neutropenia, skin and nail disorders (Palmar-plantar erythrodysesthesia) and fluid retention [13].

Though differences exist between the pharmacology and pharmacokinetics of paclitaxel and docetaxel, both taxanes have improved disease-free survival (DFS) and overall survival (OS) in breast cancer patients, especially when combined with anthracycline-based regimes [8,19,21].

Other types of MI agents include the vinca alkaloids, vinblastine and vincristine, extracted over 40 years ago from the leaves of *Catharanthus roseus* [22]. These drugs demonstrate bone marrow suppression and have anti-leukemic effects [23]. Initially, they were used as single-agents for the treatment of hematological malignancies and were particularly effective in treating childhood leukemia [24]. Further development of these drugs has lead to the introduction of semi-synthetic derivatives, including vinorelbine [25,26], vinflunine [27] and vindesine which are now clinically used in the treatment of non-small-cell lung cancer (NSCLC), leukemia, lymphoma and breast cancer [28]. The main side-effects of these drugs include peripheral neuropathy and reversible myelosuppression [29].

3. Microtubules as targets for anti-mitotic drugs

Microtubules consist of long, filamentous protein polymers having important functions in cellular activities such as, maintenance of cell shape, cellular movement, cell signaling, division and mitosis [24]. These roles make microtubules a highly effective cancer target as evidenced by the diversity of available microtubule inhibitory agents including the taxanes, vinca alkaloids and novel taxanes, such as the taxol-like agents, epothilones [24,30].

3.1. Tubulin and microtubule structure

Microtubules are hollow cylindrical cores composed of α and β tubulin heterodimers, as illustrated (Fig. 2a and b) [31]. Each α and β-tubulin monomer structure consists of three major functional domains [32]. The N-terminal domain (residues 1–206) which forms a structure known as a Rossman fold, with six alternating parallel β-sheets (S1–S6) and helices (H1–H6), is involved in nucleotide (GDP/GTP) binding [32]. The central domain (residues 207–384) is formed by four mixed β-sheets (S7–S10) and three helices (H8–H10) and is involved in the lateral/longitudinal contact between α and β-tubulin monomers forming protofilaments [32]. The C-terminal domain consists of two anti-parallel helices (H11 and H12) starting at residue 385 (Fig. 2b) which have been implicated in the binding of both microtubule-associated proteins (MAPs), such as tau, MAP2, stathmin, CENP-E (also known as kinesin-7) and the mitotic kinesin Eg5 [also known as kinesin spindle protein (KSP)] [32,33].

When microtubules are being formed, α and β tubulin heterodimers associate together in a head-to-tail fashion to form a

microtubule nucleus (Fig. 3a). The microtubule nucleus elongates linearly into protofilaments which then associate laterally to form microtubules (Fig. 3b) [24]. In vivo, a microtubule is composed of 13 protofilaments with a diameter of 25-nm [34]. The way in which protofilaments are arranged gives polarity to the microtubule, making the exposed β-tubulin, the plus (+) end and the exposed α-tubulin at the other end of the microtubule, the minus (−) end (Fig. 3b) [24]. The plus (+) end is more capable of rapid growth and is more kinetically dynamic than the minus (−) end [24]. Therefore, net growth occurs at the plus (+)

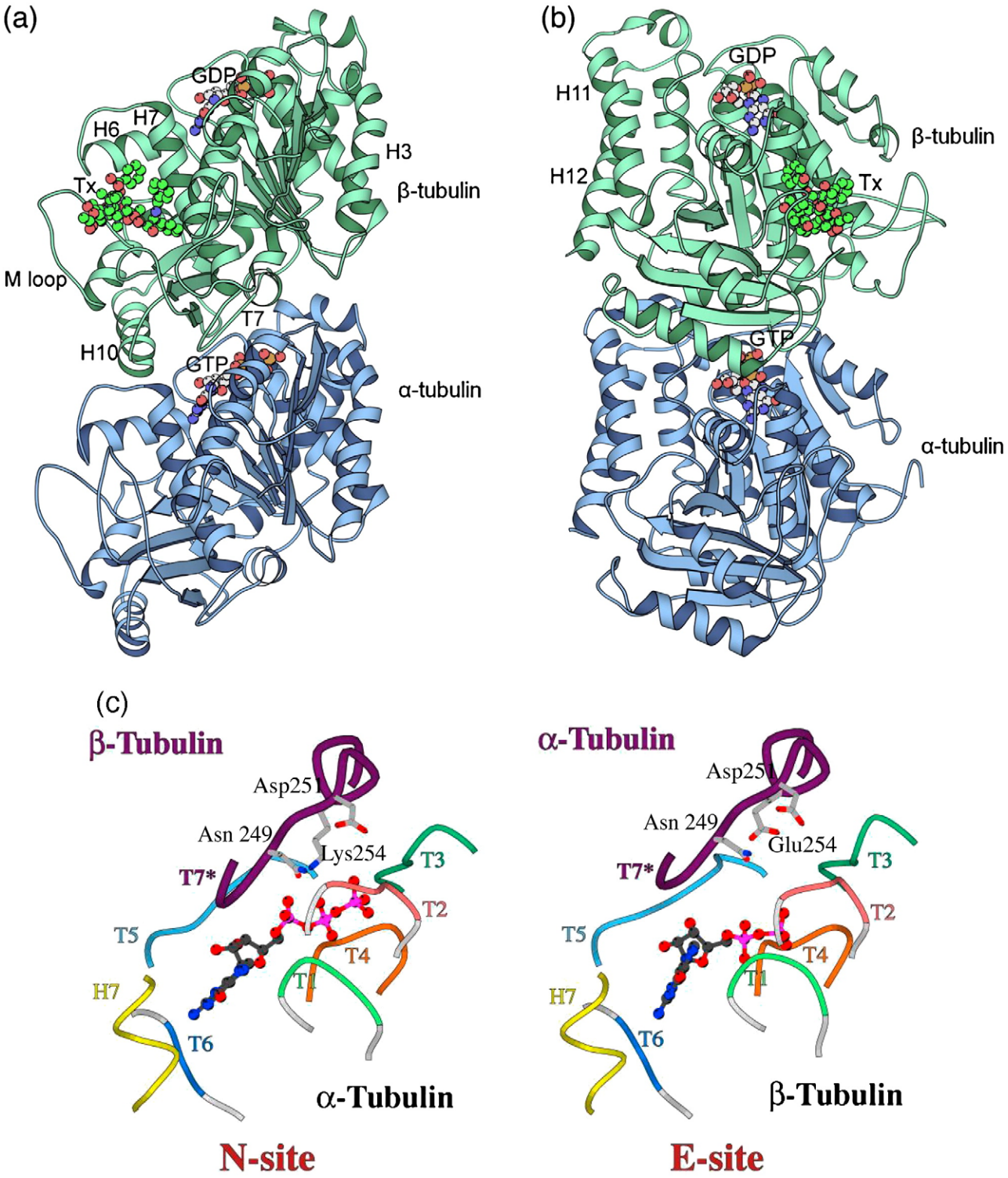


Fig. 2. Ribbon diagrams of the tubulin dimer/paclitaxel-binding site. (a) Ribbon diagram of the tubulin dimer consisting of upper β-tubulin subunit and lower α-tubulin subunit. The nucleotides (GDP, GTP) and paclitaxel (Tx) are indicated as ball-and-stick structures on the tubulin dimer. Other features include helices H3, H7, H10 and M loop. (b) Lateral view of tubulin dimer indicating helices H11 and H12 (c) The structure of the E-site and N-sites on α and β-tubulin where the nucleotides GTP and GDP bind (Both figures printed with permission Downing and Nogales [31]) (Figure printed with permission Snyder et al. [62]).

end and net shortening at the minus (−) end [35]. Microtubules are organized with the plus (+) ends free in the cytoplasm located toward the periphery (plasma membrane), whereas the minus (−) ends are associated with the microtubule-organizing center (MTOC) or centrosome (Fig. 4) [32]. The centrosome is characterized by two centrioles and the pericentriolar material (PCM) [36]. One component of the PCM is γ-tubulin, a protein highly homologous to the α/β tubulins [37]. γ-tubulin forms ring-like structures on the PCM [38] providing a primer template from which α/β tubulins can polymerize, forming microtubules extending from the centrosome [39]. Therefore, the centrosome and its components are critical in accurately monitoring microtubule growth and dynamics.

3.2. Microtubule polymerization dynamics

The polymerization and depolymerization dynamics of microtubules are important in cellular function [9]. Polymerization of microtubules occur when each monomer of α and β tubulin binds to a GTP molecule at the nucleotide exchangeable site (E-site) in β-tubulin and the non-exchangeable site (N-site) in α-tubulin (Fig. 2c) [31]. Hydrolysis only occurs when dimers have GTP in their E-site [40]. Following hydrolysis of GTP to GDP+Pi, the conformation of the β-tubulin changes to a curved form (D-form), causing tubulin-GDP to lock into the core of the microtubule [24,41]. Once GTP is hydrolyzed it becomes non-exchangeable, which accommodates the addition of the next tubulin dimer to the plus (+) end of the microtubule [24,41,42]. In addition, the growing end of the microtubule is capped with GTP or (GDP+Pi) attached to β-tubulin (Fig. 3c). This configuration favors growth of the microtubule and stabilizes the GDP-tubulin core by helping to maintain the association between the protofilaments (Fig. 3c) [43]. Upon depolymerization, the GTP cap is lost, allowing the GDP-containing polymers to relax and the protofilaments peel outwards (Fig. 3d). Subsequently, the microtubules depolymerize releasing the α/β tubulin heterodimers into the cytoplasm [32]. The GDP attached to free β-tubulin can now exchange to GTP at the E-site before another cycle of polymerization begins (Fig. 3d) [22]. The GTP cap model explains the phenomenon whereby the GTP or GDP+Pi allows the microtubule to stabilize enabling growth at the plus (+) end, while in contrast, when the GTP cap is lost, the GDP-tubulin dimers dissociate from the shortening microtubule [43].

Microtubules display two forms of dynamic behavior. These are dynamic instability and treadmilling [44]. Dynamic instability is a form of nonequilibrium dynamics [44]. This process describes the association and dissociation of α/β tubulin heterodimers from the microtubules resulting in their alternative lengthening and shortening [24]. Microtubule dynamics can change dramatically, undergoing rapid lengthening or shortening. Alternatively, they can reach a steady state, where growth of the microtubule polymer is balanced by microtubule polymer shrinkage [41]. The transition from a sudden change in microtubule growth to shortening is termed 'catastrophe', whereas the transition from shortening to growth is termed 'rescue' [45]. The term 'dynamicity' refers to the overall rate of exchange of tubulin dimers at the microtubule ends, while "treadmilling" describes the net growth at the plus (+) end of the microtubule concomitant with net shortening at the minus (−) end [22]. This balanced type of dynamic behavior is affected by the exchange of tubulin subunits from the plus (+) end of the microtubules to the minus (−) end, due to differing subunit concentrations of free tubulin in equilibrium with the microtubule ends [24].

Dynamic instability and treadmilling occur in all living cells and are regulated by a number of microtubule-associated proteins (MAPs) including, tau, MAP2 and MAP4 which bind to stabilize the microtubules (Fig. 3b) [40]. Phosphorylation of these MAPs is an important regulatory post-translational modification. In general, MAP phosphorylation leads to their dissociation from the microtubule and/or tubulin, leading to microtubule instability [40]. Kinases such as Cdc2 kinases (controlling centromere localization) [46], mitogen-activated protein kinases ERK, JNK (controlling cell migration) [47] and the main serine/threonine phosphatases, type 1 (PP1) and type 2A (PP2A) [48] have a critical role to play in the regulation of MAPs and therefore, microtubule dynamics [40]. Another factor which promotes microtubule destabilization is oncoprotein 18 (op18)/stathmin [49]. Stathmin regulates the mitotic spindle by binding to the microtubule ends, increasing the catastrophe rate at both ends of the microtubule [40,50].

3.3. Microtubule dynamics in mitosis and proliferating cells

The dynamic nature of microtubules, that is their ability to polymerize and depolymerize, is essential for cellular division and chromosome segregation during mitosis [41]. In interphase the microtubules radiate outwards from the microtubule-organizing centre (MTOC) and act as stable protein structures for protein and vesicle transport (Fig. 4) [33]. With the onset of mitosis, the interphase microtubule network depolymerizes and is replaced by microtubules that are up to 100 times more dynamic [9]. These microtubules form the bipolar mitotic spindle critical for the segregation of sister chromatids during mitosis [33]. Thus, microtubules are fundamental to the normal functioning of the cell.

During interphase microtubule turnover is relatively slow, with half-lives ranging from several minutes to several hours [24]. During G_1 to S phase the two centrioles of the centrosome separate and duplicate and by late G_2 phase have enlarged in size but remain attached together until mitosis begins (Fig. 4) [41]. In eukaryotic cells, mitosis is initiated when the regulatory subunit cyclin B1 complexes with the cyclin-dependent kinase (cdk1) $p34^{cdc2}$ forming the maturation promoting factor (MPF) (Fig. 4) [51].

In early prophase, centrosomes move apart, forming the spindle poles, and the chromosomes condense [Fig. 4 (1)]. One of the most studied kinesin spindle proteins, kinesin Eg5 (KSP), functions by mediating the separation of centrosomes and the formation of the bipolar spindle, critical for intracellular trafficking along the microtubule [52]. In eukaryotes, the mitotic spindle is attached to the two opposing spindle poles (centrosomes), with the minus (−) end of the microtubules attaching to the poles, and the plus (+) ends extending away from them [Fig. 4 (1)] [40]. The nuclear envelope

is still intact [40]. Upon nuclear envelope breakdown, chromosomes and nuclear factors enter the cytoplasm and the microtubule (MT) dynamics dramatically increase [40].

In prometaphase, the plus (+) ends of the spindle microtubules extend from each pole and probe the cytoplasm until they attach to the kinetochore on the sister chromatids [Fig. 4 (2)] [9]. This is the hallmark of the 'Search-and-Capture' model underlying spindle assembly [40]. Three subtypes of mitotic microtubules have been characterized, these are; (1) kinetochore microtubules (MTs) whose plus (+) ends attach to the chromatids at the kinetochore, (2) interpolar MTs, which overlap MTs from the opposite poles at the midzone of the spindle, and (3) astral MTs which extend away from the poles to the periphery of the cell [Fig. 4 (2)]. The spindle can attach to the kinetochore of sister chromatids in a variety of ways [53]. The most stable attachment 'amphitelic attachment', or bi-orientation, occurs when both sister kinetochores attach simultaneously to microtubules from opposite centrosomes [Fig. 4 (3)] [53]. Such microtubules, which grow up to 5–10 μm in length, shorten completely then re-grow until they attach fully to the kinetochore. Kinetochores stabilize their associated microtubules forming mature stabilized kinetochore microtubules (K fibers), which contain up to 25 microtubules in a metaphase cell [54]. In

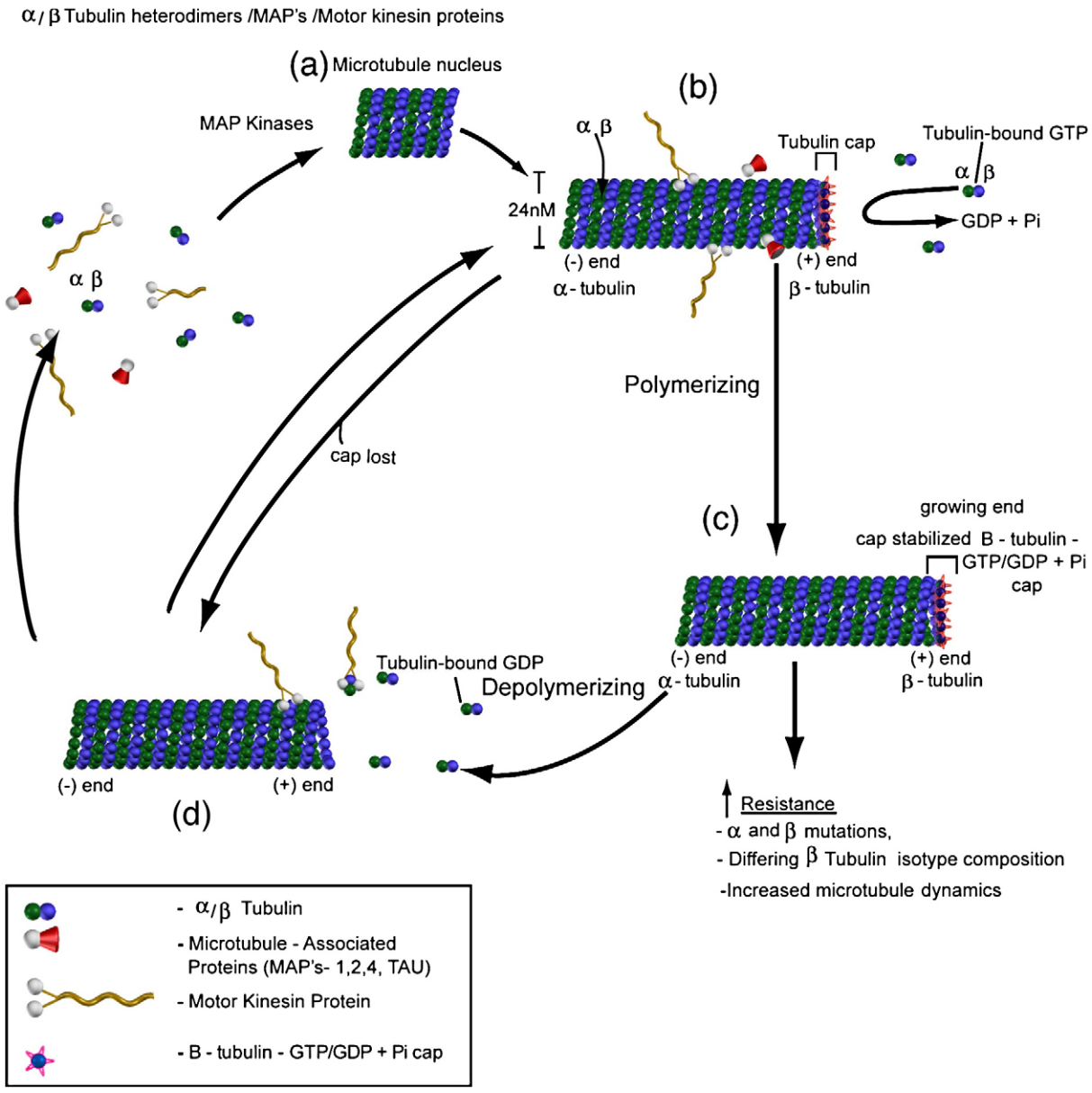


Fig. 3. Polymerization and depolymerization microtubule dynamics. (a) Microtubule nucleus forms when α/β heterodimers associate together in a head-to-tail fashion. (b) Microtubules consisting of α/β heterodimers elongate to form cylindrical microtubules of 13 protofilaments with a plus (+) end and minus (−) end. Tubulin-bound GTP binds to plus (+) end of microtubule and GTP is hydrolyzed to GDP+Pi (inorganic phosphate) forming a GTP cap. (c) The GTP cap stabilizes the microtubules plus (+) end and stabilization is further enhanced by addition of the taxanes, paclitaxel and docetaxel, which bind to β-tubulin sites causing polymerization of microtubules. (d) Depolymerization occurs when the tubulin−GTP/GDP+Pi cap is lost, Pi is released from tubulin, destabilization occurs and the tubulin-bound GDP dissociates from the plus (+) end causing depolymerization (effects of vinca alkaloids) (Figure adapted with permission from Jordan and Wilson [24]).

prometaphase, the spindle assembly checkpoint (SAC) remains activated until all kinetochore microtubules have attached correctly to kinetochores with the appropriate tension [55].

During metaphase, the sister chromatids attach to the microtubules at their kinetochore. These then oscillate back and forth in order to create the appropriate tension needed for silencing the SAC thereby generating a signal which causes sister chromatids to separate and drive mitosis into anaphase [Fig. 4 (3)] [56]. Specifically, the SAC ensures correct alignment and segregation of chromosomes at the metaphase/anaphase

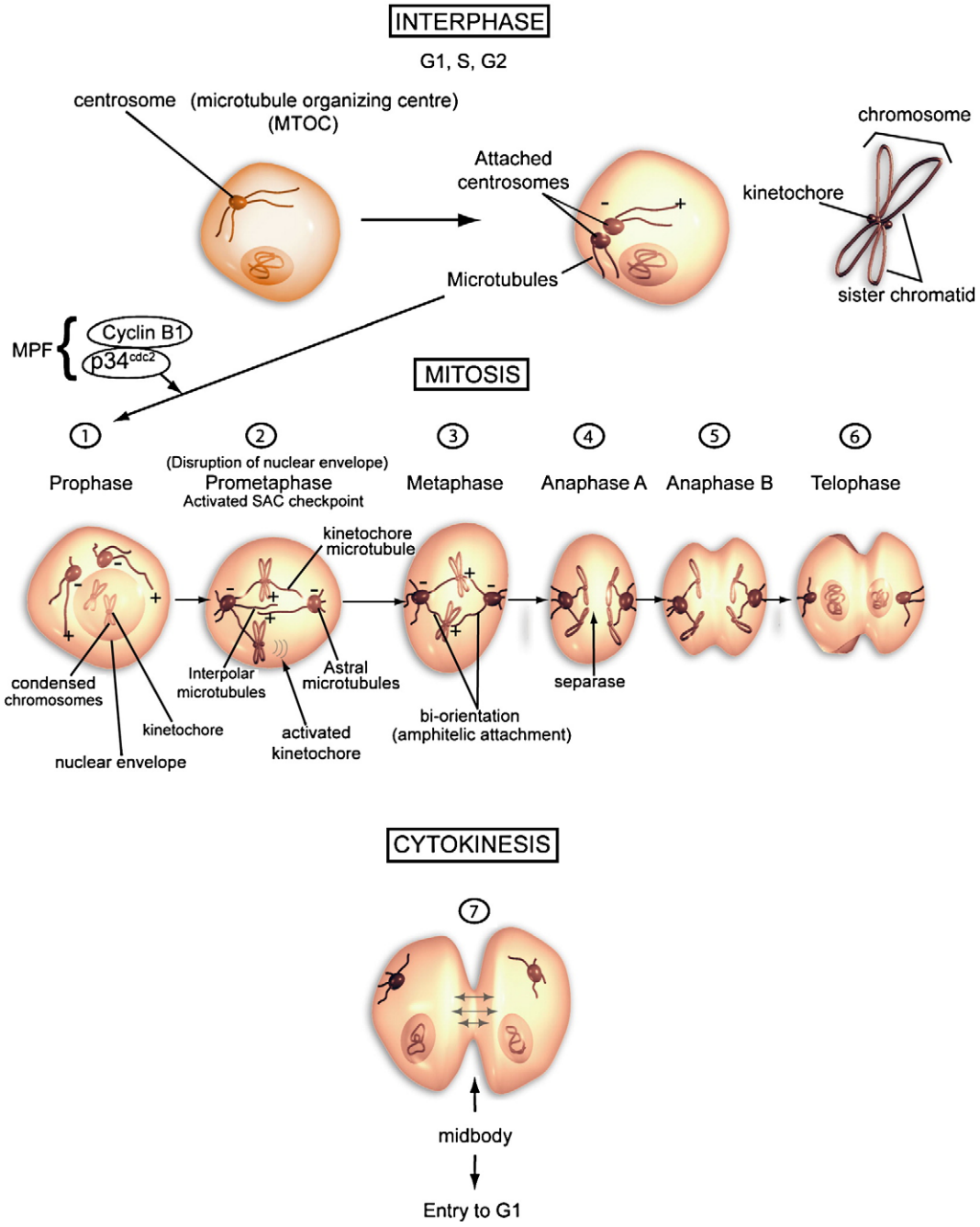


Fig. 4. Cell division and microtubule dynamics. During interphase the centrosomes are duplicated and DNA is replicated in S phase. Mitosis is divided into six different phases; (1) In prophase centrosomes move apart, forming the spindle poles, and subsequently condense. (2) In prometaphase the nuclear envelope breaks down and the microtubules begin to elongate from each spindle pole enabling attachment to the kinetochores of each sister chromatid. This activates the spindle assembly checkpoint (SAC) until all sister chromatids have attached fully. When amphitelic (bi-orientation) of each sister chromatid to the microtubules has occurred (3) the checkpoint is silenced at metaphase. (4) During anaphase (A) the duplicated sister chromatids are pulled apart and in (5) anaphase (B) further separation occurs with microtubule shortening. (6) In telophase the chromosomes decondense and a new nuclear envelope forms around each new daughter cell. (7) During cytokinesis bundling of microtubules occurs around the midbody, which allows for a membrane to form between each new daughter cell. (Figure adapted with permission from Mollinedo and Gajate [41]).

transition in mitosis [57]. There are a number of checkpoint proteins affecting chromosomal alignment at the metaphase stage. One of these is the kinetochore-associated kinesin microtubule motor protein, CENP-E, required for the stabilization of the kinetochore-MT attachment [58]. Another is the mammalian centrosome-associated kinesin (MCAK), a catastrophe factor [33] required to correct abnormal kinetochore attachments that can interfere with chromosome alignment along the metaphase plate [59].

During anaphase, the K fibers established during prometaphase, depolymerize and allow chromosomal segregation to take place [Fig. 4 (4)] [40]. It is at this stage in mitosis that the sister chromatids are pulled apart, due to cleaving by separase of the cohesions between sister chromatids [Fig. 4 (4)]. Concurrently, the overlapping interpolar microtubules depolymerize at the spindle midzone and the sister chromatids are pulled apart [Fig. 4 (5)]. Finally, in telophase the chromosomes decondense with a new nuclear envelope forming around each new daughter cell [Fig. 4 (6)]. This leads to cytokinesis and entry into the G_1 phase as duplicated daughter cells [Fig. 4 (7)] [40]. Microtubule dynamics are highly controlled during cell division and accurate monitoring by microtubule-regulating proteins, nuclear factors and cell cycle-related proteins are critical [40].

4. β-tubulin the cellular target for taxanes and other anti-mitotic agents

The cellular target for paclitaxel is the taxane site on β-tubulin (Fig. 2a) [31]. Docetaxel shares the same binding site as paclitaxel, though with greater affinity [60]. The taxane-binding site on microtubules is only present in assembled tubulin [61]. Paclitaxel binds to the intermediate domain on β-tubulin as determined by electron crystallography (Fig. 2a) [31]. This pocket for paclitaxel lies within a hydrophobic cleft near the surface of β-tubulin and allows for paclitaxel to interact with proteins via hydrogen bonding and hydrophobic contact [62]. The binding of paclitaxel causes lateral polymerization and microtubule stability [61]. Photoaffinity labeling methods have located the sites of interaction between paclitaxel and β-tubulin corresponding to amino acid residues 217–233 of β-tubulin [63]. The molecular docking model of paclitaxel and β-tubulin as described by Snyder et al. demonstrates a T-shaped paclitaxel conformation within the β-tubulin site [62]. Segments of α-helices H1, H6, H7 and the loop between H6 and H7 interact hydrophobically with paclitaxel [62]. In addition, paclitaxel comes in contact with β-strands, B8 and B10. Other drugs that bind to the taxane site include, epothilones (isolated from myxobacterium *Sorangium cellulosum*), discodermolide (originally isolated from marine sponge *Discodermia dissolute*) and eleutherobin (originally isolated from the coral species *Eleutherobia*) [22].

The vinca alkaloids, vinblastine and vincristine, and the semisynthetic analogs, vinflunine and vinorelbine, bind to the vinca domain on microtubules (Fig. 5a) [22]. This domain is located adjacent to the exchangeable GTP-binding site on β-tubulin at the plus (+) end of the microtubule (Fig. 5a) [22]. Vinca alkaloids bind with high-affinity to the plus (+) end of the microtubule and with low affinity to the sides of the microtubule [9,41].

Colchicine was originally isolated from the meadow saffron *Colchicum autumnale* [22]. It is not used in the treatment of cancer because of its increased toxicity to normal tissue. Nevertheless, colchicine has played a role in elucidating the function and properties of microtubules [64]. The colchicine domain is located between the β-tubulin and α-tubulin interface (Fig. 5b) [22]. Other drugs which bind to the colchicine site include, combretastatin and 2-methoxy-estradiol (2ME2) [22,65]. The latter is a metabolite of estradiol, which has been shown to exert inhibitory growth effects in cancer cells in vitro [22]. Combretastatin (isolated from the South African willow *Combretum caffrum*) binds to tubulin, inhibiting microtubule polymerization, and leads to mitotic arrest and subsequent apoptosis [65].

4.1. Mechanism of action and pharmacology of anti-mitotic drugs

Microtubule-targeting drugs function in suppressing spindle microtubule dynamics, thus inhibiting the metaphase anaphase transition, blocking mitosis and inducing apoptosis (Fig. 4) [24]. These drugs are arbitrarily divided into two main groups. The first is the microtubule-stabilizing agents (MSAs), including the taxanes (paclitaxel and docetaxel), epothilones A and B, discodermolide, and eleutherobin [24]. These agents stabilize microtubules by binding to polymeric tubulin thus preventing disassembly [22]. Monastrol is another microtubule-stabilizing agent which functions as a mitotic kinesin protein (Eg5/KSP) inhibitor causing mitotic arrest [52,66].

The second group of microtubule-targeting drugs is the microtubule-destabilizing agents including, the vinca alkaloids (vincristine, vinblastine), colchicines, podophyllotoxin and nocodazole [24,67]. These agents destabilize microtubules by preventing the attachment of microtubules to the kinetochores inhibiting microtubule assembly [32]. The binding of these destabilizing agents does not affect the binding of taxanes [24].

Paclitaxel suppresses spindle microtubule dynamics by allowing microtubule attachment but altering the tension across the kinetochore in mitosis [67,68]. With docetaxel, centrosome organization is disrupted affecting the late S phase, G_2 and M phases which results in incomplete mitosis, accumulation of cells in the G_2M phase and cell death [13].

The cytotoxic effects of paclitaxel and docetaxel are expressed using IC_{50} values (concentration that reduces an effect by 50%) and vary depending on the parameters under investigation and the particular cell lines being studied [69,70]. The IC_{50} values relate to parameters such as dynamicity, shortening rate of microtubules and clonogenic survival [9,71]. In vitro, it has been demonstrated that the cytotoxic effects of paclitaxel and docetaxel are time and drug concentration dependent [69,70]. In addition, paclitaxel and docetaxel have been found to have an antiproliferative effect with IC_{50} values in the nanomolar range in a variety of cell lines, with docetaxel being 2- to 4-fold more cytotoxic than paclitaxel [72]. The cytotoxicity of paclitaxel has been studied with in vitro clonogenic survival assays in a number of cell lines including the HeLa, MCF-7 and A549 cell lines [73]. In this study [73], the paclitaxel IC_{50} ranged between 2.5 and 7.5 nM for all the

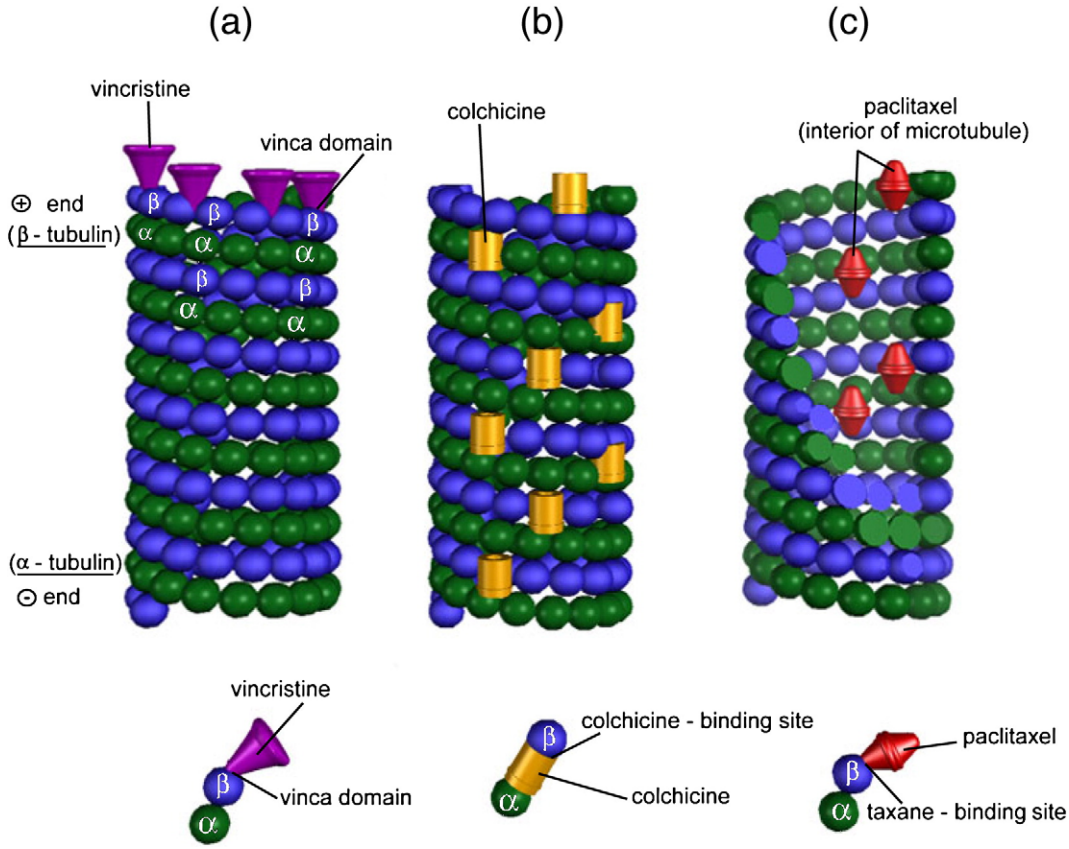


Fig. 5. Antimitotic drugs bind to different sites on the tubulin heterodimers (a) Vincristine binds to the vinca domain at the microtubule plus (+) ends leading to depolymerization. (b) Colchicine complexes with tubulin dimers at the colchicine domain located between the β-tubulin and α-tubulin interface suppressing microtubule dynamics (c) Paclitaxel binds to the interior surface of the microtubule at the taxane-binding site, suppressing microtubule dynamics. (Figure adapted with permission from Jordan & Wilson [24]).

cell lines investigated with a paclitaxel IC_{50} of 2.6 nM for the HeLa cell line specifically. Increasing paclitaxel concentration above 50 nM-, induced no additional cytotoxicity after 24 h drug exposure [73], although increasing the exposure of cells to paclitaxel from 24 to 72 h increased cytotoxicity from 5- to 200-fold in the cell lines investigated [73]. This would imply that cytotoxicity due to paclitaxel is dependent on exposure of the cells to this drug and increases in concentrations above 50 nM are unlikely to result in increased tumor response [73]. The cellular effects of paclitaxel in HeLa cells at paclitaxel concentrations (-<10 nM-) include suppression of microtubule dynamics without affecting microtubule mass, leading to mitotic arrest and apoptosis [9]. HeLa cells also exhibit aberrant mitosis containing multipolar spindles resulting in aneuploidy [74]. As the paclitaxel concentrations increases (>100 nM), microtubules assemble into stabilized bundles and the mass of microtubule polymers increase rapidly as tubulin is recruited to the microtubule [9]. High concentrations of paclitaxel block mitosis, and lead to G_2/M cell cycle arrest and apoptosis [75]. Similarly, the cytotoxicity of docetaxel has been studied using clonogenic assays in a number of ovarian carcinoma cell lines including the SKOV-3, CAOV-3 and UTOC-1 cell lines [71]. In this study [71], the docetaxel IC_{50} ranged between 0.23 and 2.3 nM for the cell lines investigated. The cellular effects of docetaxel in MCF-7 cell lines at low docetaxel concentrations (4 nM) induce aberrant mitosis followed by aneuploidy [70], while higher concentrations (100 nM) induce sustained mitotic arrest and mitotic slippage [70]. In addition, docetaxel has similar cellular cytotoxic effects to paclitaxel inducing apoptosis and cell death in a dose-dependent manner in the ovarian cancer cell lines A2780, H134, IGROV-1 (wild-type for p53) and OVCAR-3 (mutant for p53) [76]. Moreover, docetaxel has been shown in MCF-7 cells and PC-3 prostate cancer cells to be 10- to 100-fold more potent than paclitaxel in phosphorylating Bcl-2, which may account for the increased cytotoxicity associated with docetaxel compared to paclitaxel [77].

Vinca alkaloids inhibit microtubule dynamics by inhibiting microtubule assembly [9]. Vinca alkaloids bind to high-affinity binding sites at the ends of microtubules, suppressing microtubule dynamics without depolymerizing microtubules, thereby inducing apoptosis in a manner similar to paclitaxel [9]. In fact the vinca alkaloid, vinblastine, has been shown to have a stabilizing effect on microtubule dynamics at low concentrations in the absence of microtubule depolymerization [9]. However, at higher concentrations vinca alkaloids bind to both the high-affinity binding sites and the low-affinity binding sites on tubulin, destabilizing spindle microtubules and arresting cells in mitosis hence preventing spindle assembly occurring [22].

4.2. Cellular response to anti-mitotic agents

Several outcomes are associated with the use of anti-mitotic chemotherapeutic agents [56,78]. Firstly, cells can undergo sustained or chronic mitotic arrest until the drug is cleared [56]. This enables cells to survive and continue dividing as diploid cells. Alternatively, mitotic slippage or adaptation can occur, when cells exit mitosis without engaging in anaphase or cytokinesis, producing tetraploid (4N) multinucleated G_1 cells without chromosomal segregation [53]. The determination of cell fate following mitotic slippage is complex with a number of possible associated outcomes [56,78]. Adapted cells can survive and continue dividing as tetraploid (4N) cells (Adaption I). Alternatively, adapted cells can exit G_1 undergoing senescence or apoptosis as tetraploid (4N) cells (Adaption II). Furthermore, cells can escape to G1 overriding mitotic checkpoint signaling leading to apoptosis in interphase (Adaption III). Finally, cells can undergo cell death directly in mitotic arrest [78].

Clinically, modes of cell death, other than apoptosis, may contribute to the overall therapeutic response of tumor cells to treatment with anti-mitotic agents [79,80]. This is supported by evidence that the correlation between therapeutic response and apoptosis does not always correspond [81]. Mitotic catastrophe is another form of cell death that occurs following anti-mitotic drug treatment [82]. It has been shown that mitotic catastrophe occurs during mitosis, as a result of DNA damage or abnormal spindle formation in combination with deficient checkpoint control mechanisms [83]. Mitotic catastrophe in HeLa cells exhibits signs of apoptosis including chromatin condensation, release of pro-apoptotic cytochrome *c*, activation of caspase-3 and degradation of DNA [83]. In one study, mitotic catastrophe was shown to be the predominant form of cell death associated with docetaxel treatment in the breast cancer cell lines, MCF-10A, MCF-7 and MDA-MB-231 [82]. Both the taxanes and vinca alkaloids can induce cellular damage leading to mitotic catastrophe [84].

It is evident that the fate of tumor cells associated with the use of anti-mitotic agents depends on factors such as the mechanism of drug action, drug dosage and the genetic signature of the tumor cells. Taken together, these factors, in combination with cellular resistance mechanisms will influence the response of tumor cells to drug therapy ultimately leading to tumor cell death or tumor cell survival and chemoresistance.

5. Markers predictive of taxane resistance

Intrinsic and acquired drug resistance to taxanes are common [22]. Multiple factors underlying taxane resistance include, mutations in both α and β tubulin [85], differing β-tubulin isotype compositions [86], P-glycoprotein (Pg) overexpression [87] and increased microtubule dynamics associated with altered microtubule-associated protein (MAP) expression [88]. Moreover, functional aberrations in multiple molecular pathways, such as cell cycle control, growth promotion and apoptosis can all contribute to chemoresistance [89]. Elucidating the complex role played by some or all of these factors in taxane resistance is crucial to the better understanding of taxanes and their use in the clinical setting.

5.1. β tubulin mutations

Most tubulin mutations identified to date occur at the HM40 βI-tubulin isotype (Table 1) [85]. Mutations in β-tubulin can cause changes in microtubule dynamics and stability [90] and can also alter the binding of anti-mitotic drugs, such as paclitaxel to β-tubulin subunits leading to resistance [85]. It has been shown that cancer cell lines adapt to anti-mitotic drug treatment by acquiring tubulin mutations at important sites of drug interaction, weakening the interactions between β-tubulin and paclitaxel [91–94]. This is subsequently followed by a loss of heterozygosity (LOH) of the wild-type tubulin allele after prolonged exposure to the anti-mitotic agents such as paclitaxel and epothilones resulting in a highly resistant phenotype [95]. In vitro, β-tubulin mutations have been found at the leucine residue (215) in the H6–H7 loop region (Fig. 2a), an area close to the paclitaxel-binding site, giving rise to reduced tubulin levels, destabilized microtubule assembly, and increased resistance to paclitaxel [96]. In another study, two paclitaxel-resistant ovarian carcinoma cell lines, 1A9PTX10 and 1A9PTX22 established from the parental cell line A2780, exhibited a 24-fold increased resistance to paclitaxel compared to the parent line [97]. On cDNA sequencing two point mutations located within the intermediate domain of the HM40 βI-tubulin gene encoded by part of exon 4 were identified. The 1A9PTX10 cells had a P270V substitution while the 1A9PTX22 had an A364T substitution with the former located in the taxane-binding region. Other groups have proposed that mutations in the paclitaxel resistant 1A9 parent cell lines could have arisen as a result of a functionally inactive p53 gene (a property associated with the 1A9 cell line) [98]. Moreover, lack of p53 could prevent expression of the hMSH2 (mismatch repair gene) leading to a higher frequency of β-tubulin mutations [99].

Sequencing analysis of exons 1 and 4 of 49 formalin-fixed paraffin-embedded (FFPE) non-small cell lung carcinomas (NSCLCs) identified 19 β-tubulin mutations within 16 of the 49 patients within this cohort [100]. Apart from 2 of these mutations, all of them resided within exon 4. None of the patients with β-tubulin mutations had responses to platinum and paclitaxel treatment, whereas in the group of patients without β-tubulin mutations, 13/33 (39.4%) had complete or partial responses. The median survival for the 16 patients with β-tubulin mutations was 3 months compared to 10 months in those with no mutations ($p=0.0001$) [100]. However, the PCR primers used were subsequently discovered to amplify both wild-type β-tubulin genes and β-tubulin pseudogenes perhaps explaining why so many β-tubulin mutations were identified originally. Conversely, other studies have found that somatic mutations of β1-tubulin are rare in breast cancer patients [101,102]. In summary, β-tubulin mutations in vitro have been shown to play an important role in paclitaxel resistance leading to compromised paclitaxel binding [97] or altered microtubule dynamics [90]. However, there is little clinical evidence in vivo to indicate that β-tubulin mutations have a role to play in taxane resistance.

Paclitaxel resistance has also been associated, but to a lesser extent, with α-tubulin mutations, increased levels of MAP4 and

Table 1
β-tubulin isotypes

Class	Human isotype	Expression
I	M40	Major constitutively expressed β-tubulin isotype
II	hβ9	Major neuronal isotype, expressed mainly in the brain, but at low concentrations in various cell types
III	hβ4	Minor neuronal isotype, expressed only in neurons and the brain, at lower concentrations than class II isotype
IVA	hβ5	Neural-specific
IVB	hβ2	Constitutive
VI	hβ1	Haemopoietic-specific

Reprinted with permission from Elsevier (Berrieman et al. [85]).

phosphorylated stathmin protein in the A549 NSCLC cell line [94]. Mutations in α-tubulin may affect MAP binding and therefore confer resistance to MI agents.

5.2. β-tubulin isotypes

There are seven isotypes of β-tubulin in humans (Table 1) [85]. βV tubulin has only recently been cloned and expression profiled in human cell lines [103]. Structurally, the β-tubulin isotype composition differs within the 15–20 C-terminal amino acids [86]. The C-terminal region is the putative binding site for many microtubule-associated proteins (MAPs) [104]. The different β-tubulin isotypes show varying distribution within tissues (Table 1), suggesting that differential isotype expression may have functional significance [85]. βI and βIVb-tubulin isotypes are constitutively expressed while the other isotypes are tissue specific [105]. For example, non-neuronal tissue often expresses βI-tubulin, whereas neuron-specific tissue predominantly expresses the class βIII-tubulin [85].

Interestingly, it has been shown that dimers composed of βIII-tubulin are less stable with an increased tendency toward depolymerization compared to other β-tubulin isotypes [106,107]. In vitro studies have demonstrated that isotypically pure microtubules have unique dynamic properties [108]. Specifically, microtubules composed entirely of βIII-tubulin exhibit increased dynamicity, reflected by an increased overall rate of exchange of tubulin dimers with microtubule ends [106]. The effects of tubulin isotype composition on paclitaxel-induced microtubule stability have also been studied [107]. Isotypically pure βIII and βIV microtubules have been found to require, a higher ratio of bound paclitaxel to induce microtubule stability [107]. Subsequently, it was shown that a serine/arginine substitution had occurred at position 277 in the βIII-tubulin sequence which affected paclitaxel binding [109]. This substitution was absent in the βI-tubulin sequence. These data suggest that paclitaxel response is affected by the β-tubulin isotype composition within microtubules with increased expression of βIII and βIV-tubulin being a possible mechanism of paclitaxel resistance. Further contribution for this is highlighted by knockdown experiments in the paclitaxel-resistant cell line A549-T24, where downregulation of βIII-tubulin by antisense oligonucleotides results in a 39% increase in paclitaxel sensitivity [110].

In other studies, upregulation of βI and βIII-tubulin in ovarian tumors mRNA has been associated with paclitaxel resistance [111,112] with similar findings for βIII-tubulin in advanced breast cancer [113]. In NSCLC, expression of βIII-tubulin has also been associated with poor response to treatment with taxanes/vinorelbine [114–116]. However, despite the correlation between βIII-tubulin overexpression and paclitaxel resistance in vitro [110,117], no correlation has been found between βIII-tubulin expression levels and paclitaxel resistance in a cohort of 12 ovarian carcinoma xenografts created from patient's samples before and after paclitaxel treatment [117]. This suggests that in vitro analysis of βIII-tubulin may not correlate with in vivo expression levels. Moreover, in vitro selection methods using high concentrations of MI drugs could alter the tubulin isotype composition, biasing these methods and may not reflect the action of MI drugs in the clinical setting.

5.3. Microtubule-associated proteins (MAPs), tau, stathmin and MAP4

MAPs, which regulate microtubule dynamics by interacting with tubulin polymers and microtubules, play a role in cellular sensitivity to MI drugs by modulating microtubule stability [32]. MAPs consist of subtypes MAP1A, MAP1B, MAP2, MAP4 and tau proteins [40]. MAP2, MAP4 and tau have three or four repeat proteins in their C-terminal microtubule-binding domain [40]. Each MAP has several isoforms reflecting differences in the number of microtubule-binding repeats [118]. Tau is predominantly a neuronal MAP, although it can be expressed in epithelial and glial cells. Tau exists in six different isoforms derived from alternative splicing and post-translational modifications [118]. It binds to microtubules via either three- or four-repeat microtubule-binding domains in the tau protein (termed 3R and 4R, respectively). Tau functions primarily by enabling tubulin assembly and microtubule stabilization [119]. The number of repeats affects tau's ability to stabilize microtubules, with the 4R tau protein having a greater stabilizing effect than the 3R tau protein [120]. The tau protein binds longitudinally to the inner and outer surface of the microtubule, between two adjacent tubulin dimers on the protofilaments, and has been found to bind to the paclitaxel-binding site on the inner surface of the microtubule [118], [121]. Tau activity is controlled by phosphorylation of the microtubule-binding domain and various kinases have been shown to phosphorylate/dephosphorylate tau, including the type 2A phosphatases (PP2A) [122].

Tau differentially binds to microtubules depending on the presence or absence of paclitaxel [123]. In the absence of paclitaxel, tau binds strongly to microtubules and dissociates slowly, whereas in the presence of paclitaxel tau binds moderately to microtubules, enhancing paclitaxel-induced polymerization and dissociates rapidly [119]. When microtubules assemble in the presence of low concentrations of tau there is increased paclitaxel binding compared to microtubules that form in the presence of higher levels of tau. In another study of breast cancer patients receiving combinatorial treatment with

paclitaxel, 5-fluorouracil, doxorubicin and cyclophosphamide (P/FAC), low levels of tau expression were associated with a greater response to treatment [124]. Conversely high levels of tau were associated with residual tumor and resistance to treatment [124]. However, half of the patients in this study with low tau expression were resistant to treatment, indicating that paclitaxel resistance is multifactorial in nature and may not be predicted by tau expression levels alone. In another study of estrogen-receptor (ER)-positive breast cancer patients, high tau mRNA expression levels were associated with tamoxifen sensitivity and taxane resistance, whereas low tau mRNA levels were associated with a poor prognosis in patients receiving tamoxifen alone [125]. This therefore suggested a potential benefit for the addition of a taxane-containing regime in breast cancers with low tau mRNA expression levels [125]. Clearly evaluation of tau expression may be an important factor in predicting response to anti-mitotic drugs such as paclitaxel. Standardized approaches in evaluating tau expression need consideration, as tubulin binding and microtubule dynamics are affected by the numerous splice variants and different tau isoforms, each having a number of phosphorylation sites [124].

The oncoprotein 18 (op18)/stathmin is a microtubule-destabilizing phosphoprotein which alters the microtubule dynamics within the cell during interphase and mitosis [126]. Stathmin is ubiquitously expressed in all cell types [127]. In vitro, stathmin is involved in mitotic spindle regulation and binds to microtubules, increasing the catastrophe rate (transition from a sudden change in microtubule growth to shortening) at both the plus (+) and minus (−) ends of the microtubule [40,45,50]. Thus, dysregulation of stathmin may lead to reduced mitotic spindle functioning and taxane resistance. Structurally, stathmin has two functional domains consisting of an N-terminal domain with catastrophe-promoting ability and a C-terminal domain, which sequesters tubulin [40]. The ability of stathmin to effect microtubule stabilization is controlled by phosphorylation, preventing it from binding to tubulin [40,127]. Phosphorylation of stathmin is mediated by a number of protein kinases including Cdc2 (Cdk1) family kinases [128] and the p21-associated kinases (PAKs) [129]. Furthermore, expression of the non-phosphorylated mutant stathmin leads to defects in the spindle assembly checkpoint (SAC) and G_2M cell cycle arrest, which may be a possible mechanism underlying paclitaxel resistance [49]. Overexpression of stathmin has been found in both breast cancer [130,131] and in [132] leukemia and is thought to increase the rate of catastrophe, decreasing microtubule polymerization and decreasing paclitaxel binding, leading to increased paclitaxel resistance [131]. Moreover, functional knockdown of stathmin using siRNA results in increased sensitivity to paclitaxel [133].

MAP4 is ubiquitously found in all cell types [40]. It stabilizes microtubules by increasing the rescue frequency (transition from a sudden change in microtubule shortening to growth) and has been postulated to be involved in the regulation of microtubule dynamics in mitosis [45,118]. The activity of MAP4 within the cell is controlled by phosphorylation [40]. When MAP4 is phosphorylated/inactivated it dissociates from the microtubule allowing mitosis to proceed [134]. Thus, theoretically, downregulation or inactivation of MAP4 may increase the dynamicity of microtubules therefore having effects on paclitaxel resistance [32]. Interestingly, in the paclitaxel-resistant ovarian carcinoma cell lines 1A9PTX10 and 1A9PTX22, MAP4 remains bound to microtubules [134]. Finally, in vitro, MAP4 overexpression correlates with increased resistance to paclitaxel [135,136].

5.4. Multidrug resistance (MDR)

Multidrug resistance (MDR) is a phenomenon whereby resistance to one drug can result in cross-resistance to other structurally unrelated drugs leading to the multidrug resistant (MDR) phenotype [105]. A key mechanism underlying multi-drug resistance relates to the expression of the ATP-dependent transporter family known as the ATP-binding cassette (ABC) family [137]. One of the first members of these energy-dependent drug efflux pumps to be described was the P-glycoprotein (P-gp) encoded by the MDR-1 gene, localized to chromosome 7 [138,139]. P-gp protein functions by increasing the efflux of drugs out of the cell, thereby decreasing intracellular levels of the drug leading to drug resistance [140]. When drugs bind to P-gp, one of the ATP-binding domains is activated and hydrolysis of ATP causes a conformational change in P-gp, causing drugs to be released into the extracellular space [141]. P-gp can bind to a wide variety of hydrophobic drugs including paclitaxel, doxorubicin, vincristine and vinblastine [142]. Moreover, paclitaxel is a P-gp substrate [142]. The role of P-gp in multidrug resistance has been defined through in vitro cell culture work. Gene transfer experiments using MDR-1 cDNAs induced drug-sensitive cultured cells to become drug-resistant [143]. The National Cancer Institute (NCI) evaluated MDR-1 expression levels in a 60-cell line drug-screening panel using quantitative polymerase chain reaction (PCR) and found that the lower the MDR-1 expression level, the greater the sensitivity to paclitaxel in the cell lines [144].

Evidence that P-gp may have a role to play in drug resistance has been extensively reviewed [137]. The relevance of MDR1/P-gp gene expression in breast cancer has been investigated in a number of studies [138,145]. One study showed that increased MDR1/P-gp expression levels were significantly ($p=0.0433$) associated with shortened disease-free survival (DFS) in chemotherapy-naive breast cancer patients, compared to patients that were MDR1/P-gp negative [146]. In contrast, other studies have found no significance between MDR1/P-gp expression and response to either paclitaxel or docetaxel treatment in breast cancer patients [147–149]. In non-small cell lung carcinoma (NSCLC) patients treated with paclitaxel, 28/28 (100%) patients with no MDR-1/P-gp expression responded well to treatment whereas 15/22 (68%) patients with increased MDR-1/P-gp expression ($p<0.05$) were refractory to treatment [150].

In ovarian cancer, MDR-1/P-gp overexpression has been found to be a significant predictor of survival and paclitaxel response ($p=0.004$) [151]. Specifically, strongly positive MDR1/P-gp immunostaining was associated with stage III

tumors in ovarian cancer patients with a median survival of 9.8 months [152]. In contrast, patients with negative MDR1/P-gp immunostaining had prolonged survival, and had not yet reached their median survival at 5.8 years [152]. The possible reasons for the disparity between studies may relate to a variety of factors including, differences in the analytical technique used, antibodies employed, poorly designed studies with high sample variability and contamination of results due to the expression of efflux pumps in non-tumor cells within tumor samples [153].

With regard to P-gp inhibitors, verapamil and cyclosporin A have been shown to reverse the MDR-1 phenotype in a variety of paclitaxel-resistant human ovarian cancer cell lines [154]. However, early clinical trials found that the high plasma concentrations of P-gp inhibitors needed to reverse the MDR phenotype increased patient toxicity and failed to restore treatment response in MDR-1 expressing tumors [155,156]. Other P-gp inhibitors have also been developed including second generation inhibitors, biricodar (VX-710), valspodar (PSC833) and third generation inhibitors tariquidar (XR9576), laniquidar (R101933) and zosuquidar (LY335979) [153]. The P-gp inhibitor valspodar (PSC-833), an analogue of cyclosporine D, is highly potent with less nephrotoxicity and immunosuppressive effects compared to first-generation P-gp inhibitors, such as verapamil and cyclosporin A [157]. In relation to the third generation inhibitor tariquidar, this P-gp inhibitor has undergone phase I and II trials [153]. One phase II trial combining vinorelbine and tariquidar was halted early in patients being treated for chemoresistant advanced breast cancer as patients were not expected to achieve a positive benefit: risk ratio with the addition of tariquidar [158], whilst another third generation P-gp inhibitor, ONT-093 was found to adversely interact with paclitaxel [159]. Therefore, P-gp inhibitor therapy has shown little clinical value overall in restoring tumor sensitivity, with adverse side-effects associated with many P-gp inhibitors in these settings [158,159]. Other potential approaches in modulating MDR-1/P-gp expression have come from short interfering double stranded RNAs (siRNA) [160] and short hairpin RNA (shRNAi) targeted degradation of MDR-1, with the latter study effectively restoring sensitivity in a paclitaxel doxorubicin-resistant cell line [161].

Another class of drug efflux pumps implicated in taxane resistance is the multiresistance-associated protein (MRP) family of transport proteins also referred to as ABCC (adenosine triphosphate-binding cassette C group) transporters, which include several subfamily members [162,163]. One such member is MRP1 which codes for a 190 kDa protein and is associated with the cell membrane and the intracellular membrane [164]. MRP1 was originally identified through its overexpression in a doxorubicin-resistant lung cancer cell line in the absence of MDR1/P-gp expression [164]. MRP1 expression confers resistance to a variety of drugs including anthracyclines, vincristine and, to a lesser extent, vinblastine [165]. Finally, targeted chemotherapies, such as Gefitinib (Iressa) [166] and Gleevec (Imatinib) [167], are substrates for P-gp and MRP1 which may further compound drug resistance associated with expression of these drug efflux pumps [153]. Not withstanding this, the use of epidermal growth factor receptor (EGFR) inhibitor, Gefitinib has been shown in vitro to have a chemosensitizing effect on MDR cells [166]. More specifically, reversal of P-gp-mediated resistance to paclitaxel and docetaxel was observed in MCF-7/Adr breast cancer and PC-6/PTX small-cell lung cancer cell lines overexpressing P-gp. Indeed Gefitinib has been shown to restore sensitivity to doxorubicin in MCF-7/Adr cell lines [168].

5.5. C-erbB-2/HER2-neu

Human epidermal growth factor receptor 2 (HER2) is a 185 kD transmembrane receptor protein encoded by the proto-oncogene HER2/neu [169]. HER2 is a member of the erbB epidermal growth factor receptor family of tyrosine kinases and regulates cellular proliferation [170]. Overexpression of HER2/neu occurs in approximately 20–30% of primary breast cancer patients and is clinically associated with a more aggressive disease and poorer prognosis [171,172].

Overexpression of HER2/neu in breast cancer cell lines has been shown to confer resistance to paclitaxel with at least two mechanisms identified [173]. Yu et al. induced paclitaxel resistance by transfecting the receptor tyrosine kinase $p185^{ErbB2}$ into the breast cancer cell line (MDA-MB-435) [173]. They proposed that overexpression of HER2/neu transcriptionally upregulates $p21^{WAF1/Cip1}$ which associates with the kinase $p34^{cdc2}$, thereby inhibiting paclitaxel-induced $p34^{cdc2}$ activation and apoptosis at the G_2/M phase leading to drug resistance (Fig. 7) [173]. Subsequently, Tan et al. showed that overexpression of ErbB2 receptor tyrosine kinase (RTK) inhibits $p34^{cdc2}$ activation in primary breast tumors and the breast cancer cell line (MDA-MB-435) [174]. Moreover, the ErbB2 kinase domain was shown to directly phosphorylate Cdc2 tyrosine (Y) 15 (Cdc2-Y15-p) making the cells resistant to paclitaxel-induced apoptosis and delaying entry into M phase [174]. From other data the increased resistance to paclitaxel, doxorubicin and 5-fluorouracil has been attributed to the HER2/PI3K/Akt pathway [175]. In that study [175], MCF-7 breast cancer cells were transfected with HER2/neu resulting in phosphorylation and activation of Akt. Importantly, the observed drug resistance could be reversed and cells re-sensitized using dominant-negative expression vectors for PI3K/Akt [175].

Clinically, in a retrospective study of metastatic breast cancer ($n=141$) [176], HER2/neu expression has been evaluated using the polyclonal HercepTest (Dako, Carpinteria, CA) and the monoclonal antibody CB-11 (Biogenex, San Ramon, CA) [176]. No association was found between HER2/neu IHC staining and clinical response to taxanes using either the HercepTest or CB11 in either univariate or multivariate analysis [176]. However, IHC staining for the phosphorylated/activated form of HER2/neu using the PN2A monoclonal antibody, has been shown to be associated with increased resistance to single-agent taxane treatment in a cohort of metastatic breast cancers ($n=114$) [177]. In addition, disease progression was significantly associated with PN2A-positive tumors compared to PN2A-negative tumors (42% vs. 18%, $p=0.046$) [177].

Interestingly, in some preclinical studies, taxane resistance has been reversed with the use of trastuzumab in HER-2/neu overexpressing tumors [178]. Trastuzumab (Herceptin® Genentech Inc, South San Francisco, CA) is a humanized monoclonal antibody, which selectively binds to the extracellular domain of the human epidermal growth factor receptor 2 (HER2) [179]. Combinatorial regimes with trastuzumab and paclitaxel are highly active in HER2-overexpressing human breast tumor xenograft models [169]. In addition, phase III clinical trials incorporating both trastuzumab and paclitaxel have resulted in a beneficial effect, with trastuzumab having synergistic antitumor effects when used in combination with docetaxel and other drugs such as platinum salts [180,181].

6. The spindle assembly checkpoint (SAC) and MI treatment

The spindle assembly checkpoint (SAC), also known, as the mitotic checkpoint, monitors the attachment of spindle microtubules to the kinetochore of each sister chromatid [182]. This ensures that the correct tension is generated across sister chromatids enabling accurate chromosomal segregation to occur in mitosis (Fig. 6) [182]. The integrity of the genome is maintained by the attachment of all sister chromatids to the kinetochore microtubules prior to mitosis [183]. Essentially, the spindle checkpoint impedes cell cycle progression in prometaphase until all kinetochores have bi-orientated microtubule attachment [184]. Considering that anti-mitotic drugs target microtubules, correct functioning of the SAC would seem crucial for an appropriate drug response.

The SAC was first described in experiments using anti-mitotic destabilizing agents on budding yeast [185,186]. Subsequent research in yeast and *Xenopus* revealed a number of checkpoint proteins required for the proper functioning of this checkpoint. SAC proteins include mitotic arrest deficient proteins (1–3) MAD 1–3, budding uninhibited by benzimidazoles proteins (BUB1-3), Bub1-related protein kinase, BubR1 [183,185,186] and monopolar spindle 1 (Mps1) [187,188]. All of these SAC proteins locate transiently to kinetochores during mitosis [183,189]. Additional proteins involved in the regulation of the SAC include the microtubule motor proteins cytoplasmic dynein [190], dynein-associated proteins such as, dynactin and cytoplasmic linker protein (CLIP) 170 [190,191], CENP-E [192], CENP-F [193], rough deal (ROD)- zeste white-10 (ZW1)-ZWILCH (RZZ) complex [194] and Hec1 [195]. Other proteins include the $p31^{comet}$ protein (previously known as CMT2) involved with SAC regulation at metaphase (Fig. 6b) [196] and the chromosomal passenger proteins, survivin [197] and Aurora B [198], which are responsible for responding to a lack of tension at the kinetochore microtubule interface and also have anti-apoptotic functions [199].

The spindle checkpoint is activated when unattached kinetochores in prometaphase generate a 'wait' signal until the spindle microtubules have attached to all vacant kinetochores in metaphase (Fig. 6a) [55]. The activation of the SAC ensures that only one copy of each pair of duplicated sister chromatids segregates correctly into two daughter cells, thus preventing aneuploidy [200]. One of the first events to occur in the activation of the SAC is the recruitment of Mad1 to the kinetochores, which subsequently recruits Mad2 (Fig. 6a) [201]. Mad2 attaches via two mechanisms [201–203]. In the first instance, mad1 can act as a kinetochore receptor for Mad2, attaching in prometaphase (Fig. 6a) [201–203]. Alternatively, Mad2 can also be recruited to the kinetochore as part of a complex with Mad1 (Fig. 6a) [204–206]. The interaction between MAD1 and MAD2 is facilitated by means of two leucine zipper motifs positioned at amino acids 501–522 and 557–571 [57]. Overall, the spindle checkpoint delays anaphase onset by inhibiting the Anaphase Promoting Complex/cyclosome (APC/C) (Fig. 6a). This is an E3 ubiquitin ligase which associates with the activator Cdc20, the target of the SAC required for progression through anaphase, and inhibits the degradation of the maturation promoting factor (MPF) cyclin B1/$p34^{cdc2}$ kinase complex, thereby inhibiting cell cycle progression (Fig. 6b) [207–210].

When kinetochores are unattached by spindle microtubules, checkpoint proteins such as BubR1, Bub1, Bub3, Mad1, Mad2 and Mps1 are recruited to the kinetochore prior to anaphase and inhibit the degradation of the APC/C complex (Fig. 6a) [56,189]. During checkpoint activation Mad2, Bub3, BubR1 and Cdc20 are continuously recruited and released from the kinetochores [209,210]. In contrast, Mad1 and Bub1 remain securely bound to the kinetochores (Fig. 6a) [205], [206]. Mad2, BubR1, Bub3 and Cdc20 form the MCC (mitotic checkpoint complex) [211] which binds the APC/C complex rendering it unable to degrade securin and cyclin B1 (Fig. 6a) [207], [212–215].

A number of models have been proposed to explain how Mad1 catalyses the formation of the Cdc20: Mad2 complex referred to as the Two-state Mad2 model [216] and the "Mad2 Template" model [209]. These models have been comprehensively reviewed [209,210,217,218]. In metaphase the SAC is silenced when kinetochore microtubules attach to the kinetochore surface (Fig. 6b). SAC proteins detach from the kinetochores and Cdc20 activates APC/C causing the polyubiquitylation of the two key substrates cyclin B1 and securin (Fig. 6b) [210,218]. Securin activates separase, which cleaves the cohesion links between sister chromatids leading to the onset of telophase while cyclin B1 inactivates cyclin-dependent kinase 1 (cdk1) initiating exit from mitosis (Fig. 6c) [200].

The importance of the SAC and associated-checkpoint proteins in monitoring cellular division and safeguarding against chromosomal instability (CIN) has been demonstrated using a variety of studies in mice. Specifically, homozygous deletions of the mitotic checkpoint proteins Mad2 [219], BubR1 [220] and Bub3 [221] lead to early embryonic death, with mice having increased CIN and enhanced tumor development [222,223]. In vitro studies have further established that alterations in key spindle checkpoint proteins, such as MAD2, BUBR1, BUB3 and Aurora kinase A promote CIN, compromising the SAC and conferring resistance to anti-mitotic drugs, such as paclitaxel, vincristine and the destabilizing agent nocodazole [224–226]. In humans, defects in the spindle

checkpoint leading to aneuploidy and chromosomal CIN have been frequently associated with different types of cancer including breast [227], lung [228], colon [229] and nasopharyngeal carcinoma [230]. More specifically, colorectal carcinoma (CRC) is frequently associated with CIN, coupled with a defective spindle checkpoint regulator, namely the adenomatous polyposis coli (APC) gene product [231]. APC has a role to play in monitoring chromosomal separation and is commonly mutated in CRCs harboring CIN [231]. In addition, cell lines derived from colorectal tumors with CIN undergo less efficient mitotic arrest after spindle damage compared to near-diploid cell lines with microsatellite instability (MSI+) [226]. Hence, CRCs with defective spindle checkpoints and CIN are less likely to respond to microtubule-targeted agents, such as taxanes [231].

To further support the role of the spindle assembly checkpoint (SAC) in taxane resistance, RNA interference (RNAi)-based genomic screening libraries have been used [232–234]. In one study, siRNA knockdown of BUB1B and Aurora kinase B, antagonized the paclitaxel response in the HCT-116 colon carcinoma, MDA-MB-231 (MDA) breast adenocarcinoma and A549 non-small-cell lung carcinoma (NSCLC) cell lines [232]. Moreover, in the HCT-116 cancer cell line, BUB1B and Aurora kinase B knockdown resulted in polyploidy, multinucleation and centrosomal abnormalities in the absence of paclitaxel [232]. In a paclitaxel-dependent synthetic lethal screen other interesting proteins involved in microtubule dynamics and function have been identified, using short interfering RNA (siRNA) reverse transfection in an NCI-H1155 NSCLC cell line [233]. One such protein is the γ-tubulin ring complex (γ-TuRC) protein, an element of the microtubule-organizing center (MOC) that nucleates microtubules extending from the centrosome [39]. Moreover, γ-TuRC knockdown results in the formation of multipolar spindles in the presence of paclitaxel [233]. Finally, the knockdown of the spindle checkpoint protein, MAD2 is also known to lead to an aberrant spindle assembly checkpoint, the accumulation of micronucleated cells and mitotic slippage [233].

6.1. Spindle checkpoint proteins

6.1.1. MAD1/MAD2/BUBR1/BUB proteins

It is known that partial downregulation of either MAD1 or MAD2 confers resistance to the microtubule-destabilizing agent nocodazole [67]. In the case of the microtubule-stabilizing agents, paclitaxel and monastrol, partial downregulation of MAD2, but not MAD1 leads to increased resistance. These findings suggest that apoptosis induced by microtubule-destabilizing drugs require both Mad1 and Mad2 activity. Alternatively, microtubule-stabilizing drugs have a requirement for MAD2 to induce apoptosis, independent of MAD1 [67].

Using siRNA targeted knockdown of MAD2 in a human gastric carcinoma cell line (SGC7901), Du et al. observed that following treatment of these cells with either a microtubule stabilizing or destabilizing drug, upregulation of the anti-apoptotic protein, Bcl-2 and reduction of the apoptotic markers cytochrome *c* and cleaved caspase-3 occurred [235]. This suggests that when MAD2 is down regulated in cells exposed to anti-mitotic drugs, the anti-apoptotic effect is a result of the downstream regulation of the mitochondrial apoptotic signaling pathway [235].

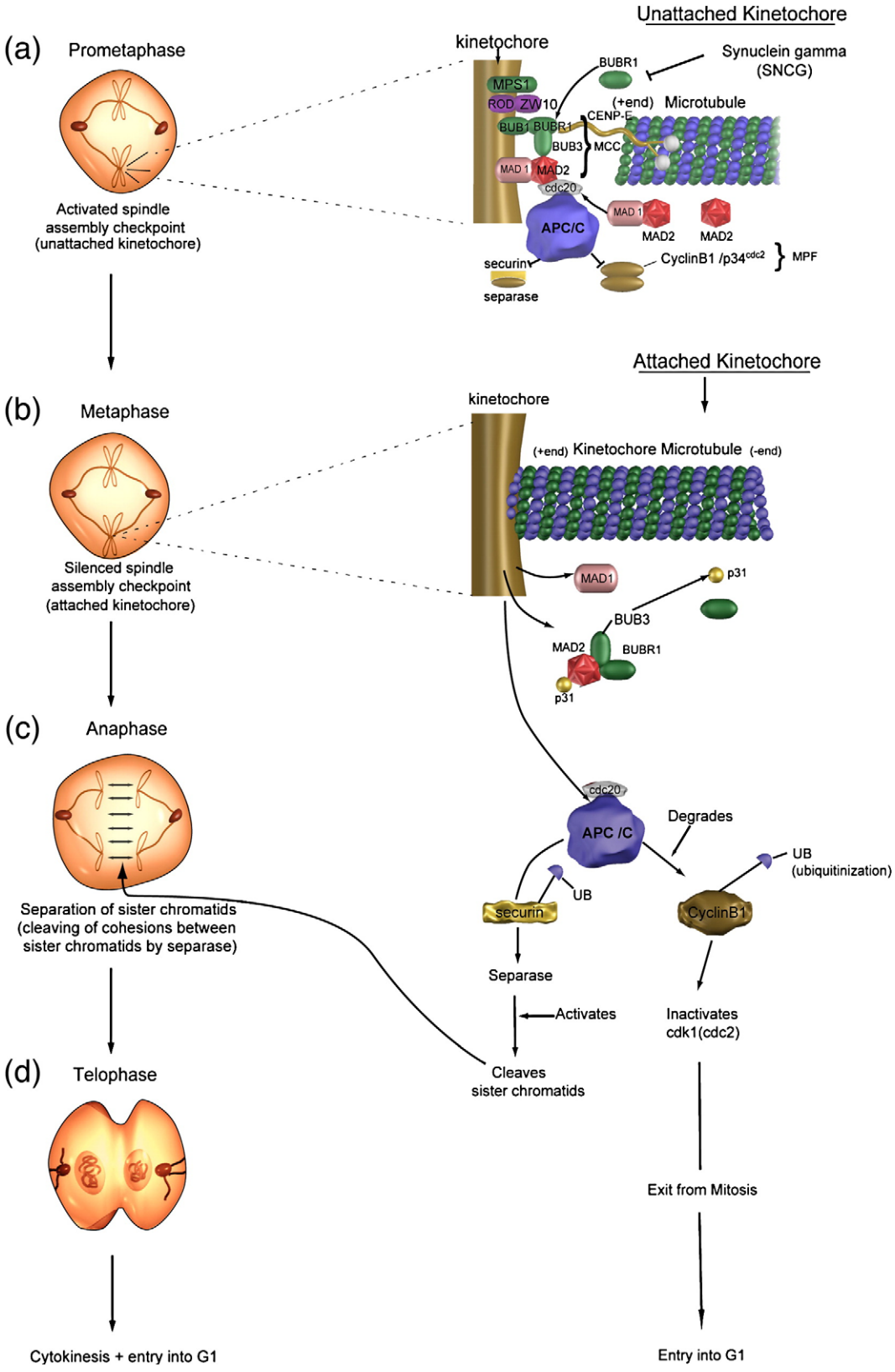
Other studies support the suggestion that decreased mitotic checkpoint function can lead to increased taxane resistance [224,236]. Indeed suppression of the two spindle assembly checkpoint genes MAD2 and BUBR1 in MCF-7 breast cancer cell lines results in resistance to paclitaxel with corresponding reduced cyclin-dependent kinase-1 (cdk1) $p34^{cdc2}$ activity [224]. Cdk1 is required for the regulation of mitosis [237] and is important in paclitaxel-induced cell death [238]. This suggests that abrogation of either MAD2 or BUBR1 critically affects spindle checkpoint function [224]. It is also known that downregulation of BUB3 and BUB2-like proteins enable cells with damaged microtubules to exit from mitosis leading to mitotic slippage contributing to cell survival and drug resistance [236]. Interestingly, in Adult T-cell leukemia (ATL) the HTLV-1 Tax protein affects subcellular localization of MAD1 and MAD2 leading to an incompetent checkpoint response and subsequent resistance to the anti-mitotic drugs, vincristine and nocodazole [239]. These studies reiterate the importance of the SAC proteins MAD1, MAD2, BUBR1 and BUB3 in microtubule function and highlight how reduced SAC protein levels can result in a compromised spindle checkpoint and anti-mitotic drug resistance. It should also be noted that increased protein mRNA expression of the checkpoint proteins BUB1B, BUB1 and MAD2 has been documented in a panel of twelve breast cancer cell lines and primary breast cancers [240]. It has been postulated that high expression levels of these mitotic spindle proteins may represent a compensation for other spindle checkpoint defects.

6.1.2. Survivin/Aurora kinases A and B

Survivin, Aurora A and Aurora B are chromosomal passenger proteins which regulate microtubule dynamics, monitor the presence of misaligned chromosomes and control bipolar spindle formation [241]. Survivin is overexpressed in

Fig. 6. The mammalian spindle assembly checkpoint — activation and silencing (a) In early prometaphase checkpoint proteins, MAD1, MAD2, BUB1, BUBR1, BUB 3, MPS1, Rod/ZW10 and CENP-E, among other proteins, are recruited to unattached kinetochores, as seen above. These checkpoint proteins help assembling microtubules to attach to kinetochores correctly ensuring that there is proper microtubule attachment and tension generated across each sister kinetochore. The interactions between these checkpoint proteins have not been fully elucidated and are thought to involve the phosphorylation of BUBR1 (checkpoint kinase), which binds to the motor protein CENP-E and helps stabilize microtubule capture. The complex interactions between MAD1, MAD2, BUBR1, and Cdc20 also known as the mitotic checkpoint complex (MCC) inhibit APC/C ubiquitin ligase activation until all kinetochores of sister chromatids have biorientated attached microtubules. (b) In metaphase when all microtubules have attached to the kinetochores, the checkpoint proteins leave the attached kinetochores and Cdc20 activates the APC/C ubiquitin ligase. (c) In anaphase after APC/C^{cdc20} is activated this causes ubiquitinization of securin which activates separase causing sister chromatids to cleave and separate leading to anaphase onset. Ubiquitinization and degradation of cyclin B1 inactivates cdk1 allowing exit from mitosis to occur (Figure adapted from Kops et al. [200]).

a wide variety of human cancers and is one of the most commonly upregulated transcripts expressed in tumors compared to normal tissue [242]. Two distinct pools of survivin exist, with 20% of the protein localized to the nucleus [243] and 80% associated with the cytoplasmic microtubules. Specifically, survivin becomes attached to the kinetochore at metaphase, the central spindle midzone at anaphase and the midbody at telophase [244] and associates



with the inner centromere protein (INCENP) and Aurora B [245].

Aurora B [246] and $p34^{cdc2}$ (cdk1) [247] function by monitoring the tension created across kinetochores, and are both mitotic kinases which phosphorylate survivin [197,248]. Both survivin and Aurora B, are required for activation of the spindle checkpoint following treatment with paclitaxel (microtubule-stabilizing drug), but not required following treatment with nocodazole (microtubule-destabilizing drug) [197,247]. When survivin is targeted by siRNA in mouse fibroblast cells, NIH-3T3 and human osteosarcoma cells U2OS, BUBR1 and MAD2 are displaced prematurely from the kinetochore and cells fail to respond to treatment with paclitaxel and monastrol but responded to microtubule-destabilizing drug treatment [197]. The data also show that kinetochore-associated BUBR1 largely depends on the expression of survivin [197]. Moreover, survivin is required for sustained mitotic arrest following paclitaxel treatment and therefore is an important regulator of the spindle checkpoint [249].

Aurora A, a serine/threonine kinase, is located in the centrosome and is overexpressed in 10%–60% of breast cancers [250]. Aberrant expression has been associated with spindle checkpoint dysfunction and increased resistance to the anti-mitotic agents, paclitaxel and docetaxel [225,250]. High expression levels of Aurora A interfere with the Mad2-Cdc20 signal in mitosis, overriding the mitotic checkpoint even in the presence of defective spindle formation [225]. In addition, Aurora A overexpression promotes prolonged mitosis and decreased post-mitotic G1 arrest, due to inactivation of the p53 checkpoint by phosphorylation of serine residues ser-215 and 315 resulting in a loss of p53 G1-checkpoint control [251]. In a breast cancer study, increased Aurora A mRNA levels were associated with reduced response rate (41%) to docetaxel compared to breast cancers with low Aurora A mRNA levels (71%) [250]. In addition, the association between overexpression of Aurora A and increased docetaxel resistance has been demonstrated in estrogen-receptor (ER)-negative tumors (33% vs. 83%) but not in ER-positive tumors (46% vs. 56%) [250]. This finding suggests that Aurora A expression levels may be clinically useful in predicting response to docetaxel treatment particularly for hormone-negative breast cancer.

Novel Aurora kinase inhibitors, such as MLN8054, Hesperadin and VX-680 have been developed, that inhibit both Aurora A and Aurora B kinase activity [39]. Although some cell-based assays have shown that Hesperadin [252] and VX-680 specifically inhibit the function of Aurora B alone [39]. These agents function by enabling tumor cells to proceed through the cell cycle without dividing (no cytokinesis) thereby forming multinucleated, polyploid cells [39].

6.1.3. Synuclein-gamma (SNCG)

Synuclein-gamma (SNCG) proteins are primarily expressed in brain tissue in the presynaptic terminals [253] and are involved in normal neuronal development and function [254]. The CpG island in exon 1 of the SNCG gene is hypermethylated in tissue other than neuronal tissue [255]. However, during cancer development, the SNCG gene can become demethylated leading to aberrant SNCG expression, particularly in breast and ovarian tumors [255,256]. SNCG is not expressed in normal breast tissue, but has been found to be overexpressed in the majority of invasive and metastatic breast cancers [257], identifying it as a potential tumor specific marker for this tumor type. Specifically, SNCG expression is associated with increased cellular motility and metastasis in both breast and ovarian cancer [258]. Functionally, SNCG overexpression has been shown to induce ERK1/2 activation and reduce JNK activity, inhibiting apoptosis and inducing increased survival after treatment with the anti-mitotic drugs, paclitaxel and vincristine [259]. In addition, SNCG binds to the mitotic checkpoint protein BubR1, resulting in downregulation of BubR1, compromising the mitotic checkpoint and conferring resistance to anti-mitotic drug-induced apoptosis [257]. SNCG-positive breast cancer cell lines also exhibit increased resistance to paclitaxel compared to SNCG-negative breast cancer cell lines ($p<0.01$) and downregulation of SNCG expression increases the effectiveness of anti-mitotic drugs in these cell lines [260]. Interestingly, inhibition of SNCG activity using a novel SNCG-binding peptide (ANK), disrupting the SNCG-BubR1 interaction, enhanced increased sensitivity to paclitaxel and nocodazole [261]. This suggests that SNCG overexpression may contribute to increased resistance to the anti-mitotic drugs paclitaxel, vincristine and nocodazole perhaps through its association with the spindle checkpoint kinase BubR1 and downstream signaling pathways.

6.2. Cell cycle-related proteins

6.2.1. Breast cancer susceptibility gene 1 (BRCA1)

BRCA1 is a tumor suppressor gene with pleiotropic functional roles including DNA repair, transcriptional regulation and maintenance of genomic stability [262]. It functions by sensing cellular stress and transducing signals either to the cell cycle or the apoptotic machinery initiating mitotic arrest and apoptosis [263]. BRCA1 is associated with the stress-response pathway p38/MAPK [264] and can activate the G_2M and spindle checkpoints in response to MI drugs that disrupt the mitotic spindle [264]. BRCA1 also induces apoptosis by activating the C-Jun N-terminal kinase/stress-activated protein kinase JNK/SAPK pathway [265] and additionally through enhanced activation of Fas and caspase 9 [266]. Following paclitaxel treatment BRCA1 activates the upstream regulator MEKK3 of both the p38/MAPK and JNK/MAPK pathways inducing apoptosis. However the exact mechanism by which BRCA1 activates MEKK3 is unclear [267] and both pathways have been implicated in mediating paclitaxel-induced apoptosis [268–270]. BRCA1 also activates $p21^{WAF1/Cip1}$ contributing to cell cycle arrest at G_1/S phase (Fig. 7) [271] and has been shown to transcriptionally regulate GADD45 at the G2/M checkpoint sequestering, cdk1 and inhibiting kinase activity, thereby, identifying GADD45 as an important effector in BRCA1 mediated cell cycle control (Fig. 7) [264].

The important role played by BRCA1 in modulating cellular stress response has implications for chemoresistance to MI drugs, which affect the mitotic spindle. It has been shown that

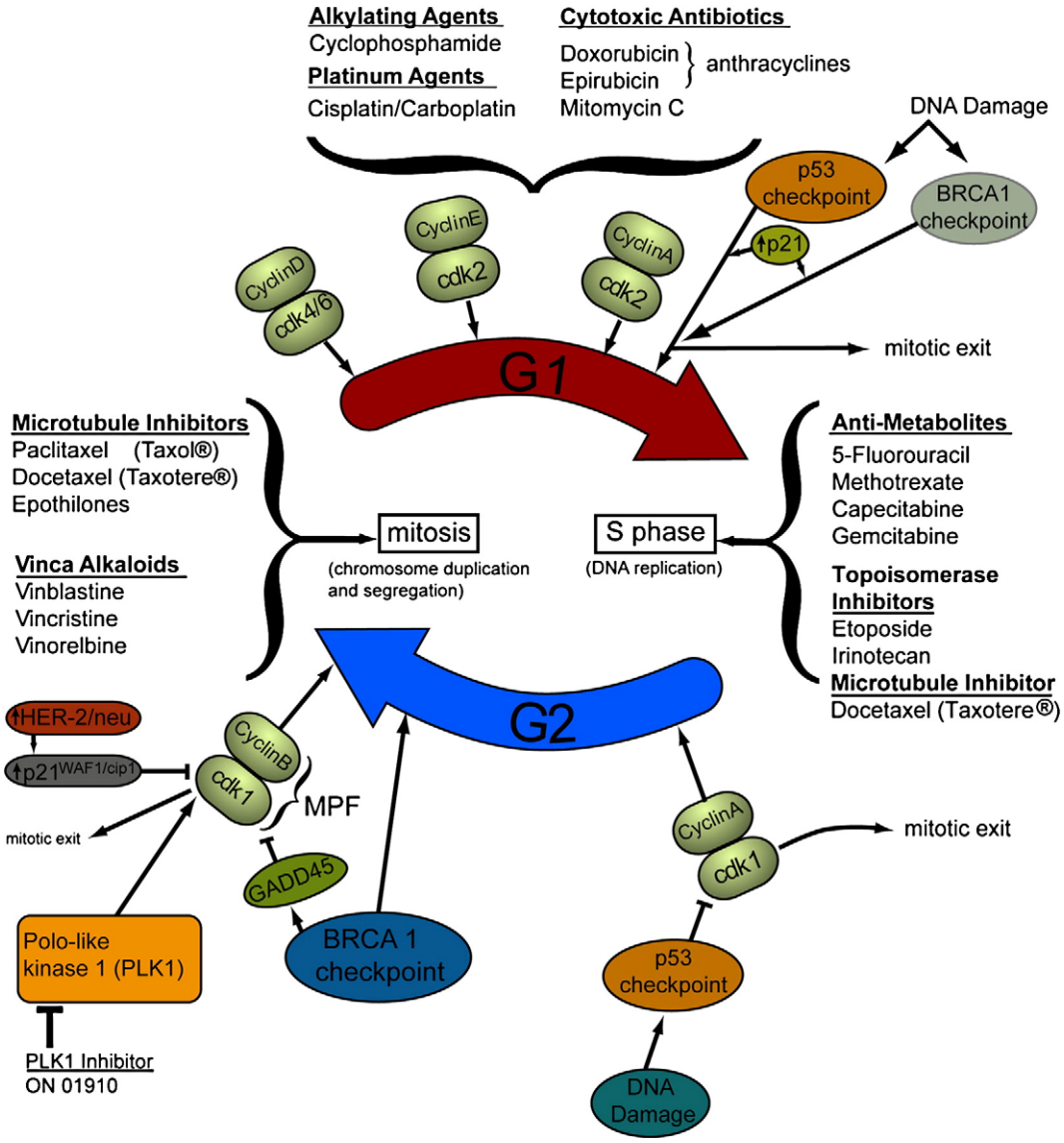


Fig. 7. Cell cycle regulation and drug action. The cell cycle is regulated by cyclin-dependent kinases (CDKs) — cdk1 (cdc2), cdk2, cdk4/6 that bind to various cyclins during cell cycle progression. The CDK complexes are regulated by phosphorylation and are involved in the regulation of transcription of a variety of genes. Different chemotherapeutic agents target different parts of the cell cycle, as shown, initiating the transduction of downstream signaling pathways. For example, anti-mitotic drugs target the G_2/M phase of mitosis, whereas other commonly used drugs target G_1/G_2 and S phase of the cell cycle interfering with DNA replication.

BRCA1 sensitizes breast cancer cells to apoptosis induced by both paclitaxel and vinorelbine [272] and reduced BRCA1 activity confers resistance to these MI agents [273,274]. In MCF-7 cells, BRCA1 targeted knockdown increases resistance to paclitaxel evidenced by premature sister-chromatid separation after paclitaxel treatment, and implicating BRCA1 in spindle checkpoint control. Moreover, BubR1 is transcriptionally regulated by BRCA1 [275] and BRCA1 also regulates the expression of MAD2 [276] through interaction with the transcription factor Oct1 [271]. Finally, BRCA1 also co-immunoprecipitates with γ-tubulin in mitosis thus having a potential role in centrosomal function [277]. The importance of BRCA1 and centrosome regulation has been observed in a mouse model, where BRCA1 disruption resulted in centrosomal amplification [278]. Importantly, the role played by BRCA1 in the regulation of the SAC proteins MAD2 and BubR1 suggests that BRCA1 activity may have a potential role to play in predicting response to MI drugs.

6.2.2. p53 expression

p53 plays a pivotal role in cell cycle arrest and apoptosis [263]. It is therefore not surprising that more than 50% of all cancers harbor p53 mutations [279]. Following DNA damage, cells with wild-type p53, induce G_1 and G_2 cell cycle arrest whereas p53-mutant cells bypass these checkpoint controls and progress into G_2M (Fig. 7) [135]. p53 is also a transcription factor for numerous genes including $p21^{WAF1/cip1}$ [280], Mdm2, Bcl-2, and BAX [281]. The $p21^{WAF1/cip1}$ inhibitory factor binds to promoter regions of several genes downregulating their transcription [282]. These genes include BRCA1,

$p34^{cdc2}$, stathmin, cyclin B1, cyclin D, cyclin E and MAP4 among others [282].

The role of p53 in taxane resistance has produced conflicting results [283,284]. Some studies suggest that paclitaxel-induced apoptosis is independent of p53 [285,286], while other studies have found that lack of p53 activity confers increased chemosensitivity to paclitaxel with increasing G_2M arrest and apoptosis [287,288]. There are a number of possible reasons for increased paclitaxel sensitivity in p53-mutated tumors. Firstly, mutant-p53 is associated with increased microtubule-associated protein 4 (MAP4) activity [136]. This increased MAP4 activity induces increased microtubule polymerization, which in turn increases the sensitivity of cells to paclitaxel [135]. Secondly, mutant p53 can override the G_1S checkpoint control leading to increased cell cycle progression into mitosis (G_2M) where anti-mitotic drugs, such as paclitaxel exert their action (Fig. 7) [230]. In contrast, however, mutant p53 has also been associated with increased taxane resistance. Specifically, mutant p53 cannot upregulate the pro-apoptotic protein BAX, thereby reducing apoptosis and leading to taxane resistance [289]. Moreover, mutations in p53 have been shown to alter spindle checkpoint control, increasing resistance to damage exerted by taxanes [290]. From a clinical perspective, paclitaxel and cisplatin chemotherapy have shown effectiveness in p53 mutant ovarian cancers compared to p53 wild-type cancers [291], while other data have no such association in this tumor type [292]. For breast cancer patients, tumors harboring wild-type p53 were unlikely to respond to paclitaxel treatment [293], a finding borne out by others [294]. Clearly, these studies present ambiguities in the significance of p53 status to taxane chemosensitivity and further investigations are warranted.

In summary there are sufficient data indicating that the cell cycle and the SAC are directly involved in mitotic cell death and subsequent apoptosis induced after microtubule damage exerted either by abrogating microtubule attachment (vincristine and nocodazole) or by inhibiting microtubule dynamics and generation of tension (paclitaxel, monastrol). Therefore, chemoresistance may in part be due to a compromised mitotic checkpoint, inappropriate signaling pathways and/or altered cell cycle-regulating proteins. However, the exact mechanisms linking the SAC and dysfunctional cell cycle control to the apoptotic pathway remain unclear and need to be further investigated.

7. Apoptosis, signaling pathways and MI drug resistance

MI drugs induce apoptosis in cancer cells through multiple signaling pathways, which are not yet fully elucidated and remain an area of much interest and debate. Among the many MI drugs discovered to date, paclitaxel remains the most actively researched compound in the discovery of signaling pathways induced after MI treatment [2,41,230,295].

The two main pathways that lead to cell death by apoptosis are the intrinsic pathway, also termed the mitochondrial pathway, and the extrinsic pathway, also termed the death receptor pathway [296]. The intrinsic pathway is activated by a variety of diverse stimuli including DNA damage and cell cycle deregulation [297]. These stimuli trigger the caspase cascade, causing the release of cytochrome *c* from the mitochondria into the cytosol due to increased membrane permeability [298,299]. Cytochrome *c* binds to apoptosis protease activating factor-1 (Apaf-1), forming a complex with procaspase-9 [300,301] which is activated to caspase-9 triggering downstream caspase-3 and 7 and inducing cell death through cleavage of death substrates [301]. Other pro-apoptotic effectors include SMAC/Diablo, released from the mitochondria following death signaling [300]. The inhibitor of apoptosis (IAP) proteins, including survivin and X-linked IAP (XIAP), bind to caspase-9 inhibiting their release [302]. SMAC/Diablo enables caspase-9 to be released from IAP proteins, which can then form complexes with Apaf-1 and cytochrome *c* leading to apoptosis [300].

The extrinsic pathway involves the activation of plasma membrane receptors by the binding of ligands such as, FasL, tumor necrosis factor (TNFα) and (TNF)-related apoptosis-inducing ligand (TRAIL) to the death receptors Fas (CD95/APO-1), DR4 (TRAIL-R1) and DR5 (TRAIL-R2) on the cell membrane [263]. This recruits caspase 8 through the adaptor protein Fas-associated death domain (FADD) forming a death-inducing signaling complex (DISC) [295]. Activated caspase-8 competes with the intracellular protein FADD-like interleukin-1β-converting enzyme inhibitory protein (c-FLIP) for binding to FADD and inhibits caspase-8 activation [296]. C-FLIP over-expression has been found in a number of in vitro studies to inhibit death receptor-induced apoptosis [303,304] and downregulation of the c-FLIP splice forms with siRNA enhances chemotherapy-induced and death-ligand-induced apoptosis in colorectal cancer (CRC) cell lines [305]. Activated caspase-8 can also activate the intrinsic pathway by cleaving cytosolic Bid (a Bcl-2 family protein associated with the extrinsic pathway), which becomes truncated (tBid) and induces pro-apoptotic signaling by binding to the pro-apoptotic proteins Bak or Bax [295].

The Bcl-2 family of proteins are key regulators of the intrinsic pathway and localize to the mitochondria, controlling apoptosis [306]. The Bcl-2 family consists of pro-apoptotic proteins including Bax and Bak which share three of the four homology domains with Bcl-2 (BH1–BH3) [306,307] and the BH3-domain subfamily of pro-apoptotic proteins including Bid, Bim, Bad, and PUMA among others [41]. The anti-apoptotic subfamily which includes Bcl-2, Bcl-x_L and McL-1, functions by blocking the release of pro-apoptotic molecules into the cytosol by heterodimerizing with Bax, Bid, Bim or Bad via their BH1–BH3 domains [193,307]. This inhibits apoptosis by inhibiting changes in mitochondrial membrane potential associated with cytochrome *c* release [295]. The relative concentrations of pro-apoptotic vs. anti-apoptotic Bcl-2 protein family determine whether cells survive or undergo apoptosis suggesting that altered phosphorylation and/or expression of these proteins may be involved in resistance to MI-induced cell death.

7.1. Apoptotic signaling

Paclitaxel has been shown to activate the JNK/SAPK signaling pathway in cancer cells through the activation of the apoptosis signal-regulating kinase (ASK1) and/or the GTP-

binding protein Ras [308,309]. It is thought that JNK activation occurs in the early stage of paclitaxel-induced apoptosis and JNK-independent pathways are activated at a later stage (up to 48 h) [309]. JNK phosphorylates and inactivates Bcl-2 at the G_2M phase of the cell cycle as demonstrated by the inhibition of paclitaxel-induced phosphorylation of Bcl-2 using dominant-negative mutants of JNK and ASK1 [310].

The Raf/MEK/ERK pathway is a survival pathway which regulates various proteins involved in apoptosis [311]. Pro-apoptotic proteins such as, Bim, Bax, Bad and anti-apoptotic proteins including Bcl-2 and Bcl-xl, are phosphorylated via this pathway leading to their inactivation [311]. Phosphorylation of these proteins interferes with their ability to bind to respective partners [312]. For example, pro-apoptotic Bim is phosphorylated on S69 by ERK [313]. Bim is then ubiquitinized and degraded causing dissociation from the anti-apoptotic protein Bcl-2. Bcl-2 then binds to Bax, preventing Bax: Bax homodimerization and Bax: Bim association leading to inhibition of apoptosis [314]. In addition, Bad phosphorylation on S112 results in its dissociation from the mitochondrial membrane and sequestering by 14-3-3 proteins, which enables Bcl-2 to homodimerize generating an anti-apoptotic response [315]. Controversially, it has been shown that phosphorylated Bcl-2 can induce increased anti-apoptotic activity [316]. This is in contrast to other studies showing that inactivation of Bcl-2 by phosphorylation leads to apoptosis [317]. Additionally, ERK activation is thought to have a protective role against MI-induced cell death and inhibition of MEK (the kinase upstream of ERK) with chemical inhibitors such as U0126 has been shown to increase paclitaxel-induced cell death [318]. Other studies have also shown that inhibition of ERK signaling in cancer cells increases paclitaxel-induced apoptosis [318,319]. Clinically, a number of cancers have upregulated ERK activity, which could be targeted by MEK inhibitors such as CI-1040 [320,321]. Such inhibitors are currently undergoing phase I and II trials [320,321]. Potentially, tumors overexpressing ERK could be targeted with such inhibitors in combinatorial therapy with taxanes thereby possibly resulting in improved response rates.

The protein tyrosine kinases (PTKs) exert their effects on drug resistance through regulation of the anti-apoptotic signaling pathway phosphatidylinositol-3-kinase/Akt (PI3K/Akt) [322]. The PI3K/Akt pathway modulates the function of various substrates involved in cell survival, cell growth and cell cycle progression and the pathway is frequently dysregulated in cancer, suggesting a possible role in tumor response to chemotherapy [322–324]. The PI3K/Akt signaling pathway phosphorylates molecules downstream from Akt including p27, mTOR and mdm2 which promote cell growth [322]. Furthermore, AKT phosphorylates other effector proteins including the pro-apoptotic protein Bad, the forkhead-related transcription factors (FKHRs) and the IkB kinase complex (IKK) promoting a pro-survival effect [322]. The PI3K/AKT is activated when PI3K catalyzes the generation of secondary messengers, phosphatidylinositol 3,4 (PIP_2) and phosphatidylinositol 3,4,5-trisphosphate (PIP_3) [311]. These bind to Akt resulting in its translocation to the plasma membrane, where it becomes phosphorylated at residues T308 and S473 by phosphoinositol-dependent kinase-1 (pdk-1) [311]. Once activated, Akt phosphorylates downstream targets such as Bim, Bad, and caspase-9, inhibiting their pro-apoptotic function.

Increased activation of the PI3K/Akt signaling pathway has been described in a variety of cancers and may induce resistance to MI treatment [175,325]. In ovarian cancer, increased activation of AKT can confer resistance to paclitaxel in different cell lines [326]. Interestingly, both in vitro and in vivo inhibitions of the PI3K/Akt pathway have been shown to sensitize cancer cells to MI-induced apoptosis [327,328] and the potential of targeting this pathway therapeutically is being increasingly investigated [322,324,329]. PI3K/Akt inhibitors include wortmannin and LY294002, both of which have been shown to enhance MI-induced apoptosis in vitro [329]. In one study, LY294002 was associated with increased sensitivity to paclitaxel in both lung and esophageal cancer cell lines [330]. Therefore, overactivation of the PI3K/Akt pathway may increase cancer cell survival and induce increased resistance to MI drugs [322].

One of the most frequently mutated tumor suppressor genes associated with the PI3K/Akt pathway is phosphatase and tensin homologue deleted on chromosome 10 (PTEN) [331]. PTEN inhibits Akt by dephosphorylating PIP3, and acts as a negative regulator of PI3K/Akt signaling [322]. Overexpression of PTEN has been found in a variety of cell lines and acts by inhibiting cell growth and promoting apoptosis [332]. However, loss of PTEN activity leads to increased cell growth and overactivation of the PI3K/Akt pathway [322]. In vitro studies have found that by inhibiting the PI3K/Akt pathway, chemosensitivity to agents such as gemcitabines and paclitaxel was achieved [330,333]. Interestingly, in one study of HER2-overexpressing tumors, immunohistochemical PTEN overexpression correlated with increased sensitivity to trastuzumab [334]. In the same study the trastuzumab-resistant breast cancer cell line SKBR3/R was found to have reduced PTEN levels and increased activation of the Akt signaling pathway [334]. Therefore, reduced PTEN expression in association with overexpression of other kinases in the PI3K/Akt pathway may be involved with increased resistance to MI drugs and be a potential target for inhibitory regulation [322,324,329].

7.2. Apoptosis-related proteins

7.2.1. Bcl-2/Bcl-xl phosphorylation

Phosphorylation of the Bcl-2 oncoprotein occurs following treatment with anti-mitotic drugs through various signaling pathways, leading to the inactivation of Bcl-2 and induction of apoptosis [312,317]. The precise mechanism in which Bcl-2 is inactivated by phosphorylation remains controversial. A variety of kinases have been implicated in drug-induced phosphorylation of Bcl-2 including cdc2 (cdk1) [335], Raf-1 [336], mTOR kinase [337] and JNK [310]. Various studies have demonstrated that paclitaxel-induced mitotic arrest initiates phosphorylation of Bcl-2 on the serine and threonine amino acid residues S70, S87 and T69 [295]. This is supported by studies in which amino acid residues S70, S87 and T69 on Bcl-2 were substituted for alanine resulting in an increased anti-apoptotic effect following

paclitaxel treatment [310]. It is proposed that phosphorylation of these sites, specifically S70 [317], inside the unstructured "loop region" (LR) of Bcl-2, acts as a checkpoint during mitosis which may induce apoptosis [310]. In this regard, Bcl-2 is often referred to as the "guardian of microtubule integrity" [77]. Interestingly, Bcl-2 has been identified as a paclitaxel-binding protein following screening of a library of phage-displayed peptides [338]. Specifically, the C-13 side chain of paclitaxel binds to the LR of Bcl-2, further implicating the role of paclitaxel-induced apoptosis through interactions and phosphorylation of Bcl-2 [339]. Clinically, breast tumors showing increased phosphorylated Bcl-2 expression have increased sensitivity to both paclitaxel and docetaxel, compared to tumors with reduced phosphorylated Bcl-2 expression [340]. Events inhibiting phosphorylation of Bcl-2 can also lead to increased resistance to MI drugs. For example, the growth factor signaling molecule insulin receptor substrate protein (IRS-1) can bind to the "loop region" (LR) of Bcl-2 [341]. This may hinder access of phosphorylation kinases to Bcl-2 and Bcl-xl molecules thereby inhibiting phosphorylation and apoptosis with implications for anti-mitotic drug resistance [341].

7.2.2. Inhibitors of apoptosis (IAPs) — Survivin/XIAP

Survivin is known experimentally to protect normal and transformed cells from apoptosis [342,343]. It has been proposed that survivin localizes to the mitochondria and is released into the cytoplasm in response to a cell-death signal, which in turn complexes with XIAP molecules inhibiting caspase-9 activity and preventing apoptosis [241]. Following paclitaxel treatment, when mitotic arrest and mitotic slippage occur, survivin is downregulated [344] and Aurora B inactivated [345], enabling apoptosis to occur in G1. Overexpression of survivin has been shown to be associated with increased resistance to paclitaxel-induced cell death [346] and inhibition of survivin by mitotic inhibitors such as oxaliplatin, increase paclitaxel-induced apoptosis and cell death in colonic carcinoma cells [347]. Improved prognosis and response to chemotherapy has also been correlated with reduced survivin expression [346,348], identifying survivin as a possible marker of chemoresistance to drugs including anti-mitotic agents.

7.2.3. NF_kB

NF_kB is a transcription factor involved in promoting cell proliferation and inhibiting apoptosis [349]. It consists of two subunits, p50 and p65, which are negatively regulated by the repressor I_kB, which binds to NF_kB in the cytoplasm [2]. When I_kB is phosphorylated by the I_kB kinase (IKK) complex, NF_kB translocates to the nucleus where it activates the transcription of numerous genes, including a number of anti-apoptotic proteins such as, c-FLIP, Bcl-2, and Bcl-xl [349]. Akt signaling regulates NF_kB, which explains its pro-survival function [349]. In vitro cell line data have shown that NF_kB is upregulated following exposure to various chemotherapeutic agents including 5-FU, doxorubicin, cisplatin and paclitaxel [350] resulting in reduced chemotherapy-induced apoptosis [351]. It has also been shown that NF_kB inhibition sensitizes cells to paclitaxel and cisplatin treatment [352,353]. A specific inhibitor of I_kB phosphorylation, BAY 11-7085, increases paclitaxel-induced apoptosis in the ovarian cell line CAOV-3 [353]. However, other studies have suggested a pro-apoptotic role for NF_kB in paclitaxel-induced apoptosis [354].

8. Clinical trials and taxane treatment — How far have we come?

8.1. Paclitaxel and docetaxel in the adjuvant/neoadjuvant setting

Chemotherapy has an important role to play in the adjuvant and neoadjuvant setting for patients with operable and metastatic breast cancer (MBC) [355]. Reductions in breast cancer mortality have been seen in both settings using anthracycline-based (doxorubicin/epirubicin) regimes in combination with cyclophosphamide, methotrexate and 5-fluorouracil (CMF) [3]. Taxanes have been incorporated into these drug regimes and have lead to the first and second generation of clinical trials evaluating their efficacy. A summary of the main clinical trials is given below.

In the phase III MD Anderson Cancer Center (MDACC) trial the adjuvant and neoadjuvant administration of FAC (5-fluorouracil, doxorubicin and cyclophosphamide) alone vs. FAC followed by paclitaxel (P) in both advanced and early-stage breast cancer was addressed [356] (Table 2). Although slight increases in both disease-free survival (DFS) and overall survival (OS) were seen in the FAC-P arms compared to FAC alone, these increases were not statistically significant. Overall survival results of this trial are pending.

The first large prospective trial to examine the addition of paclitaxel to an anthracycline-based (doxorubicin(A)/cyclophosphamide(C)) regime in node-positive women was undertaken by the Cancer and Leukemia Group B Trial (CALGB 9344) (Table 2) [8]. This study was designed to determine whether increasing the dose of doxorubicin with or without the addition of paclitaxel to a standard chemotherapy regime improved DFS or OS in a cohort of node-positive breast cancer patients. There was no improvement in either DFS or OS with increasing doxorubicin dose while statistically significant increases ($p<0.0001$) in toxicity were seen with each doxorubicin dose escalation. The addition of paclitaxel to AC significantly improved DFS 70% vs. 65% ($p=0.0023$) and OS 80% vs. 77% ($p=0.0064$). The benefits of paclitaxel addition, however, indicated efficacy only for estrogen-receptor (ER) negative patients (hazard ratio [HR] recurrence 0.68; 95% CI 0.55–0.85), with no benefits for ER-positive patients.

The Cancer and Leukemia Group B Trial (CALGB 9344) also undertook a retrospective study using tissue blocks (approximately 2800) to evaluate HER2/neu status [8]. In this study, HER2/neu was evaluated using fluorescent in-situ hybridization (FISH) and immunohistochemistry (IHC) (Herceptest and monoclonal antibody CB-11) from the same cohort of 3121 node-positive women. The blocks were divided into group 1 ($n=643$) and group 2 ($n=679$). Cox proportional hazard models and Kaplan–Meier analysis were used to compare the 5-year DFS of each group. Both groups demonstrated increased benefit in DFS in HER2/neu-positive tumors compared to HER2/neu-negative tumors, regardless of ER status [357]. This

Table 2
Randomized clinical trials of paclitaxel as adjuvant/neoadjuvant therapy

Study	Accrual	Design		Results	*P*-values	Conclusion
MDACC	350	1st arm	FAC (500/50/500 mg/m^2) every 3 wks×8 cycles	*Median follow-up 60 months*		No advantage to substituting anthracycline component
adjuvant/neoadjuvant	(adjuvant)	2nd arm	P (250 mg/m^2) every 3 wks×4 cycles	FAC vs. P→FAC		of neoadjuvant chemotherapy with paclitaxel.
clinical trial			→FAC (500/50/500 mg/m^2) every 3 wks×4 cycles	4 yr DFS; 83% vs. 86%	0.09 (ns)	Although trend toward addition of paclitaxel
Operable	174	3rd arm	P (250 mg/m^2) every 3 wks×4 cycles→locoregional surgery	cCR: 79.3% vs. 80.2%	0.85 (ns)	independent of hormonal receptor status.
breast cancer	(neoadjuvant)		→FAC (500/50/500 mg/m^2) every 3 wks×4 cycles	Overall survival is pending		
(Buzdar et al.		4th arm	FAC (500/50/500 mg/m^2) every 3 wks×4 cycles→locoregional surgery			
[356])			→FAC (500/50/500 mg/m^2) every 3 wks×4 cycles			
			Sequential protocol			
CALGB 9344	1551	1st arm	AC (60, 75 or 90/600/mg/m^2) every 3 wks×4 cycles	*Median follow-up 69 months*		Addition of paclitaxel to adjuvant anthracycline
Phase III adjuvant			→P (175 mg/m2) every 3 wks×4 cycles	DFS AC+P/AC; 70% vs. 65%	0.0023 (s)	therapy improved DFS and OS in this cohort
clinical trial			TAM given after adjuvant therapy	Reduction hazard rate of recurrence 17%		of LN + MBC patients. Toxicities minimal.
LN+ MBC	1570	2nd arm	AC (60, 75 or 90/600 mg/m^2 every 3 wks×4 cycles	OS AC+P/AC; 80% vs. 77%	0.0064 (s)	The benefits of paclitaxel addition suggested to only
(Henderson et al.			TAM given after adjuvant therapy	Reduction hazard rate of death 18%		affect ER-negative patients [HR] for reccurence 0.68
[8])			Sequential protocol	*5 yr DFS and 5 yr OS for increasing AC dose*		(95% CI 0.55–0.85) with no benefits for ER+ patients.
				Not statistically significant	(ns)	Increased hematological toxicities assoc. with increased
						doxorubicin dose.
NSABP (B-28)	3060	1st arm	AC (60/600 mg/m^2) every 3 wks×4 cycles	*Median follow-up 64.6 months*		Addition of paclitaxel to adjuvant anthracycline therapy
Phase III adjuvant			TAM given concomitantly for 5 yrs	5 yr DFS AC+P vs. AC; 76% vs. 72%	0.008 (s)	improved 5 yr DFS significantly but not 5 yr OS.
clinical trial		2nd arm	AC (60/600 mg/m2) every 3 wks×4 cycles	Reduction hazard rate of recurrence 17%		Side-effects; frequently associated with paclitaxel
≥1+LN MBC			→P (225 mg/m^2) every 3 wks×4 cycles	5 yr OS AC+P vs. AC; both 85%	0.46 (ns)	were grade 3 toxicites (% patients) — neurotoxicity 19%,
(Mamounas et al.			TAM given concomitantly for 5 yrs	Reduction hazard rate of death 7%		myalgia 11%, febrile neutropenia 2%. Frequently
[361])			Sequential protocol			associated with AC — granulocytopenia 8%, febrile
						neutropenia 7% and nausea 6%.
CALGB 9741	2005	1st arm	A (60 mg/m^2) every 3 wks×4 cycles g P (175 mg/m^2) every 3 wks×4 cycles	*Median follow-up 36 months*		Dose-dense regime with the addition of paclitaxel
Phase III adjuvant trial			→C (600 mg/m^2) every 3 wks×4 cycles	Dose-dense vs. Conventional		to anthracycline therapy improved 6 yr DFS
clinical trial		2nd arm	A (60 mg/m^2) every 2 wks×4 cycles→P (175 mg/m^2) every 2 wks×4 cycles	3 yr DFS 85% vs. 81% [RR], 0.74	0.01 (s)	significantly and subset analysis suggested more
LN+, MBC			→C (600 mg/m^2) every 2 wks×4 cycles+filgrastim (days 3–10 each cycle)	26% reduction in risk of relapse		benefit in ER− vs. ER+ patients.
(Citron et al.		3rd arm	AC (60/600 mg/m^2) every 3 wks×4 cycles→P (175 mg/m^2) every 3 wks×4	3 yr OS 92% vs. 90% [RR], 0.69	0.014 (s)	Side-effects — ↓grade 4 neutropenia occurred in
[363])		4th arm	AC (60/600 mg/m^2) every 2 wks×4 cycles→P (175 mg/m^2) every 2 wks×4	31% reduction in risk of death		dose-dense vs. conventional regime and ↑grade 2
			+filgrastim (days 3–10 each cycle)	Subset analysis ER− vs. ER+		anemia in dose-dense regime.
			Sequential/concurrent protocol	[HR]: 0.76 no significance for ER+ patients	0.014 (s)	

Abbreviations: MDDACC = M.D. Anderson Cancer Center; CALGB = Cancer and Leukaemia Group B; NASBP = National Surgical Adjuvant Breast and Bowel Project; LN = lymph node; MBC = Metastatic breast Cancer; FAC = 5-fluorouracil/doxorubicin/cyclophosphamide; Wks = weeks; P = paclitaxel; TAM = tamoxifen; DFS = disease-free survival; OS = overall survival; RR = risk ratio; HR = hazard ratio; ER = estrogen receptor; CI = confidence interval; AC = doxorubicin/cyclophosphamide.

finding suggests that stratification of patients based on HER2 status, and not ER status, as found in the first part of this trial, may be more informative when deciding on treatment regimes [357]. Further investigation into molecular subtype stratification is warranted [358–360].

The National Surgical Adjuvant Breast and Bowel Project (NSABP) B28 trial was undertaken to determine whether the addition of paclitaxel to an anthracycline-based regime improved overall survival in lymph node-positive metastatic breast cancer patients (Table 2) [361]. Although the addition of paclitaxel to adjuvant anthracycline therapy in this trial improved 5-year DFS regardless of tumor grade, histological type, age of patient or number of positive lymph nodes, there was no improvement in 5-year OS.

While the NSABP B28 and the CALGP 9344 trials both demonstrated improvement in DFS with addition of paclitaxel to adjuvant anthracycline regimes, the CALGP 9344 was the only trial to demonstrate improvement in OS. The discordant outcomes found in these trials may be explained by differences in drug dosage and patient selection. Paclitaxel doses differed between the trials with a higher dose being used in the NSABP B28 trial (225 mg/m^2 vs. 175 mg/m^2). In addition, increasing doses of doxorubicin were used in the CALGB trial (60, 75 and 90 mg/m^2) compared to a consistent dose of anthracycline being used in the NSABP B28 trial (60 mg/m^2). Moreover, ER-positive patients in the NSABP B28 trial were given tamoxifen concomitantly with chemotherapy as opposed to sequentially in the CALGB study. Tamoxifen administered concurrently with adjuvant anthracycline-based chemotherapy has been shown to cause impaired disease-free survival when compared with delaying tamoxifen administration until chemotherapy is completed [362].

In the Cancer and Leukemia Group B trial 9741 trial, doxorubicin (A), cyclophosphamide (C) and paclitaxel (P) administration was compared in sequential (A→P→C) versus concurrent (AC→P) regimes in the setting of either conventional three-weekly administration or dose-dense two-weekly administration. 2005 women with node-positive metastatic breast cancer were randomly assigned to one of four treatment arms illustrated in Table 2 [363].

Statistically significant improvements were seen in DFS, OS, relapse risk and mortality risk with dose-dense scheduling. The dose-dense regime was also associated with a reduced occurrence of contralateral breast cancer 0.3 vs. 1.5% ($p=0.0004$) [364]. There was no significant difference in DFS or OS between concurrent and sequential chemotherapy (Hazard Ratio HR=1.04; $p=0.65$). It is worth considering however, that the dose-dense arm was associated with increased grade 2 anemias. In addition filgrastim (growth factor support) was required to reduce hematological toxicities associated with dose-dense treatment [365].

In conclusion, improved outcomes have been associated with the addition of taxanes (paclitaxel or docetaxel) to the standard chemotherapy regimes in a number of phase III randomized clinical trials. Results from the CALGB 9344 [8] and NSABP B28 [361] trials have shown statistical significance with the addition of paclitaxel adjuvantly to AC regimes in node-positive metastatic breast cancers in terms of DFS and OS in the CALGB trial and DFS for the NSABP B28 trial. Moreover, a combined analysis of the 20-year experience with the CALGB trial found benefits for node-positive ER-negative breast cancer patients as a result of improved chemotherapy regimes over the past two decades [366]. The MD Anderson Cancer Center (MDACC) trial showed a trend toward improved relapse-free survival with the addition of paclitaxel, however statistical significance was not achieved as the sample size was small [356]. The CALGB 9741 trial [363] found that the addition of paclitaxel to an AC regime in a dose-dense manner (administered every two-weeks instead of every three-weeks) improved outcome compared to conventional scheduling. In addition, neoadjuvantly the pathological complete response (pCR) rates were better for those who received weekly vs. three-weekly treatment with paclitaxel [16]. This suggests that dose-dense regimes may be more efficacious when administering paclitaxel to breast cancer patients. However, whether this approach will improve overall survival warrants further investigation.

The BCIRG 001 (Breast Cancer International Research Group) (Table 3) [19] and the Programme Adjuvant Cancer du Seins (PACS) 01 (Table 3) [21] were important in providing information regarding the benefits of incorporating adjuvant docetaxel (T) either sequentially or concomitantly in operable node-positive breast cancer patients. In the BCIRG 001 women with axillary node-positive breast cancer were randomized into one of two treatment arms (TAC vs. FAC) illustrated in Table 3. Tamoxifen was given for 5 years to all hormone-receptor positive tumors after completion of adjuvant chemotherapy. A significant reduction in 5-year DFS and OS was seen with TAC compared with FAC in patients with 1–3 positive lymph nodes only. This study demonstrated the benefit of the addition of docetaxel irrespective of HER2-neu status or ER status and supports the value of incorporating taxanes into the adjuvant treatment of operable node-positive breast cancers.

In the PACS 01 trial [21] 1999 patients with operable node-positive breast cancers were randomized to receive either 6 cycles of FEC100 (5-fluorouracil, epidoxorubicin and cyclophosphamide) or FEC100 for 3 cycles followed by 3 cycles of docetaxel (Table 3). A significant reduction in 5-year DFS and OS was demonstrated for FEC100 followed with docetaxel compared to FEC alone.

The NSABP B-27 trial was designed to determine the effects of the addition of docetaxel after 4 cycles of neoadjuvant AC (doxorubicin/cyclophosphamide) on the pathological and clinical response rates (pCR and cCR) and on DFS and OS of women with operable breast cancer (Table 3) [367]. This trial randomized 2411 women with stage I and II breast cancer into one of three treatment arms as illustrated. Women receiving the sequential AC/docetaxel neoadjuvant regime (2nd arm) showed statistically significant improvements in overall clinical response rate (ORR), pCR and cCR rates ($p<0.001$). Lymph node down staging was observed with the addition of preoperative docetaxel ($p<0.001$). However, there was no significant difference in disease-free survival (DFS) or overall survival (OS) between each of the three groups. The design of this trial has been criticized because the preoperative regimes were of different durations (4 vs. 8 cycles of treatment)

Table 3
Randomized clinical trials of docetaxel as adjuvant/neoadjuvant therapy

Study	Accrual	Design		Results	*P*-values	Conclusion
BCIRG 001	745	1st arm	TAC (75/50/500 mg/m^2) every 3 wks × 6 cycles	*Median follow-up 55 months*		Significant reduction in 5 yr DFS and OS for
Randomized adjuvant			(TAM after chemotherapy for 5 yrs)	TAC vs. FAC		TAC vs. FAC. Although, febrile neutropenia was
Phase III clinical trial.	746	2nd arm	FAC (500/50/500 mg/m^2) every 3 wks × 6 cycles	5 yr DFS 75% vs. 68%;	0.001 (s)	more assoc. with TAC; 25% vs. 2.5%, $p \leq 0.05$,
Operable LN+ breast			(TAM after chemotherapy for 5 yrs)	28% reduction in risk of relapse		G-CSF should be administered for TAC.
cancer (1997–1999)			Concomitant regime	5 yr OS 87% vs. 81%;	0.008 (s)	
(Martin and Pienkowski [19])				30% reduction in risk of death		
				Subset analysis; 1–3 LN+ HR, 0.61	<0.001 (s)	
PACS (01)	1999	1st arm	FEC 100 (500/100/500 mg/m^2) every 3 wks × 6 cycles	*Median follow-up 59.7 months*		Significant reduction in 5 yr DFS and OS for FEC100 → T
Adjuvant phase III		2nd arm	FEC 100 (500/100/500 mg/m^2) every 3 wks × 3 cycles	FEC100 → T vs. FEC100		vs. FEC100 alone. Toxicity profile for both regimes were
Clinical trial.			→ docetaxel (100 mg/m^2) × 3 cycles	5 yr DFS 78.3% vs. 73.2%; [HR], 0.83	0.041 (s)	acceptable. Subset analysis found that patients aged
Operable LN+ breast			Sequential regime	5 yr OS 90.7% vs. 86.7%; [HR], 0.77	0.05 (s)	≥ 50 years and patients with 1–3+ LNs benefited more
cancer (1997–2000)						from the addition of docetaxel to FEC100. Therefore
(Roche et al. [21])						FEC100 should be replaced with FEC100→T for adjuvant
						treatment of node-positive breast cancer.
NSABP B-27	804	1st arm	AC (60/600 mg/m^2) every 3 wks × 4 cycles → surgery (TAM given concomitantly)	*Clinical and pathological response rates*		Patients with operable breast cancer that received
Randomized phase III	805	2nd arm	AC (60/600 mg/m^2) every 3 wks × 4 → T (100 mg/m^2) every 3 wks × 4 cycles	AC → T → surgery vs. AC → surgery/T		preoperative AC → T achieved higher cCR, pCR
Neoadjuvant clinical			→ surgery (TAM for 5 yrs)	cCR: 63% vs. 40.1%	<0.001 (s)	and ORR compared to patients that received
Trial (1995–2000)	802	3rd arm	AC (60/600 mg/m^2) every 3 wks × 4 cycles → surgery	ORR: 90.7% vs. 85.5%	<0.001 (s)	preoperative AC alone. No significant difference in DFS
Operable breast cancer			→ T (100 mg/m^2) every 3 wks × 4 cycles (TAM given concomitantly for 5 yrs)	pCR: 26.1% vs. 13.7%	<0.001 (s)	and OS between 3 groups was found. Best to give all
(Bear and Anderson [367])			Sequential regime			chemotherapy uninterrupted to maintain dose density.
				AC → surgery → T vs. AC → surgery		Increased grade 4 toxicity assoc. with docetaxel arm
				DFS HR; 0.91	0.32 (ns)	(23.4%) compared to AC alone (10.3%) with febrile
						neutropenia being main side-effect.
University of Aberdeen	162	1st arm	CVAP (100/50/1.5/100 mg/m^2) every 3 wks × 8 cycles → surgery	*Median follow-up 65 months*		Addition of a taxane to anthracycline chemotherapy
Phase II neoadjuvant		2nd arm	CVAP (100/50/1.5/100 mg/m^2) every 3 wks × 4 cycles	CVAP → T → surgery vs. CVAP → surgery		neoadjuvantly was more beneficial than anthracycline
Clinical trial.			→ T (100 mg/m^2) every 3 wks × 4 cycles → surgery	ORR 94% vs. 66%	0.001 (s)	therapy alone in this cohort of locally advanced breast
Locally advanced			Sequential regime	pCR 34% vs. 16%	0.04 (s)	cancer patients.
breast cancer						
(Smith and Heys [368])						

Abbreviations: BCIRG = Breast Cancer International Research Group; PACS = Programme Adjuvant Cancer du Seins; TAC = docetaxel/doxorubicin/cyclophosphamide; FAC = 5-fluorouracil/doxorubicin/cyclophosphamide; FEC100 = 5-fluorouracil/epidoxorubicin/cyclophosphamide; T = docetaxel; TAM = tamoxifen; CVAP = cyclophosphamide/vincristine/doxorubicin/prednisolone; DFS = disease-free survival; OS = overall survival; AC = doxorubicin/cyclophosphamide; LN = lymph node; HR = hazard ratio; cCR = clinical complete response; ORR = overall clinical response rate; pCR = pathologic complete response; assoc = associated.

Table 4
Randomized clinical trials of trastuzumab in combination with paclitaxel/docetaxel as adjuvant therapy in patients with early-stage breast cancer

Study	Accrual	Design		Results	*P*-values	Conclusion
NCCTG N9831	807	1st arm	AC (60/600 mg/m^2) every 3 wks×4 cycles→P (80 mg/m^2/week) for 12 wks	Interim data from combined NCCTG N9831		From the combined results there was a statistically significant difference in groups where trastuzumab was added to paclitaxel with a reduction of recurrence risk of 52% in the paclitaxel and trastuzumab arms. The number of deaths were significantly reduced when trastuzumab was combined with paclitaxel with a 33% relative reduction in deaths after 3 years. After 4 years 90% of trastuzumab recipients had no recurrences compared to 74% of the paclitaxel only groups. These trials demonstrate the benefits of adding trastuzumab to a paclitaxel and anthracycline-based regime in patients with HER2-overexpressing tumors.
intergroup trial		2nd arm	AC (60/600 mg/m^2) every 3 wks×4 cycles→P (80 mg/m^2/week) for 12 wks	*(1st and 3rd arms) and NSABP B-31(Both arms)*		
Operable breast cancer			→tras (2 mg/kg/week)×52 wks	3 yr DFS P+ tras vs. P alone		
Node-positive	808	3rd arm	AC (60/600 mg/m^2) every 3 wks×4 cycles→P (80 mg/m^2/week) and	87% vs. 75% (HR=0.48)	$p<0.0001$	
HER2-overexpressing			tras (2 mg/kg/week) concurrently for 12 wks	~52% reduction in risk of recurrence		
3+ IHC or FISH positive			→tras (2 mg/kg/week) for 40 wks	3 yr OS P+ tras vs. P alone		
(Romond et al. [371])			All patients received TAM for 5 yrs after adjuvant chemotherapy	62 vs. 92 deaths (HR=0.67)	$p=0.015$	
				~33% relative reduction in number of deaths		
NSABP B-31 trial	872	1st arm	AC (60/600 mg/m^2) every 3 wks×4 cycles→P (175 mg/m^2/week) every 3 wks			
Operable breast cancer			x4 cycles			
Node-positive	864	2nd arm	AC (60/600 mg/m^2) every 3 wks×4 cycle→P (175 mg/m^2/week) every 3 wks			
HER2-overexpressing			x4 cycles+tras (2 mg/kg/week) concurrently for 12 wks			
3+ IHC or FISH positive			→tras (2 mg/kg/week) for 40 wks			
chemotherapy naive						
(Romond et al. [371])						
BCIRG 006	1073	1st arm	AC (60/600 mg/m^2) every 3 wks×4 cycles→T (100 mg/m^2)every 3 wks×4 cycles	*2nd treatment arm vs. 1st treatment arm*		This trial found significant reduction in risk of recurrence (51%) in the 2nd treatment arm with docetaxel/ trastuzumab compared to the 1st arm (AC→T). There was also a significant reduction in risk of recurrence (39%) in the 3rd arm with docetaxel/ trastuzumab and carboplatin compared to control arm (AC→T). These results are highly significant and demonstrate the synergistic effects of docetaxel and trastuzumab in the treatment of patients with HER2-overexpressing tumors.
Node-positive and	1074	2nd arm	AC (60/600 mg/m^2) every 3 wks×4 cycles→T (100 mg/m^2) every 3 wks×4 cycles	51% reduction in risk of recurrence(HR=0.49)	$p<0.0001$	
high risk node-negative			+Tras (2 mg/kg/week)×12 cycles→Tras every 3 wks×13 cycles	*3rd treatment arm vs. 1st treatment arm*		
chemotherapy naive	1075	3rd arm	T (100 mg/m^2)+ Carbo (AUC=6)+tras (2 mg/m^2/week)×18 wks	39% reduction in risk of recurrence (HR=0.61)	$p=0.00015$	
(Slamon et al. [372])			→tras (2 mg/kg) every 3 wks×11 wks			

Abbreviations: NCCTG = North Central Cancer Treatment Group; NSABP = National Surgical Adjuvant Breast and Bowel Project; BCIRG = Breast Cancer International Research Group; AC = doxorubicin/cyclophosphamide; wks = weeks; tras = trastuzumab; TAM = tamoxifen; P = paclitaxel; T = docetaxel; 3 yr = 3-year; DFS = disease-free survival; HR = hazard ratio; Carbo = carboplatin; AUC = area under the concentration-time curve; OS = overall survival.

suggesting that benefits found in the neoadjuvant AC/docetaxel arm may have been due to the addition of extra cycles of treatment, rather than as a result of the addition of docetaxel. In summary, this study demonstrates no overall survival benefit with taxane addition in the neoadjuvant setting.

Conversely, the University of Aberdeen trial, comparing the addition of 8 cycles of neoadjuvant CVAP (cyclophosphamide/vincristine/doxorubicin/prednisolone) to 4 cycles of CVAP, followed by 4 cycles of docetaxel prior to surgery, has suggested that the addition of the taxane is beneficial (Table 3) [368]. The pCR rate, DFS and OS showed significant results with the addition of docetaxel. Furthermore two patients who received 8 cycles of CVAP alone developed progressive disease after responding to the first 4 cycles. This was not observed in the docetaxel-treated arm [368]. This study has generated the hope of a new "standard" neoadjuvant therapy [369] in the neoadjuvant setting.

8.2. Paclitaxel and docetaxel/trastuzumab in the adjuvant/neoadjuvant setting

Trastuzumab was approved by the FDA in 1998 for use in combination with either paclitaxel (Europe and USA) or docetaxel (Europe) for HER2/neu-positive patients with metastatic breast cancer not previously treated with chemotherapy [169]. Randomized clinical trials incorporating trastuzumab plus chemotherapy in the treatment of HER2/neu-positive operable and metastatic breast cancers have demonstrated improved OS, increased response rates and longer time to disease progression (TTP) compared to chemotherapy alone [181,370,371]. However, a major side-effect of this drug is ventricular dysfunction and New York Heart Association (NYHA) classes II–IV congestive heart failure (CHF) [169]. Interim analyses from four phase III trials in patients with operable HER2/neu-positive breast cancer have demonstrated that the addition of trastuzumab to adjuvant chemotherapy significantly improves outcomes in patients compared to those who received adjuvant chemotherapy alone [169]. These trials which include the combined analyses of the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-31 study and North Central Cancer Treatment Group (NCCTG) N9831 trial (Table 4) [371] and preliminary data from the Breast Cancer International Research Group (BCIRG 006) study are outlined in detail (Table 4) [372].

Data from clinical trials incorporating trastuzumab with either paclitaxel or docetaxel adjuvantly or neoadjuvantly in operable or MBC have confirmed laboratory findings that there is increased synergy between taxanes and trastuzumab when combined together [169,371,372]. Such large benefits have not been seen in adjuvant and neoadjuvant trials before. Thus, it is evident that HER2/neu overexpressing breast cancers benefit from a combination of taxanes and anthracycline-based chemotherapy with trastuzumab, which should now become the gold standard for treatment of early-stage and metastatic HER2/neu-positive breast cancers.

The 9th St Gallen consensus meeting (January 2005) categorized early-stage breast cancer into three groups: endocrine responsive, endocrine non-responsive and tumors of uncertain endocrine responsiveness. These categories were further subdivided according to their menopausal status. Subsequently the panel stratified patients into low-, intermediate- and high-risk groups [373]. The panelists suggested that combined anthracycline and taxane treatment was appropriate for patients with intermediate- or high-risk status with endocrine non-responsive disease whereas AC × 4 cycles alone was considered appropriate for high-risk endocrine responsive disease. These suggestions indicate that taxanes are more beneficial for estrogen-receptor negative patients as evidenced in the CALGB 9344 and CALGB 9741 trials. There are several on-going trials examining the potential benefit of adding taxanes to an anthracycline-based regime [3]. Two of the largest trials to date include the Canadian MA21 trial and the UK Taxotere as Adjuvant Chemotherapy trial (UK TACT). These trials will compare combinations of taxanes and anthracycline-based regimes with anthracycline only regimes using the same number of treatment cycles and stratifying patients according to lymph node status. Clearly there is a need to further define subgroups of breast cancer patients who will benefit specifically from the addition of taxanes to treatment protocols. Further randomized adjuvant and neoadjuvant trials with longer follow-up times will be essential to answer the many questions regarding the appropriate addition of taxanes to treatment regimes with optimal scheduling, choice of taxane and dosing regimes still unclear at this stage. A full consensus report on the use of taxanes in both early-stage and metastatic breast cancer is awaited.

9. Conclusions and perspectives

Intrinsic and acquired resistance to many chemotherapeutic agents including doxorubicin, taxanes and vinca alkaloids can be a result of alterations in drug efflux by proteins of the ABC-transporter family, leading to the multidrug resistance (MDR) phenotype [263]. With this phenotype, resistance to one drug can result in cross-resistance to other structurally unrelated drugs [105]. Moreover, altered expression of the efflux transporters, MDR1/P-glycoprotein (P-gp) and multiresistance-associated proteins (MRP) has been shown both in vitro and in vivo to cause intrinsic or acquired resistance to commonly used cytotoxic drugs, such as the anthracyclines, taxanes, anti-metabolites and platinum agents, all of which are substrates of either the MDR1/P-gp or MRP transporters [162,263,374]. Therefore, the MDR phenotype and overexpression of efflux transporters contribute greatly in conferring cellular resistance to many chemotherapies, including taxanes.

Altered expression and activity of cell cycle/apoptotic signaling regulators, such as p53, BRCA1 and Bcl-2 have also been associated with increased resistance to different chemotherapeutic agents including DNA-damaging drugs, platinum agents and taxanes [263,287,288,375,376]. Firstly, the lack of functional p53 both in vitro and in vivo contributes to increased resistance to doxorubicin, cisplatin, 5-fluorouracil (5-FU) and taxanes, due to abrogation of p53-mediated apoptosis [263]. Conversely however, other studies have found no correlation between p53 status and chemoresistance [292,377,378]. Interestingly, the cell cycle protein BRCA1 is activated in response to

both DNA-damaging agents and microtubule disruption and has been shown in vitro to be a predictive marker of response to both DNA-damaging agents and taxanes [264,379]. Moreover BRCA1 enhances sensitivity to apoptosis induced by paclitaxel and inhibits apoptosis induced by cisplatin [272]. Finally, BRCA1 is a transcriptional regulator of the spindle checkpoint proteins BUBR1 and MAD2, with possible implications for spindle checkpoint control [275,276]. Therefore, BRCA1 may be an important predictive marker of chemotherapy response to DNA-damaging agents and taxanes.

With regard to the anti-apoptotic protein Bcl-2, and its ability to predict chemoresponsiveness, data are inconclusive. Some studies support the hypothesis that Bcl-2 overexpression correlates with poor response to varying chemotherapies [378,380], while other studies have shown no association between Bcl-2 expression and drug response [263,381]. Increased levels of phosphorylated Bcl-2 have however been associated with increased sensitivity to both paclitaxel and docetaxel compared to breast tumors with reduced phosphorylated Bcl-2 expression levels [340].

Hyperactivity of the pro-signaling PI3/Akt pathway has been shown in vitro to result in paclitaxel, trastuzumab and gemcitabine chemoresistance [263]. In several ovarian cancer cell lines, increased activation of AKT conferred resistance to paclitaxel [326]. Moreover, a frequently mutated tumor suppressor gene associated with the PI3K/Akt pathway, PTEN, acts as a negative regulator of PI3K/Akt signaling [322]. In one study of HER-2 overexpressing tumors, immunohistochemical PTEN overexpression was found to correlate with increased sensitivity to trastuzumab [334]. In addition, reduced PTEN levels and increased Akt activation are associated with the trastuzumab-resistant breast cancer cell line SKBR3/R [334].

Resistance to taxanes specifically, is multifaceted, with defects in tubulin, microtubule-associated proteins (MAPs) and dysregulated cell cycle and apoptotic signaling pathways playing candidate roles in taxane resistance. To date, in vivo analysis of both tubulin mutations and isotype composition have yielded no substantial evidence identifying tubulin defects as a major contributing factor underlying taxane resistance [100,117]. On the other hand, in vitro and in vivo overexpression of the MAPs, tau and stathmin (destabilizing protein) have been shown to correlate with paclitaxel resistance [125,131]. These regulators may be a useful adjunct in predicting taxane response, with further studies warranted. More importantly, defects in the spindle assembly checkpoint (SAC), which is activated in response to taxanes, have lead to a number of promising predictive markers for taxane resistance including MAD2, BUBR1, Aurora A and Synuclein-gamma among others.

Since a robust spindle checkpoint is crucial to appropriate microtubule functioning during mitosis, it is fitting that a deficient spindle checkpoint would be detrimental to the efficacy of agents such as taxanes that function primarily by stabilizing microtubules in mitosis. Furthermore, the spindle checkpoint is important in maintaining chromosomal stability, with abnormalities in the SAC being associated with increased chromosomal instability (CIN), mitotic slippage and taxane resistance [189,200,210,231]. Therefore, establishing whether the spindle checkpoint is functioning by assessing key spindle checkpoint components in combination with specific MAP expression levels may identify taxane responders from non-responders. Furthermore, it will be clinically important to incorporate immunohistochemical analysis of these proteins into clinical trials in an effort to optimize individualized drug therapy.

In the clinical setting, targeting different oncogenic signaling pathways with combinations of chemotherapeutic agents is generally more effective than single-agent therapies. Data from several large breast cancer clinical trials suggest that the addition of taxanes to anthracycline-based combinatorial regimes is beneficial in certain subgroups of patients [8,357,366]. These clinical findings have been validated using gene expression profiling in which basal-like estrogen-receptor (ER)-negative and HER2-positive breast cancer molecular subtypes demonstrated increased sensitivity to a combination of paclitaxel and doxorubicin chemotherapy compared to the luminal (ER-positive) and "normal-like" subgroups [382]. Moreover, combining taxanes with targeted therapies, such as trastuzumab, have been found to dramatically improve overall survival (OS) and disease-free survival (DFS) in breast cancer patients, suggesting a possible synergistic effect between these agents [169]. Novel taxanes have been developed to overcome the "resistant phenotype" in taxane-resistant cell lines [383]. The orally active docetaxel analogue MST-997 has potent in vitro and in vivo efficacy in paclitaxel and docetaxel-resistant models and has now entered phase I clinical trials [383]. Furthermore, novel taxanes can inhibit multidrug transporters, overcoming the multidrug resistance phenotype, with a number of these agents currently undergoing clinical trials [384]. Finally, epothilones are taxol-like microtubule-stabilizing agents that appear to be responsive in taxane-resistant models [385]. For example, epothilone B (ixabepilone) has been shown in phase II clinical trials to induce responses in taxane-refractory breast cancer [385].

Resistance to chemotherapeutic agents is a very challenging and complex phenomenon, orchestrated by a number of complex mechanisms in a single cell. Our increased understanding of the molecular pathways and mechanisms contributing to drug resistance will enable the development of more patient-tailored therapies. Combining clinical trials data with gene expression microarrays and predictive markers, will make it possible to classify breast cancers more accurately and help define patient response to chemotherapeutic regimes, with a hope to improving overall survival and disease-free survival for cancer patients.

Acknowledgements

We would like to thank Dr. K. Downing, Dr. G. Kops, Dr. M.A. Jordan, Dr. J.P. Snyder, Dr. F. Mollinedo and Dr. F.A. Fitzpatrick for their permission to adapt and use figures in this review. Special thanks to Robert McGrogan and graphics artist Ms. Sharon Phelan.

References

[1] L. O'Driscoll, M. Clynes, Biomarkers and multiple drug resistance in breast cancer, Curr. Cancer Drug Targets 6 (2006) 365–384.

[2] D.T. Bergstralh, J.P.-Y. Ting, Microtubule stabilizing agents: their molecular signaling consequences and the potential for enhancement by drug combination, Cancer Treat. Rev. 32 (2006) 166–179.
[3] E. Ring, Taxanes in the treatment of early breast cancer, Cancer Treat. Rev. 31 (2005) 618–627.
[4] R. Paridaens, L. Biganzoli, P. Bruning, J.G.M. Klijn, T. Gamucci, S. Houston, R. Coleman, J. Schachter, A. Van Vreckem, R. Sylvester, A. Awada, J. Wildiers, M. Piccart, Paclitaxel versus doxorubicin as first-line single-agent chemotherapy for metastatic breast cancer: a European Organization for Research and Treatment of Cancer Randomized Study with cross-over, J. Clin. Oncol. 18 (2000) 724–733.
[5] M. Tubiana-Hulin, How to maximize the efficacy of taxanes in breast cancer, Cancer Treat. Rev. 31 (2005) S3–S9.
[6] B. Fisher, C. Redmond, D. Wickerham, D. Bowman, H. Schipper, N. Wolmark, R. Sass, E. Fisher, P. Jochimsen, S. Legault-Poisson, Doxorubicin-containing regimens for the treatment of stage II breast cancer: the National Surgical Adjuvant Breast and Bowel Project experience, J. Clin. Oncol. 7 (1989) 572–582.
[7] J.M. Nabholtz, T. Pienkowski, Phase III trial comparing TAC (docetaxel, doxorubicin, cyclophosphamide) with FAC (5-fluorouracil, doxorubicin, cyclophosphamide) in the adjuvant treatment of node positive breast cancer (BC) patients: interim analysis of the BCIRG 001 study, Proc. Am. Soc. Clin. Oncol. 21 (2002) Abstract 36.
[8] I.C. Henderson, D.A. Berry, G.D. Demetri, C.T. Cirrincione, L.J. Goldstein, S. Martino, J.N. Ingle, M.R. Cooper, D.F. Hayes, K.H. Tkaczuk, G. Fleming, J.F. Holland, D.B. Duggan, J.T. Carpenter, E. Frei III, R.L. Schilsky, W.C. Wood, H.B. Muss, L. Norton, Improved outcomes from adding sequential paclitaxel but not from escalating doxorubicin dose in an adjuvant chemotherapy regimen for patients with node-positive primary breast cancer, J. Clin. Oncol. 21 (2003) 976–983.
[9] M.A. Jordan, Mechanism of action of antitumour drugs that interact with microtubules and tubulin, Curr. Med. Chem. 2 (2002) 1–17.
[10] F.A. Fitzpatrick, R. Wheeler, The immunopharmacology of paclitaxel (Taxol(R)), docetaxel (Taxotere(R)), and related agents, Int. Immunopharmacol. 3 (2003) 1699–1714.
[11] F. Gueritte, General and recent aspects of the chemistry and structure-activity relationships of taxoids, Curr. Pharmacol. Des. 7 (2001) 1229–1249.
[12] V. Roy, E.A. Perez, New therapies in the treatment of breast cancer, Semin. Oncol. 33 (2006) S3–S8.
[13] J. Gligorov, J.P. Lotz, Preclinical pharmacology of the taxanes: implications of the differences, Oncologist 9 (2004) 3–8.
[14] W.J. Gradishar, S. Tjulandin, N. Davidson, H. Shaw, N. Desai, P. Bhar, M. Hawkins, J. O' Shaughnessy, Phase III trial of nanoparticle albumin-bound paclitaxel compared with polyethylated castor oil-based paclitaxel in women with breast cancer, J. Clin. Oncol. 23 (2005) 7794–7803.
[15] J.J. Lee, S.M. Swain, Development of novel chemotherapeutic agents to evade the mechanisms of multidrug resistance (MDR), Sem. Oncol. 32 (2005) S22–S26.
[16] M.C. Green, A.U. Buzdar, T. Smith, N.K. Ibrahim, V. Valero, M.F. Rosales, M. Cristofanilli, D.J. Booser, L. Pusztai, E. Rivera, R.L. Theriault, C. Carter, D. Frye, K.K. Hunt, W.F. Symmans, E.A. Strom, A.A. Sahin, W. Sikov, G.N. Hortobagyi, Weekly paclitaxel improves pathologic complete remission in operable breast cancer when compared with paclitaxel once every 3 weeks, J. Clin. Oncol. 23 (2005) 5983–5992.
[17] A.D. Seidman, D. Berry, C. Cirrincione, L. Harris, L. Dressler, H. Muss, L. Norton, E. Winer, C. Hudis, CALGB 9840:phase II study of weekly (W) paclitaxel (P) via 1-hour (h) infusion versus standard (S) 3 (h) infusion every third week in the treatment of metastatic breast cancer (MBC), with trastuzumab (T) for HER2 positive MBC and randomized for T in HER2 normal MBC, Proc. Am. Soc. Clin. Oncol. 23 (2004) Abstract 512.
[18] I. Ringel, S.B. Horwitz, Studies with RP 56976 (Taxotere). A new semisynthetic analogue of taxol, J. Natl. Cancer Inst. 83 (1989) 288–291.
[19] M. Martin, T. Pienkowski, Adjuvant docetaxel for node-positive breast cancer, N. Engl. J. Med. 352 (2005) 2302–2313.
[20] J.R. Mackey, K.S. Tonkin, S.L. Koski, A.G. Scarfe, M.G. Smylie, A.A. Joy, H.J. Au, D.M. Bodnar, D. Soulieres, S.W. Smith, Final results of phase II clinical trial weekly docetaxel in combination with capecitabine in anthracycline-pretreated metastatic breast cancer, Clin. Breast Cancer 5 (2004) 287–292.
[21] H. Roche, P. Fumoleau, M. Spielmann, J.-L. Canon, T. Delozier, D. Serin, M. Symann, P. Kerbrat, P. Soulie, F. Eichler, P. Viens, A. Monnier, A. Vindevoghel, M. Campone, M.-J. Goudier, J. Bonneterre, J.-M. Ferrero, A.-L. Martin, J. Geneve, B. Asselain, Sequential adjuvant epirubicin-based and docetaxel chemotherapy for node-positive breast cancer patients: the FNCLCC PACS 01 trial, J. Clin. Oncol. 24 (2006) 5664–5671.
[22] J. Zhou, P. Giannakakou, Targeting microtubules for cancer chemotherapy, Curr. Med. Chem. Anticancer Agents 5 (2005) 65–71.
[23] I.S. Johnson, H.F. Wright, G.H. Svoboda, J. Vlantis, Antitumor principles derived from Vinca rosea Linn: I. Vincaleukoblastine and leurosine, Cancer Res 20 (1960) 1016–1022.
[24] M.A. Jordan, L. Wilson, Microtubules as a target for anticancer drugs, Nat. Rev. Cancer 4 (2004) 253–265.
[25] R. Bartsch, C. Wenzel, G. Altorjai, U. Pluschnig, T. Bachleitner-Hoffmann, G. Locker, M. Rudas, R. Mader, C. Zielinski, G. Steger, Results from an observational trial with oral vinorelbine and trastuzumab in advanced breast cancer, Breast Cancer Res.Treat. 102 (2007) 375–381.
[26] L.W. Chow, A.Y. Yip, B.H. Lang, A phase II trial of vinorelbine and pegylated liposomal doxorubicin in patients with pretreated metastatic breast cancer, Am. J. Clin. Oncol. 30 (2007) 133–138.
[27] J. Bennouna, J.L. Breton, J.M. Tourani, C. Ottensmeier, M. O'Brien, P. Kosmidis, T.E. Huat, M.C. Pinel, C. Colin, J.Y. Douillard, Vinflunine — an active chemotherapy for treatment of advanced non-small-cell lung carcinoma previously treated with a platinum-based regime: results of phase II study, Br. J. Cancer 94 (2006) 1383–1388.
[28] A. Duflos, A. Kruczynski, J.M. Barret, Novel aspects of natural and modified vinca alkaloids, Curr. Med. Chem. Anticancer Agents 2 (2002) 55–70.
[29] C.E. Gidding, S.J. Kellie, W.A. Kamps, S.S. De Graaf, Vincristine revisited, Crit. Rev. Oncol./Haematol. 29 (1999) 267–287.
[30] K.-H. Altmann, M. Wartmann, T. O'Reilly, Epothilones and related structures — a new class of microtubule inhibitors with potent in vivo antitumour activity, Biochim. Biophys. Acta 1470 (2000) M79–M91.
[31] K.H. Downing, E. Nogales, Crystallographic structure of tubulin: implications for dynamic and drug binding, Cell Struct. Funct. 24 (1999) 269–275.
[32] G. Orr, P. Verdier-Pinard, Mechanisms of taxol resistance related to microtubules, Oncogene 22 (2003) 7280–7295.
[33] D.T. Miyamoto, Z.E. Perlman, T.J. Mitchison, M. Shirasu-Hiza, Dynamics of the mitotic spindle — potential therapeutic targets, Prog. Cell Cycle. Res. 5 (2003) 349–360.
[34] L.G. Tilney, J. Bryan, D.J. Bush, K. Fujiwara, M.S. Mooseker, D.B. Murphy, D.H. Snyder, Microtubules: evidence of 13 protofilaments, J. Cell Biol. 59 (1973) 267–275.
[35] R. Walker, E. O' Brien, N. Pryer, M. Soboeiro, W. Voter, H. Erickson, E. Salmon, Dynamic instability of individual microtubules analyzed by video light microscopy: rate constants and transition frequencies, J. Cell Biol. 107 (1988) 1437–1448.
[36] E.A. Nigg, Origins and consequences of centrosome aberrations in human cancers, Int. J. Cancer 119 (2006) 2717–2723.
[37] B.R. Oakley, An abundance of tubulins, Trends Cell Biol. 10 (2000) 537–542.
[38] S.D. Fuller, B.E. Gowen, S. Reinsch, A. Sawyer, B. Buendia, R. Wepf, E. Karsenti, The core of the mammalian centriole contains gamma-tubulin, Curr. Biol. 5 (1995) 1384–1393.
[39] S.L. Warner, P.J. Gray, D.D. Von Hoff, Tubulin-associated drug targets: Aurora kinases, Polo-like kinases, and others, Sem. Oncol. 33 (2006) 436–448.
[40] S. Honore, E. Pasquier, D. Braguer, Understanding microtubule dynamics for improved cancer therapy, Cell. Mol. Life Sci. 62 (2005) 3039–3056.
[41] F. Mollinedo, C. Gajate, Microtubules, microtubule-interfering agents and apoptosis, Apoptosis 8 (2003) 413–450.
[42] E. Nogales, Microtubule function, Annu. Rev. Biophys. Biomol. Struct. 30 (2001) 397–420.
[43] D. Panda, H.P. Miller, L. Wilson, Determination of the size and chemical nature of the stabilizing "cap" at microtubule ends using modulators of polymerization dynamics, Biochemistry 41 (2002) 1609–1617.

[44] T.J. Mitchison, M. Kirschner, Dynamic instability of microtubule growth, Nature 312 (1984) 237–242.
[45] A.A. Hyman, E. Karsenti, Morphogenetic properties of microtubules and mitotic spindle assembly, Cell 84 (1996) 401–410.
[46] F. Verde, M. Dogterom, E. Stelzer, E. Karsenti, S. Leibler, Control of microtubule dynamics and length by cyclin A- and cyclin B- dependent kinases in *Xenopus* egg extracts, J. Cell Biol. 118 (1992) 1097–1108.
[47] H. Yoshida, C.J. Hastie, H. Mc Lauchlan, P. Cohen, M. Goedert, Phosphorylation of microtubule-associated protein tau by isoforms of c-Jun N-terminal kinase (JNK), J. Neurochem. 90 (2004) 352–358.
[48] P.T. Cohen, Novel protein serine/threonine phosphatases: variety is the spice of life, Trends Biochem. Sci. 22 (1997) 245–251.
[49] L. Cassimeris, The oncoprotein 18/stathmin family of microtubule destabilizers, Curr. Opin. Cell Biol. 14 (2002) 18–24.
[50] T. Manna, D. Thrower, H.P. Miller, P. Curmi, L. Wilson, Stathmin strongly increases the minus end catastrophe frequency and induces rapid treadmilling of bovine brain microtubules at steady state in vitro, J. Biol. Chem. 28 (2006) 2071–2078.
[51] R. Ohi, K.L. Gould, Regulating the onset of mitosis, Curr. Opin. Cell Biol. 11 (1999) 267–273.
[52] N. Jiang, X. Wang, Y. Yang, W. Dai, Advances in mitotic inhibitors for cancer treatment, Mini-Rev. Med. Chem. 6 (2006) 885–895.
[53] C.L. Rieder, H. Maiato, Stuck in division or passing through: what happens when cells cannot satisfy the spindle assembly checkpoint, Dev. Cell 7 (2004) 637–651.
[54] J.R. Mc Intosh, E.L. Grishchuk, Chromosome-microtubule interactions during mitosis, Ann. Rev. Cell Dev. Biol. 18 (2002) 193–219.
[55] C. Rieder, R. Cole, A. Khodjakov, G. Sluder, The checkpoint delaying anaphase in response to chromosome monoorientation is mediated by an inhibitory signal produced by unattached kinetochores, J. Cell Biol. 130 (1995) 941–948.
[56] B.A. Weaver, D.W. Cleveland, Decoding the links between mitosis, cancer, and chemotherapy: the mitotic checkpoint, adaption, and cell death, Cancer Cell 8 (2005) 7–12.
[57] Y. Iwanga, K.T. Jeang, Characterization of regions in hsMAD1 needed for binding hsMAD2. A polymorphic change in an hsMAD1 leucine zipper affects MAD1–MAD2 interaction and spindle checkpoint function, J. Biol. Chem. 277 (2002) 31005–31013.
[58] B.F. Mc Ewen, G.K.T. Chan, B. Zubrowski, M.S. Savoian, M.T. Sauer, T.J. Yen, CENP-E is essential for reliable bioriented spindle attachment, but chromosome alignment can be achieved via redundant mechanisms in mammalian cells, Mol. Biol. Cell 12 (2001) 2776–2789.
[59] P.D. Andrews, Y. Ovechkina, Aurora B regulates MCAK at the mitotic centromere, Dev. Cell 6 (2004) 253–268.
[60] J.F. Diaz, J.M. Valpuesta, P. Chacon, G. Diakun, J.M. Andreu, Changes in microtubule protofilament number induced by taxol binding to an easily accessible site. Internal microtubule dynamics. J. Biol. Chem. 273 (1998) 33803–33810.
[61] M. Abal, J.M. Andreu, Taxanes: microtubule and centromere targets, and cell cycle dependent mechanism of action, Curr. Cancer Drug Targets 3 (2003) 193–203.
[62] J.P. Snyder, J.H. Nettles, B. Cornett, K.H. Downing, E. Nogales, The binding conformation of taxol in beta-tubulin: a model based on electron crystallographic density, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 5312–5316.
[63] S. Rao, G.A. Orr, A.G. Chaudhary, D.G.I. Kingston, S.B. Horwitz, Characterization of the taxol binding site on the microtubule, J. Biol. Chem. 270 (1995) 20235–20238.
[64] G.G. Borisy, E.W. Taylor, The mechanism of action of colchicine: colchicine binding to sea urchin eggs and the mitotic apparatus, J. Cell Biol. 34 (1967) 535–548.
[65] A. Kiselyov, K.V. Balakin, S.E. Takachenko, N. Savchuk, A.V. Ivachtchenko, Recent progress in discovery and development of antimitotic agents, Anti-Cancer Agents Med. Chem. 7 (2007) 189–208.
[66] J.C. Cochran, J.E. Gatial III, T.M. Kapoor, S.P. Gilbert, Monastrol inhibition of the mitotic kinesin Eg5, J. Biol. Chem. 280 (2005) 12658–12667.
[67] A. Kienitz, C. Vogel, I. Morales, R. Muller, H. Bastians, Partial down-regulation of MAD1 causes spindle checkpoint inactivation and aneuploidy, but does not confer resistance towards taxol, Oncogene 24 (2005) 4301–4310.
[68] J. Kelling, K. Sullivan, L. Wilson, M.A. Jordan, Suppression of centromere dynamics by taxol in living osteosarcoma cells, Cancer Res. 63 (2003) 2794–2801.
[69] K. Torres, S.B. Horwitz, Mechanisms of taxol-induced cell death are concentration dependent, Cancer Res. 58 (1998) 3620–3626.
[70] H. Hernandez-Vargas, J. Palacios, G. Moreno-Bueno, Telling cells how to die, Cell Cycle 6 (2007) 780–783.
[71] P. Engblom, V. Rantanen, J. Kulmala, J. Heiskanen, S. Grenman, Taxane sensitivity of ovarian carcinoma in vitro, Anticancer Res. 17 (1997) 2475–2479.
[72] B.J. Braakhuis, B.T. Hill, M. Dietel, L.R. Kelland, M.S. Aapro, W. Zoli, P. Lelieveld, In vitro antiproliferative activity of docetaxel (Taxotere), paclitaxel (Taxol) and cisplatin against human tumour and normal bone marrow cells, Anticancer Res. 14 (1994) 205–208.
[73] J.E. Liebmann, J.A. Cook, C. Lipschultz, D. Teague, J. Fisher, J.B. Mitchell, Cytotoxic studies of paclitaxel (Taxol) in human tumour cell lines, Br. J. Cancer 68 (1993) 1104–1109.
[74] J.G. Chen, S.B. Horwitz, Differential mitotic responses to microtubule-stabilizing and destabilizing drugs, Cancer Res. 63 (2002) 1935–1938.
[75] M.A. Jordan, K. Wendell, S. Gardiner, W.B. Derry, H. Copp, L. Wilson, Mitotic block induced in HeLa cells by low concentration of paclitaxel (Taxol) results in abnormal exit and apoptotic cell death, Cancer Res. 56 (1996) 816–825.
[76] G.M. Kolfschoten, T.M. Hulscher, M.C.A. Duyndam, H.M. Pinedo, E. Boven, Variation in the kinetics of caspase-3 activation, Bcl-2 phosphorylation and apoptotic morphology in unselected human ovarian cancer cell lines as a response to docetaxel, Biochem. Pharmacol. 63 (2002) 733–743.
[77] S. Haldar, A. Basu, C.M. Croce, Bcl-2 is the guardian of microtubule integrity, Cancer Res. 57 (1997) 229–233.
[78] M.V. Blagosklonny, Mitotic arrest and cell fate, Cell Cycle 6 (2007) 70–74.
[79] R. Schimming, K.A. Mason, N. Hunter, M. Weil, K. Kishi, L. Milas, Lack of correlation between mitotic arrest or apoptosis and antitumour effect of docetaxel, Cancer Chemother. Pharmacol. 43 (1999) 165–172.
[80] B.-D. Chang, E.V. Broude, M. Dokmanovic, H. Zhu, A. Ruth, Y. Xuan, E.S. Kandel, E. Lausch, K. Christov, I.B. Roninson, A senescence-like phenotype distinguishes tumor cells that undergo terminal proliferation arrest after exposure to anticancer agents, Cancer Res. 59 (1999) 3761–3767.
[81] B.G. Wouters, A.J. Giaccia, N.C. Denko, J.M. Brown, Loss of p21(Waf1/Cip1) sensitizes tumors to radiation by an apoptosis-independent mechanism, Cancer Res. 57 (1997) 4703–4706.
[82] D.L. Morse, H. Gray, C.M. Payne, R.J. Gillies, Docetaxel induces cell death through mitotic catastrophe in human breast cancer cells, Mol. Cancer Ther. 4 (2005) 1495–1504.
[83] M. Castedo, J.-L. Perfettine, T. Roumier, K. Andreau, R. Medema, G. Kroemer, Cell death by mitotic catastrophe: a molecular definition, Oncogene 23 (2004) 2825–2837.
[84] I.B. Roninson, E.V. Broude, B.D. Chang, If not apoptosis, then what? — treatment-induced senescence and mitotic catastrophe in tumour cells, Drug Resist. Updat. 4 (2001) 303–313.
[85] H.K. Berrieman, M.J. Lind, L. Cawkwell, Do beta-tubulin mutations have a role in resistance to chemotherapy? Lancet Oncol. 5 (2004) 158–164.
[86] C.A. Burkhart, M. Kavallaris, S. Band Horwitz, The role of beta-tubulin isotypes in resistance to antimitotic drugs, Biochim. Biophys. Acta 1471 (2001) O1–O9.
[87] J. Van Ark-Otte, G. Samelis, G. Rubio, J.B. Lopez Saez, H.M. Pinedo, G. Giaccone, Effects of tubulin-inhibiting agents in human lung and breast cancer cell lines with different multidrug resistance phenotypes, Oncol. Rep. 5 (1998) 249–255.
[88] A. Goncalves, D. Braguer, K. Kamath, L. Martello, C. Briand, S. Horwitz, L. Wilson, M.A. Jordan, Resistance to taxol in lung cancer cells associated with increased microtubule dynamics, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 11737–11742.
[89] D.J. Villeneuve, S.L. Hembruff, Z. Veitch, M. Cecchetto, W.A. Dew, A.M. Parissenti, cDNA microarray analysis of isogenic paclitaxel- and doxorubicin-resistant breast tumour cell lines reveals distinct drug-specific genetic signatures of resistance, Breast Cancer Res.Treat. 96 (2006) 17–39.

[90] M.L. Gonzalez-Garay, L. Chang, K. Blade, D.R. Menick, F. Cabral, A beta-tubulin leucine cluster involved in microtubule assembly and paclitaxel resistance, J. Biol. Chem. 274 (1999) 23875–23882.
[91] M. Kavallaris, A.S. Tait, B.J. Walsh, L. He, S.B. Horwitz, M.D. Norris, M. Haber, Multiple microtubule alterations are associated with vinca alkaloid resistance in human leukemia cells, Cancer Res. 61 (2001) 5803–5809.
[92] X.H. Hua, D. Genini, R. Gussio, R. Tawatao, H. Shih, T.J. Kipps, D.A. Carson, L.M. Leoni, Biochemical genetic analysis of indanocine resistance in human leukemia, Cancer Res. 61 (2001) 7248–7254.
[93] M. Hari, F. Loganzo, T. Annable, X. Tan, S. Musto, D.B. Morilla, J.H. Nettles, J.P. Snyder, L.M. Greenberger, Paclitaxel-resistant cells have a mutation in the paclitaxel-binding region of beta-tubulin ($Asp^{26}Glu$) and less stable microtubules, Mol. Cancer Ther. 5 (2005) 270–278.
[94] L.A. Martello, P. Verdier-Pinard, H.J. Shen, L. He, K. Torres, G.A. Orr, S.B. Horwitz, Elevated levels of microtubule destabilizing factors in a taxol-resistant/dependent A549 cell line with an {alpha}-tubulin mutation, Cancer Res. 63 (2003) 1207–1213.
[95] Y. Wang, A. O'Brate, W. Zhou, P. Giannakakou, Resistance to microtubule-stabilizing drugs involves two events, beta-tubulin mutation in one allele followed by loss of the second allele, Cell Cycle 4 (2005) 1847–1853.
[96] Y. Wang, S. Yin, K. Blade, G. Cooper, D.R. Menick, F. Cabral, Mutations at leucine 215 of beta-tubulin affect paclitaxel sensitivity by two distinct mechanisms, Biochemistry 45 (2006) 185–194.
[97] P. Giannakakou, D.L. Sackett, Y.-K. Kang, Z. Zhan, J.T.M. Buters, T. Fojo, M.S. Poruchynsky, Paclitaxel-resistant human ovarian cancer cells have mutant beta-tubulins that exhibit impaired paclitaxel-driven polymerization, J. Biol. Chem. 272 (1997) 17118–17125.
[98] S. Sale, R. Sung, P. Shen, K. Yu, Y. Wang, G.E. Duran, J.-H. Kim, T. Fojo, P.J. Oefner, B.I. Sikic, Conservation of the class I {beta}-tubulin gene in human populations and lack of mutations in lung cancers and paclitaxel-resistant ovarian cancers, Mol. Cancer Ther. 1 (2002) 215–225.
[99] C.T. Warnick, B. Dabbas, C.D. Ford, K.A. Strait, Identification of a p53 response element in the promoter region of the hMSH2 gene required for expression in A2780 ovarian cancer cells, J. Biol. Chem. 276 (2001) 27363–27370.
[100] M. Monzo, R. Rosell, J.J. Sanchez, J.S. Lee, A. O' Brate, J.L. Gonzalez-Larriba, V. Alberola, J.C. Lorenzo, L. Nunez, J.Y. Ro, C. Martin, Paclitaxel resistance in non-small-cell lung cancer associated with beta-tubulin gene mutations, J. Clin. Oncol. 17 (1999) 1786–1793.
[101] S. Hasegawa, Y. Miyoshi, C. Egawa, M. Ishtobi, Y. Tamaki, M. Monden, S. Noguchi, Mutation of the class I beta-tubulin gene in human breast cancer, Int. J. Cancer 101 (2002) 46–51.
[102] K. Maeno, K. Ito, Y. Hama, Mutation of the class I beta-tubulin gene does not predict response to paclitaxel for breast cancer, Cancer Lett. 198 (2003) 89–97.
[103] P. Verdier-Pinard, S. Shahabi, F. Wang, B. Burd, H. Xiao, G.L. Goldberg, G.A. Orr, S.B. Horwitz, Detection of human *beta*V-tubulin expression in epithelial cancer cell lines by tubulin proteomics, Biochemistry 44 (2005) 15858–15870.
[104] O. Valiron, N. Caudron, D. Job, Microtubule dynamics, Cell Mol. Life Sci. 58 (2001) 2069–2084.
[105] R.Z. Yusuf, Z. Duan, D.E. Lamendola, R.T. Penson, M.V. Seiden, Paclitaxel resistance: molecular mechanisms and pharmacologic manipulation, Curr. Cancer Drug Targets 3 (2003) 1–19.
[106] D. Panda, H. Miller, A. Banerjee, R. Luduena, L. Wilson, Microtubule dynamics in vitro are regulated by the tubulin isotype composition, Proc. Natl. Acad. Sci. U. S. A. 91 (1994) 11358–11362.
[107] W. Derry, L. Wilson, I.A. Khan, R.F. Ludena, M.A. Jordan, Taxol differentially modulates the dynamics of microtubules assembled from unfractionated and purified beta-tubulin isotypes, Biochemistry 36 (1997) 3554–3562.
[108] A. Banerjee, Increased levels of tyrosinated alpha-, beta (III), and beta (IV)-tubulin isotypes in paclitaxel-resistant MCF-7 breast cancer cells, Biochem. Biophys. Res. 293 (2002) 598–601.
[109] C. Ferlini, G. Raspaglio, S. Mozzetti, L. Cicchillitti, F. Filippetti, D. Gallo, C. Fattorusso, G. Campiani, G. Scambia, The seco-taxane IDN5390 is able to target class III beta-tubulin and to overcome paclitaxel resistance, Cancer Res. 65 (2005) 2397–2405.
[110] M. Kavallaris, C.A. Burkhart, S.B. Horwitz, Antisense oligonucleotides to class III beta-tubulin sensitize drug-resistant cells to Taxol, Br. J. Cancer 80 (1999) 1020–1025.
[111] M. Kavallaris, D.-S. Kuo, C.A. Burkhart, D.L. Regl, M.D. Norris, M. Haber, S.B. Horwitz, Taxol-resistant epithelial ovarian tumors are associated with altered expression of specific beta-tubulin isotypes, J. Clin. Invest. 100 (1997) 1282–1293.
[112] S. Mozzetti, C. Ferlini, P. Concolino, F. Filippetti, G. Raspaglio, S. Prislei, D. Gallo, E. Martinelli, F.O. Ranelletti, G. Ferrandina, G. Scambia, Class III {beta}-tubulin overexpression is a prominent mechanism of paclitaxel resistance in ovarian cancer patients, Clin. Cancer Res. 11 (2005) 298–305.
[113] A. Paradiso, A. Mangia, A. Chiriatti, S. Tommasi, A. Zito, A. Latorre, F. Schittuli, V. Lorusso, Biomarkers predictive for clinical efficacy of taxol-based chemotherapy in advanced breast cancer, Ann. Oncol. 16 (2005) 14–19.
[114] R. Rosell, G. Scagliotti, K.D. Danenberg, R.V. Lord, G. Bepler, S. Novello, J. Cooc, L. Crino, J.J. Sanchez, M. Taron, C. Boni, F. Demartinis, M. Tonato, M. Marangolo, F. Gozzelino, F. Di Costanzo, M. Rinaldi, D. Salonga, C. Stephens, Transcripts in pretreatment biopsies from a three-arm randomized trial in metastatic non-small-cell lung cancer, Oncogene 22 (2003) 3548–3553.
[115] C. Dumontet, S. Isaac, P.J. Souquet, F. Bejui-Thivolet, Y. Pacheco, N. Peloux, A. Frankfurter, R. Luduena, M. Perol, Expression of class III beta tubulin in non-small cell lung cancer is correlated with resistance to taxane chemotherapy, Bulletin Cancer 92 (2005) 25–30.
[116] P. Seve, S. Isaac, O. Tredan, P.-J. Souquet, Y. Pacheco, M. Perol, L. Lafanechere, A. Penet, E.-L. Peiller, C. Dumontet, Expression of class III {beta}-tubulin is predictive of patient outcome in patients with non-small cell lung cancer receiving vinorelbine-based chemotherapy, Clin. Cancer Res. 11 (2005) 5481–5486.
[117] M.I. Nicoletti, G. Valoti, P. Giannakakou, Z. Zhan, J.-H. Kim, V. Lucchini, F. Landoni, J.G. Mayo, R. Giavazzi, T. Fojo, Expression of {beta}-tubulin isotypes in human ovarian carcinoma xenografts and in a sub-panel of human cancer cell lines from the NCI-anticancer drug screen: correlation with sensitivity to microtubule active agents, Clin. Cancer Res. 7 (2001) 2912–2922.
[118] L. Dehmelt, S. Halpain, The MAP2/tau family of microtubule-associated proteins, Genome Biol. 6 (2004) 204.
[119] P. Wagner, B. Wang, E. Clark, H. Lee, R. Rouzier, L. Pusztai, Microtubule associated protein (MAP)-tau a novel mediator of paclitaxel in vitro and in vivo, Cell Cycle 4 (2005) 1149–1152.
[120] J.M. Bunker, L. Wilson, M.A. Jordan, S.C. Feinstein, Modulation of microtubule dynamics by tau in living cells: implications for development and neurodegeneration, Mol. Biol. Cell 15 (2004) 2720–2728.
[121] S. Kar, J. Fan, M.J. Smith, M. Goedert, L.A. Amos, Repeat motifs of tau bind to the insides of microtubules in the absence of taxol, EMBO J. 22 (2003) 70–77.
[122] S.E. Merrick, D.C. Demoise, V.M. Lee, Site-specific dephosphorylation of tau protein at Ser202/Thr205 in response to microtubule depolymerization in cultured human neurons involves protein phosphatase 2A, J. Biol. Chem. 271 (1996) 5589–5594.
[123] V. Makrides, M.R. Masic, S.C. Feinstein, J. Lew, Evidence for two distinct binding sites for tau on microtubules, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 6746–6751.
[124] R. Rouzier, R. Rajan, P. Wagner, K.R. Hess, D.L. Gold, J. Stec, M. Ayers, J.S. Ross, P. Zhang, T.A. Buchholz, H. Kuerer, M. Bgreen, B. Arun, G.N. Hortobagyi, W.F. Symmans, L. Pusztai, Microtubule associated protein tau: a marker of paclitaxel sensitivity in breast cancer, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 8315–8320.
[125] F. Andre, C. Hatzis, K. Anderson, C. Sotiriou, C. Mazouni, J. Mejia, B. Wang, G.N. Hortobagyi, W.F. Symmans, L. Pusztai, Microtubule-associated protein-tau is a bifunctional predictor of endocrine sensitivity and chemotherapy resistance in estrogen receptor-positive breast cancer, Clin. Cancer Res. 13 (2007) 2061–2067.
[126] C.I. Rubin, G.F. Atweh, The role of stathmin in the regulation of the cell cycle, J. Cell Biol. 93 (2004) 242–250.

[127] P.A. Curmi, O. Gavet, E. Charbaut, S. Ozon, S. Lachkar-Colerauer, V. Manceau, S. Siavoshian, A. Maucuer, A. Sober, Stathmin and its phosphorylation protein family: general properties, biochemical and functional interaction with tubulin, Cell Struct. Funct. 24 (1999) 345–357.
[128] X.N. Luo, B. Mookerjee, A. Ferrari, S. Mistry, G.F. Atweh, Regulation of phosphoprotein p18 in leukemic cells. Cell cycle regulated phosphorylation by $p34^{cdc2}$ kinase, J. Biol. Chem. 269 (1994) 10312–10318.
[129] G.M. Bokoch, Biology of the p21-activated kinases, Annu. Rev. Biochem. 72 (2003) 743–781.
[130] I. Bieche, S. Lachkar, V. Becette, C. Cifuentes-Diaz, A. Sobel, R. Lidereau, Overexpression of the stathmin gene in a subset of human breast cancer, Br. J. Cancer 78 (1998) 701–709.
[131] E. Alli, J. Bash-Babula, J.M. Yang, W.N. Hait, Effect of stathmin on the sensitivity to antimicrotubule drugs in human breast cancer, Cancer Res. 62 (2002) 6864–6869.
[132] R. Melham, N. Hailat, R. Kuick, S.M. Hanash, Quantitative analysis of Op18 phosphorylation in childhood acute leukemia, Leukemia 11 (1997) 1690–1695.
[133] C. Iancu, S.J. Mistry, S. Arkin, S. Wallenstein, G.F. Atwin, Use of antisense oligonucleotides to downregulate stathmin increases sensitivity to paclitaxel, J. Cell Sci. 114 (2001) 909–916.
[134] M. Poruchynsky, P. Giannakakou, Y. Ward, J.C. Bulinski, W.G. Telford, R.W. Robey, T. Fojo, Accompanying protein alterations in malignant cells with a microtubule-polymerizing drug-resistance phenotype and a primary resistance mechanism, Biochem. Pharmacol. 62 (2001) 1469–1480.
[135] C.C. Zhang, J.M. Yang, E. White, M. Murphy, A. Levine, W.N. Hait, The role of MAP4 expression in the sensitivity to paclitaxel and resistance to vinca alkaloids in p53 mutant cells, Oncogene 16 (1998) 1617–1624.
[136] M. Murphy, A. Hinman, A.J. Levine, Wild-type p53 negatively regulates the expression of a microtubule-associated protein, Genes Dev. 10 (1996) 2971–2980.
[137] M.M. Gottesman, Mechanisms of cancer drug resistance, Annu. Rev. Med. 53 (2002) 615–627.
[138] F. Leonessa, R. Clarke, ATP binding cassette transporters and drug resistance in breast cancer, Endocr. Relat. Cancer 10 (2003) 43–73.
[139] M. Filipits, Mechanisms of cancer: multidrug resistance, Drug Discov. Today 1 (2004) 229–234.
[140] C. Dumontet, B.I. Sikic, Mechanisms of action and resistance to antitubulin agents: microtubule dynamics, drug transport and cell death, J. Clin. Oncol. 17 (1999) 1061–1070.
[141] M. Ramachandra, S.V. Ambudkar, D. Chen, C.A. Hrycyna, S. Dey, M.N. Gottesman, I. Pastana, Human P-glycoprotein exhibits reduced affinity for substrates during a catalytic transition state, Biochemistry 37 (1998) 5010–5019.
[142] A.H. Schinkel, U. Mayer, E. Wagenaar, C.A. Mol, L. Van Deemter, J.J. Smit, M.A. Van Der Valk, A.C. Voordouw, H. Spits, O. Van Tellingen, J.M. Zijlmans, W.E. Fibbe, P. Borst, Normal viability and altered pharmacokinetics in mice lacking Mdr1-Type (drug-transporting) P-glycoproteins, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 4028–4033.
[143] K. Ueda, C. Cardarelli, M.M. Gottesman, I. Pastan, Expression of a full-length cDNA for the human "MDR1" gene confers resistance to colchicine, doxorubicin, and Vinblastine, Proc. Natl. Acad. Sci. 84 (1987) 3004–3008.
[144] M. Alvarez, K. Paull, A. Monks, C. Hose, J.S. Lee, J. Weinstein, M. Grever, S. Bates, T. Fojo, Generation of a drug resistance profile by quantitation of mdr-1/P-glycoprotein in the cell lines of the National Cancer Institute Anticancer Drug Screen, J. Clin. Invest. 95 (1995) 2205–2214.
[145] N. Kroger, W. Achterrath, S. Hegewisch-Becker, K. Mross, A.R. Zander, Current options in treatment of anthracycline-resistant breast cancer, Cancer Treat. Rev. 25 (1999) 279–291.
[146] F. Tsukamoto, E. Shiba, T. Taguchi, T. Sugimoto, T. Watanabe, S.J. Kim, Y. Tanji, Y. Kimoto, M. Izukura, S.I. Takai, Immunohistochemical detection of P-glycoprotein in breast cancer and its significance as a prognostic factor, Breast Cancer 25 (1997) 259–263.
[147] J. Schneider, R. Lucas, J. Sanchez, A. Ruibal, A. Tejerina, M. Martin, Modulation of molecular marker expression by induction chemotherapy in locally advanced breast cancer: correlation with the response to therapy and the expression of MDR1 and LRP, Anticancer Res. 20 (2000) 4373–4377.
[148] J. Schneider, H. Romero, Correlation of P-glycoprotein overexpression and cellular prognostic factors in formalin-fixed, paraffin-embedded tumor samples from breast cancer patients, Anticancer Res. 15 (1995) 1117–1121.
[149] A. Kanzaki, M. Toi, K. Nakayama, H. Bando, M. Mutoh, T. Uchida, M. Fukumoto, Y. Takebayashi, Expression of multidrug resistance-related transporters in human breast carcinoma, Jpn. J. Cancer Res. 92 (2001) 452–458.
[150] J.J. Yeh, W.H. Hsu, J.J. Wang, S.T. Ho, A. Kao, Predicting chemotherapy response to paclitaxel-based therapy in advanced non-small-cell lung cancer with P-glycoprotein expression, Respiration 70 (2003) 32–35.
[151] R.T. Penson, E. Oliva, S.J. Skates, T. Glyptis, Expression of multidrug resistance-1 protein inversely correlates with paclitaxel response and survival in ovarian cancer patients: a study in serial samples, Gynaecol. Oncol. 93 (2004) 98–106.
[152] D.S. Alberts, R. Taetle, E. Frutiger, D. Heddens, L. Laptalo, J. Einspahr, Multidrug resistance expression as an independent risk factor for poor survival in optimal stage III ovarian cancer, Proc. Am. Soc. Clin. Oncol. 16 (1998) abstract 1356.
[153] S. Modok, H.R. Mellor, R. Callaghan, Modulation of multidrug resistance efflux pump activity to overcome chemoresistance in cancer, Curr. Opin. Pharmacol. 6 (2006) 350–354.
[154] S.W. Johnson, R.P. Perez, A.K. Godwin, A.T. Yeung, L.M. Handel, R.F. Ozols, Role of platinum-DNA adduct formation and removal in cisplatin resistance in human ovarian cancer cell lines, Biochem. Pharmacol. 47 (1994) 689–697.
[155] B.I. Sikic, Pharmacologic approaches to reversing multidrug resistance, Semin. Haematol. 34 (1997) 40–47.
[156] D. Toppmeyer, A.D. Seidman, M. Pollak, C. Russell, K. Tkaczuk, S. Verma, B. Overmoyer, V. Garg, E. Ette, M.W. Hardu, G.D. Demetri, Safety and efficacy of the multidrug resistance inhibitor Incel in combination with paclitaxel in advanced breast cancer refractory to paclitaxel, Clin. Cancer Res. 8 (2002) 670–678.
[157] A. Archinal-Mattheis, R.W. Rzepka, T. Watanabe, N. Kokubu, Y. Itoh, N.J. Combates, K.W. Bair, D. Cohen, Analysis of the interactions of SDZ PSC 833 ([3′-keto-Bmt1]-Val2]-Cyclosporine), a multidrug resistance modulator, with P-glycoprotein, Oncol. Res. 7 (1995) 603–610.
[158] L. Pusztai, P. Wagner, N. Ibrahim, E. Rivera, R. Theriault, D. Booser, F.W. Symmans, F. Wong, G. Blumenschein, D.R. Fleming, R. Rouzier, G. Boniface, G.N. Hortobagyi, Phase II study of tariquidar, a selective P-glycoprotein inhibitor, in patients with chemotherapy-resistant, advanced breast carcinoma, Cancer 104 (2005) 682–691.
[159] K.N. Chi, S.K. Chia, R. Dixon, M.J. Newman, V.J. Wacher, B. Sikic, K.A. Gelmon, A phase I pharmacokinetic study of the P-glycoprotein inhibitor, ONT-093, in combination with paclitaxel in patients with advanced cancer, Invest. New Drugs 23 (2005) 311–315.
[160] M. Izquierdo, Short interfering RNAs as a tool for cancer gene therapy, Cancer Gene Ther. 12 (2005) 217–227.
[161] E. Yague, C.F. Higgins, S. Raguz, Complete reversal of multidrug resistance by stable expression of small interfering RNAs targeting MDR1, Gene Ther. 11 (2004) 1170–1174.
[162] P. Borst, R. Evers, M. Kool, J. Wijnholds, A family of drug transporters: the multidrug resistance-associated proteins, J. Natl. Cancer Inst. 92 (2000) 1295–1302.
[163] E. Hopper-Borge, Z.S. Chen, I. Schaveleva, M.G. Belinsky, G.D. Kruh, Analysis of the drug resistance profile of multidrug resistance protein 7 (ABCC10): resistance to docetaxel, Cancer Res. 64 (2004) 4927–4930.
[164] S.P. Cole, G. Bhardwaj, J.H. Gerlacj, J.E. Mackie, C.E. Grant, K.C. Almquist, A.J. Stewart, E.U. Kurz, A.M. Duncan, R.G. Deeley, Overexpression of a transporter gene in a multi-drug resistant human lung cancer cell line, Science 258 (1992) 1650–1654.
[165] S.P. Cole, K.E. Sparks, K. Fraser, D.W. Loe, C.E. Grant, G.M. Wilson, R.G. Deeley, Pharmacological characterization of multidrug resistant MRP-transfected human tumour cells, Cancer Res. 54 (1994) 5902–5910.
[166] T. Kitazaki, M. Oka, Y. Nakamura, J. Tsurutani, S. Doi, M. Yasunaga, M. Takemura, H. Yabuuchi, H. Soda, S. Kohno, Gefitinib, an EGFR tyrosine kinase inhibitor, directly inhibits the function of P-glycoprotein in multidrug resistant cancer cells, Lung Cancer 49 (2005) 337–343.

[167] H. Burger, H. Van Tol, A.W. Boersma, M. Brok, E.A. Wiemer, G. Stoter, K. Nooter, Imatinib mesylate (STI571) is a substrate for the breast cancer resistance proteins (BCRP)/ABCG2 drug pump, Blood 104 (2004) 2940–2942.
[168] C.-H. Yang, C.-J. Huang, C.-S. Yang, Y.-C. Chu, A.-L. Cheng, J. Whang-Peng, P.-C. Yang, Gefitinib reverses chemotherapy resistance in Gefitinib-insensitive multidrug resistant cancer cells expressing ATP-binding cassette family protein, Cancer Res. 65 (2005) 6943–6949.
[169] G.L. Plosker, S.J. Keam, Trastuzumab: a review of its use in the management of HER2-positive metastatic and early-stage breast cancer, Drugs 66 (2006) 449–475.
[170] M. Harries, I. Smith, The development and clinical use of trastuzumab (Herceptin), Endocr. Relat. Cancer 9 (2002) 75–85.
[171] D. Slamon, W. Godolphin, L. Jones, J. Holt, S. Wong, D. Keith, W. Levin, S. Stuart, J. Udove, A. Ullrich, Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer, Science 244 (1989) 707–712.
[172] A.H. Mc Cann, P.A. Dervan, M. O' Regan, M.B. Codd, W.J. Gullick, B.M.J. Tobin, D.N. Carney, Prognostic significance of c-erbB-2 and estrogen receptor status in human breast Cancer, Cancer Res. 51 (1991) 3296–3303.
[173] D. Yu, T. Jing, B. Liu, J. Yao, M. Tan, T.J. Mc Donnell, M.C. Hung, Overexpression of ErbB2 blocks taxol-induced apoptosis by upregulation of $p21^{cip1}$, which inhibits p34Cdc2 kinase, Mol. Cell 2 (1998) 581–591.
[174] M. Tan, T. Jing, K.-H. Lan, C.L. Neal, P. Li, S. Lee, D. Fang, Y. Nagata, J. Liu, R. Arlinghaus, M.-C. Hung, D. Yu, Phosphorylation on tyrosine-15 of $p34^{Cdc2}$ activation and is involved in resistance to taxol-induced Apoptosis, Mol. Cell 9 (2002) 993–1004.
[175] C. Knuefermann, Y. Lu, B. Liu, W. Jin, K. Liang, L. Wu, M. Schmidt, G.B. Mills, J. Mendelsohn, Z. Fan, HER2/PI-3K/Akt activation leads to a multidrug resistance in human breast adenocarcinoma cells, Oncogene 22 (2003) 3205–3212.
[176] C. Van Poznak, L. Tan, K.S. Panageas, C.D. Arroyo, C. Hudis, L. Norton, A.D. Seidman, Assessment of molecular markers of clinical sensitivity to single-agent taxane therapy for metastatic breast cancer, J. Clin. Oncol. 20 (2002) 2319–2326.
[177] S. Modi, M.P. Digiovanna, Z. Lu, C. Moskowitz, K.S. Panageas, C. Van Poznak, C.A. Hudis, L. Norton, L. Tan, D.F. Stern, D. Carter, A.D. Seidman, Phosphorylated/activated HER2 as a marker of clinical resistance to single agent taxane chemotherapy for metastatic breast cancer, Cancer Invest. 23 (2005) 483–487.
[178] D. Yu, B. Liu, M. Tan, J. Li, S.S. Wang, M.C. Hung, Overexpression of c-erbB-2/neu in breast cancer cells confers increased resistance to taxol via mdr-1-independent mechanisms, Oncogene 13 (1996) 1359–1365.
[179] M.M. Goldenberg, Trastuzumab, a recombinant DNA-derived humanized monoclonal antibody, a novel agent for the treatment of metastatic breast cancer, Clin. Ther. 21 (1999) 309–318.
[180] J.M. Nabholtz, T. Pienkowski, Results of two open label multicentre phase II pilot studies with Herceptin in combination with docetaxel and platinum salts (cis or carboplatin) (TCH) as therapy for advanced breast cancer (ABC) in women with tumours over-expressing the HER-2-neu proto-oncogene, Eur. J. Cancer 37 (2001) 190.
[181] D.J. Slamon, B. Leyland-Jones, Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2, N. Engl. J. Med. 344 (2001) 783–792.
[182] J. Zhou, J. Yao, H.C. Joshi, Attachment and tension in the spindle assembly checkpoint, J. Cell Sci. 115 (2002) 3547–3555.
[183] A. Musacchio, K.G. Hardwick, The spindle checkpoint structural insights into dynamic signalling, Nat. Rev. Mol. Cell Biol. 3 (2002) 731–741.
[184] H. Yu, Regulation of APC-Cdc20 by the spindle checkpoint, Curr. Opin. Cell Biol. 14 (2002) 706–714.
[185] R. Li, A.W. Murray, Feedback control of mitosis in budding yeast, Cell 66 (1991) 519–531.
[186] M.A. Hoyt, L. Trotis, B.T. Roberts, *S. cerevisiae* genes required for cell cycle arrest in response to loss of microtubule function, Cell 66 (1991) 507–517.
[187] E. Winey, B.J. Huneycutt, Centrosomes and checkpoints: the MPS1 family of kinases, Oncogene 21 (2002) 6161–6169.
[188] H.A. Fisk, M. Winey, Mps1 flies into new areas, Curr. Biol. 14 (2004) R1058–R1060.
[189] R. Bharadwaj, H. Yu, The spindle checkpoint, aneuploidy, and cancer, Oncogene 23 (2004) 2016–2027.
[190] B.J. Howell, B.F. Mc Ewen, J.C. Canman, D.B. Hoffman, E.M. Farrar, C.L. Rieder, E.D. Salmon, Cytoplasmic dynein/dynactin drives kinetochore protein transport to the spindle poles and has a role in mitotic spindle checkpoint inactivation, J. Cell Biol. 155 (2001) 1159–1172.
[191] C.-Y. Tai, D.L. Dujardin, N.E. Faulkner, R.B. Vallee, Role of dynein, dynactin, and CLIP-170 interactions in LIS1 kinetochore function, J. Cell Biol. 156 (2002) 959–968.
[192] A. Abrieu, J.A. Kahana, K.W. Wood, D.W. Cleveland, CENP-E as an essential component of the mitotic checkpoint in vitro, Cell 102 (2000) 817–826.
[193] Z.Y. Yang, J. Guo, N. Li, M. Qian, S.N. Wang, X.L. Zhu, Mitosin/CENP-F is a conserved kinetochore protein subjected to cytoplasmic dynein-mediated poleward transport, Cell Res. 13 (2003) 275–283.
[194] R. Karess, Rod-Zw10-Zwilch: a key player in the spindle checkpoint, Trends Cell Biol. 15 (2005) 386–392.
[195] S. Martin-Lluesma, V.M. Stucke, E.A. Nigg, Role of Hec1 in spindle checkpoint signaling and kinetochore recruitment of Mad1/Mad2, Science 297 (2002) 2267–2270.
[196] T. Habu, S.H. Kim, J. Weinstein, T. Matsumoto, Identification of a MAD2-binding protein, CMT2, and its role in mitosis, EMBO J. 21 (2002) 6419–6428.
[197] S.A. Lens, R.M. Wolthuis, R. Klompmaker, J. Kauw, R. Agami, T. Brummelkamp, G. Kops, R.H. Medema, Survivin is required for a sustained spindle checkpoint arrest in response to lack of tension, EMBO J. 22 (2003) 2934–2947.
[198] G. Vader, R.H. Medema, S.M.A. Lens, The chromosomal passenger complex: guiding Aurora-B through mitosis, J. Cell Biol. 173 (2006) 833–837.
[199] C. Vogel, C. Hager, H. Bastians, Mechanisms of mitotic cell death induced by chemotherapy-mediated G2 checkpoint abrogation, Cancer Res. 67 (2007) 339–345.
[200] G.J. Kops, B.A. Weaver, D.W. Cleveland, On the road to cancer: aneuploidy and the mitotic checkpoint, Nat. Rev. Cancer 5 (2005) 773–785.
[201] R.-H. Chen, A. Shevchenko, M. Mann, A.W. Murray, Spindle checkpoint protein Xmad1 recruits Xmad2 to unattached kinetochores, J. Cell Biol. 143 (1998) 283–295.
[202] L. Sironi, M. Melixetian, M. Faretta, E. Prosperini, K. Helin, A. Musacchio, Mad2 binding to Mad1 and Cdc20, rather than oligomerization, is required for the spindle checkpoint, EMBO J. 20 (2001) 6371–6382.
[203] X. Luo, Z. Tang, J. Rizo, H. Yu, The Mad2 spindle checkpoint protein undergoes similar major conformational changes upon binding to either Mad1 or Cdc20, Mol. Cell 9 (2002) 59–71.
[204] E. Chung, R.H. Chen, Spindle checkpoint requires Mad1-bound and Mad1-free Mad2, Mol. Biol. Cell 13 (2002) 1501–1511.
[205] B.J. Howell, B. Moree, E.M. Farrar, S. Stewart, G. Fang, E.D. Salmon, Spindle checkpoint protein dynamics at kinetochores in living cells, Curr. Biol. 14 (2004) 953–964.
[206] J.V. Shah, E. Botvinick, Z. Bonday, F. Furnari, M. Berns, D.W. Cleveland, Dynamics of centromere and kinetochore proteins; implications for checkpoint signaling and silencing, Curr. Biol. 14 (2004) 942–952.
[207] G. Fang, H. Yu, M.W. Kirschner, The checkpoint protein MAD2 and the mitotic regulator CDC20 form a ternary complex with the anaphase-promoting complex to control anaphase initiation, Genes Dev. 12 (1998) 1871–1883.
[208] L.H. Hwang, L.F. Lau, D.L. Smith, C.A. Mistrot, K.G. Hardwick, E.S. Hwang, A. Amon, A.W. Murrau, Budding yeast Cdc20: a target of the spindle checkpoint, Science 279 (1998) 1041–1044.
[209] A. Deantoni, V. Sala, A. Musacchio, Explaining the oligomerization properties of the spindle assembly checkpoint protein Mad2, Philos. Trans. Royal Soc. B 360 (2005) 637–648.
[210] A. Musacchio, E.D. Salmon, The spindle-assembly checkpoint in space and time, Nat. Rev. Mol. Cell Biol. 8 (2007) 379–393.
[211] V. Sudakin, G.K. Chan, T.J. Yen, Checkpoint inhibition of the APC/C in HeLa cells is mediated by a complex of BUBR1, BUB3, CDC20, and MAD2, J. Cell Biol. 154 (2001) 925–936.
[212] Z. Tang, R. Bharadwaj, B. Li, H. Yu, Mad2-independent inhibition of APC-Cdc20 by the mitotic checkpoint protein BubR1, Dev. Cell 1 (2001) 227–237.

[213] K.B. Shannon, J.C. Canman, E.D. Salmon, Mad2 and BubR1 function in a single checkpoint pathway that responds to a loss of tension, Mol. Biol. Cell 13 (2002) 3706–3719.
[214] K.G. Hardwick, R.C. Johnston, D.L. Smith, A.W. Murray, MAD2 encodes a novel component of the spindle checkpoint which interacts with Bub3p, Cdc20p, and Mad2p, J. Cell Biol. 148 (2000) 871–882.
[215] A. Poddar, P.T. Stukenberg, D.J. Burke, Two complexes of spindle checkpoint proteins containing Cdc20 and Mad2 assemble during mitosis independently of the kinetochore in *Saccharomyces cerevisiae*, Eukaryat. Cell 4 (2005) 867–878.
[216] X. Luo, Z. Tang, G. Xia, T. Wassmann, J. Rizo, H. Yu, The Mad2 spindle checkpoint protein has two distinct natively folded states, Nat. Struct. Mol. Biol. 11 (2004) 338–345.
[217] H. Yu, Structural activation of Mad2 in the mitotic spindle checkpoint: the two-state Mad2 model versus the Mad2 template model, J. Cell Biol. 173 (2006) 153–157.
[218] K. Nasmyth, How do so few control so many? Cell 120 (2005) 739–746.
[219] M. Dobles, V. Liberal, M.L. Scott, R. Benezra, P.K. Sorger, Chromosome missegregation and apoptosis in mice lacking the mitotic checkpoint protein Mad2, Cell 101 (2000) 635–645.
[220] D.J. Baker, K.B. Jeganathan, J.D. Cameron, M. Thompson, S. Juneja, A. Kopecka, R. Kumar, R.B. Jenkins, P.C. De Groen, P. Roche, J.M. Van Deursen, BubR1 insufficiency causes early onset of aging-associated phenotypes and infertility in mice, Nat. Genet. 36 (2004) 744–749.
[221] P. Kalitsis, E. Earle, K.J. Fowler, K.H. Choo, *Bub3* gene disruption in mice reveals essential mitotic spindle checkpoint function during early embryogenesis, Genes Dev. 14 (2000) 2277–2282.
[222] W. Dai, Q. Wang, T. Liu, M. Swamy, Y. Fang, S. Xie, R. Mahmood, Y.M. Yang, Slippage of mitotic arrest and enhanced tumour development in mice with BubR1 haploinsufficiency, Cancer Res. 64 (2004) 440–445.
[223] L.S. Michel, V. Liberal, A. Chatterjee, R. Kirchweggar, B. Pasche, W. Gerald, M. Dobles, P.K. Sorger, V.V. Murty, R. Benezra, MAD2 haploinsufficiency causes premature anaphase and chromosome instability in mammalian cells, Nature 409 (2001) 355–359.
[224] T. Sudo, M. Nitta, H. Saya, N.T. Ueno, Dependence of paclitaxel sensitivity on a functional spindle assembly checkpoint, Cancer Res. 64 (2004) 2502–2508.
[225] S. Anand, S. Penrhyn-Lowe, A.R. Venkitaraman, AURORA-A amplification overrides the mitotic spindle assembly checkpoint, inducing resistance to taxol, Cancer Cell 3 (2003) 51–62.
[226] D.P. Cahill, C. Lengauer, J. Yu, G.J. Riggins, J.K. Willson, S.D. Markowitz, K.W. Kinzler, B. Vogelstein, Mutations of mitotic checkpoint genes in human cancers, Nature 392 (1998) 300–303.
[227] Y. Li, R. Benezra, Identification of a human mitotic checkpoint gene: hsMAD2, Science 274 (1996) 246–248.
[228] A. Masuda, T. Takahashi, Chromosome instability in human lung cancers. Possible underlying mechanisms and potential consequences in the pathogenesis, Oncogene 21 (2002) 6884–6897.
[229] M. Shichiri, K. Yoshinaga, H. Hisatomi, K. Sugihara, Y. Hirata, Genetic and epigenetic inactivation of mitotic checkpoint genes hBUB1 and hBUBR1 and their relationship to survival, Cancer Res. 62 (2002) 13–17.
[230] T.H. Wang, H.S. Wang, Y.K. Soong, Paclitaxel-induced cell death: where the cell cycle and apoptosis come together, Cancer 88 (2000) 2619–2628.
[231] C. Swanton, I. Tomlinson, J. Downward, Chromosomal instability, colorectal cancer and taxane resistance, Cell Cycle 5 (2006) 818–823.
[232] C. Swanton, M. Marani, O. Pardo, P.H. Warne, G. Kelly, E. Sahai, F. Elustondo, J. Chang, J. Temple, A.A. Ahmed, J.D. Brenton, J. Downward, B. Nicke, Regulators of mitotic arrest and ceramide metabolism are determinants of sensitivity to paclitaxel and other chemotherapeutic drugs, Cancer Cell 11 (2007) 498–512.
[233] A.W. Whitehurst, B.O. Bodemann, J. Cardenas, D. Ferguson, L. Girard, M. Peyton, J.D. Minna, C. Michnoff, W. Hao, M.G. Roth, X.-J. Xie, M.A. White, Synthetic lethal screen identification of chemosensitizer loci in cancer cells, Nature 446 (2007) 815–819.
[234] T.F. Westbrook, F. Stegmeier, S.J. Elledge, Dissecting cancer pathways and vulnerabilities with RNAi, Cold Spring Harb. Symp. Quant. Biol. 70 (2005) 435–444.
[235] Y. Du, F. Yin, C. Liu, S. Hu, J. Wang, H. Xie, L. Hong, D. Fan, Depression of MAD2 inhibits apoptosis of gastric cancer cells by upregulating Bcl-2 and interfering mitochondrion pathway, Biochem. Biophys. Res. Commun. 345 (2006) 1092–1098.
[236] J.G. Chen, C.P. Yang, M. Cammer, S.B. Horwitz, Gene expression and mitotic exit induced by microtubule-stabilizing drugs, Cancer Res. 63 (2003) 7891–7899.
[237] E.A. Nigg, Mitotic kinases as regulators of cell division and its checkpoints, Nat. Rev. Mol. Cell Biol. 2 (2001) 21–32.
[238] S.C. Shen, T.S. Huang, S.H. Jee, M.L. Kuo, Taxol-induced p34cdc2 kinase activation and apoptosis inhibited by 12-*O*-tetradecanoylphorbol-13-acetate in human breast MCF-7 carcinoma cells, Cell Growth Differ. 9 (1998) 23–29.
[239] T. Kasai, Y. Iwanaga, I. Hidekatsu, K.-T. Jeang, Prevalent loss of mitotic spindle checkpoint in adult T-cell leukemia confers resistance to microtubule inhibitors, J. Biol. Chem. 277 (2002) 5187–5193.
[240] B. Yuan, Y. Xu, J.-H. Woo, Y. Wang, Y.K. Bae, D.-S. Yoon, R.P. Wersto, E. Tully, K. Wilsbach, E. Gabrielson, Increased expression of mitotic checkpoint genes in breast cancer cells with chromosomal instability, Clin. Cancer Res. 12 (2006) 405–410.
[241] D.C. Altieri, The case for survivin as a regulator of microtubule dynamics and cell-death decisions, Curr. Opin. Cell Biol. 18 (2006) 609–615.
[242] V.E. Velculescu, S.L. Madden, L. Zhang, A.E. Lash, J. Yu, C. Rago, A. Lal, C.J. Wang, G.A. Beaudry, K.M. Ciriello, B.P. Cook, M.R. Dufault, A.T. Ferguson, Y. Gao, T.-C. He, H. Hermeking, S.K. Hiraldo, P.M. Hwang, M.A. Lopez, H.F. Luderer, B. Mathews, J.M. Petroziello, K. Polyak, L. Zawel, W. Zhang, X. Zhang, W. Zhou, F.G. Haluska, J. Jen, S. Sukumar, G.M. Landes, G.J. Riggins, B. Vogelstein, K.W. Kinzler, Analysis of human transcriptomes, Nat. Genet. 23 (1999) 387–388.
[243] P. Fortugno, N.R. Wall, A. Giodini, D.S. O' Connor, J. Plescia, K.M. Padgett, S. Tognin, P.C. Marchisio, D.C. Altieri, Survivin exists in immunochemically distinct subcellular pools and is involved in spindle microtubule function, J. Cell Sci. 115 (2002) 575–585.
[244] P. Vagnarelli, W.C. Earnshaw, Chromosomal passengers: the four-dimensional regulation of mitotic events, Chromosoma 113 (2004) 211–222.
[245] R. Honda, R. Kormer, E.A. Nigg, Exploring the functional interactions between aurora B, INCENP, and survivin in mitosis, Mol. Biol. Cell 14 (2003) 3325–3341.
[246] S.P. Wheatley, A.J. Henzing, H. Dodson, W. Khaled, W.C. Earnshaw, Aurora-B phosphorylation *in vitro* identifies a residue of survivin that is essential for its localization and binding to inner centromere protein (INCENP) *in vivo*, J. Biol. Chem. 279 (2004) 5655–5660.
[247] S.M. Lens, R.H. Medema, The survivin/Aurora B complex: its role in coordinating tension and attachment, Cell Cycle 6 (2003) 507–510.
[248] D.S. O' Connor, D. Grossman, J. Plescia, F. Li, H. Zhang, A. Villa, S. Tognin, P.C. Marchisio, D.C. Altieri, Regulation of apoptosis at cell division by p34cdc2 phosphorylation of survivin, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 13103–13107.
[249] A. Carvalho, M. Carmena, C. Sambade, W.C. Earnshaw, S.P. Wheatley, Survivin is required for stable checkpoint activation in taxol-treated HeLa cells, J. Cell Sci. 116 (2003) 2987–2998.
[250] S. Noguchi, Predictive factors for response to docetaxel in human breast cancers, Cancer Sci. 97 (2006) 813–820.
[251] H. Katayama, K. Sasai, H. Kawai, Z.M. Yuan, J. Bondaruk, F. Suzuki, S. Fujii, R.B. Arlinghaus, B.A. Czerniak, S. Sen, Phosphorylation by aurora kinase A induces Mdm2-mediated destabilization and inhibition of p53, Nat. Genet. 36 (2004) 55–62.
[252] F. Sessa, M. Mapelli, C. Ciferri, C. Tarricone, L.B. Areces, T.R. Schneider, T. Todd Stukenberg, A. Musacchio, Mechanism of Aurora B inactivation by INCENP and inhibition by Hesperadin, Mol. Cell 18 (2005) 379–391.
[253] C. Lavedan, E. Leroy, A. Dehejia, S. Buchholtz, A. Dutra, R.L. Nussbaum, Identification, localization and characterization of the human gamma-synuclein gene, Hum. Genet. 103 (1998) 106–112.
[254] J.E. Galvin, V.M. Lee, J.Q. Trojanowski, Synucleinopathies: clinical and pathological implications, Arch. Neurol 58 (2001) 186–190.
[255] H. Liu, W. Liu, Y. Wu, Y. Zhou, R. Xue, C. Luo, L. Wang, W. Zhao, J.D. Jiang, J. Liu, Loss of epigenetic control of synuclein-gamma gene as a

molecular indicator of metastasis in a wide range of human cancers, Cancer Res. 65 (2005) 7635–7643.
[256] A. Gupta, S. Inaba, O.K. Wong, G. Fang, J. Liu, Breast cancer-specific gene 1 interacts with the mitotic checkpoint kinase BubR1, Oncogene 22 (2003) 7593–7599.
[257] S. Inaba, C. Li, Y.E. Shi, D.Q. Song, J.D. Jiang, J. Liu, Synuclein gamma inhibits the mitotic checkpoint function and promotes chromosomal instability of breast cancer cells, Breast Cancer Res. Treat 94 (2005) 25–35.
[258] W. Bruening, B.I. Giasson, A.J.P. Klein-Szanto, V.M.-Y. Lee, J.Q. Trojanowski, A.K. Godwin, Synucleins are expressed in the majority of breast and ovarian carcinomas and in preneoplastic lesions of the ovary, Cancer 88 (2000) 2154–2163.
[259] Z.Z. Pan, W. Bruening, B.I. Giasson, V.M. Lee, A.K. Godwin, Gamma-synuclein promotes cancer cell survival and inhibits stress- and chemotherapy drug-induced apoptosis by modulating MAPK pathways, J. Biol. Chem. 277 (2002) 35050–35060.
[260] Y. Zhou, S. Inaba, J. Liu, Inhibition of synuclein-gamma expression increases the sensitivity of breast cancer cells to paclitaxel treatment, Int. J. Oncol. 29 (2006) 289–295.
[261] V.K. Singh, Y. Zhou, J.A. Marsh, V.N. Uversky, J.D. Forman-Kay, J. Liu, Z. Jia, Synuclein-gamma targeting peptide inhibitor that enhances sensitivity of breast cancer cells to antimicrotubule drugs, Cancer Res. 67 (2007) 626–633.
[262] A.R. Venkitaraman, Cancer susceptibility and the functions of BRCA1 and BRCA2, Cell 108 (2002) 171–182.
[263] D.B. Longley, P.G. Johnston, M. Wilson, M. Mc Ewan, W.L. Allen, U. Mc Dermot, L. Galligan, P.G. Johnston, Molecular mechanisms of drug resistance, J. Pathol. 205 (2005) 275–292.
[264] P.B. Mullan, J.E. Quinn, P.M. Gilmore, S. Mc Williams, H. Andrews, C. Gervin, N. Mc Cabe, S. Mc Kenna, P. White, Y.-H. Song, S. Maheswaran, E. Liu, D.A. Haber, P.G. Johnson, D.P. Harkin, BRCA1 and GADD45 mediated G2/M cell cycle arrest in response to antimicrotubule agents, Oncogene 20 (2001) 6123–6131.
[265] D.P. Harkin, J.M. Bean, D. Miklos, Y.H. Song, V.B. Truong, C. Englert, F.C. Christians, L.W. Ellisen, S. Maheswaran, J.D. Oliner, D.A. Haber, Induction of GADD45 and JNK/SAPK-dependent apoptosis following inducible expression of BRCA1, Cell 97 (1999) 575–586.
[266] M. Thangaraju, S.H. Kaufmann, F.J. Couch, BRCA1 facilitates stress-induced apoptosis in breast and ovarian cancer cell lines, J. Biol. Chem. 275 (2000) 33487–33496.
[267] P.M. Gilmore, N. Mc Cabe, J.E. Quinn, R.D. Kennedy, J.J. Gorski, H.N. Andrews, S. Mc Williams, M. Carty, P.B. Mullan, W.P. Duprex, E.T. Liu, P.G. Johnston, D.P. Harkin, BRCA1 interacts with and is required for paclitaxel-induced activation of mitogen-activated protein kinase kinase kinase 3, Cancer Res. 64 (2004) 4148–4154.
[268] L.F. Lee, G. Li, D.J. Templeton, J.P.Y. Ting, Paclitaxel (taxol)-induced gene expression and cell death are both mediated by the activation of c-jun NH2-terminal kinase (JNK/SAPK), J. Biol. Chem. 273 (1998) 28253–28260.
[269] S. Wang, Z. Wang, L. Boise, P. Dent, S. Grant, Loss of the bcl-2 phosphorylation loop domain increases resistance of human leukemia cells (U937) to paclitaxel-mediated mitochondrial dysfunction and apoptosis, Biochem. Biophys. Res. Commun 259 (1999) 67–72.
[270] Y.M. Ham, J.S. Choi, K.H. Chun, S.H. Joo, S.K. Lee, The c-jun N-terminal kinase 1 activity is differentially regulated by specific mechanisms during apoptosis, J. Biol. Chem. 278 (2003) 50330–50337.
[271] P.B. Mullan, J.J. Gorski, P. Harkin, BRCA1 — a good predictive marker of drug sensitivity in breast cancer treatment? Biochim. Biophys.Acta 1766 (2006) 205–216.
[272] J.E. Quinn, R.D. Kennedy, P.B. Mullan, P.M. Gilmore, M. Carty, P.G. Johnston, P. Harkin, BRCA1 functions as a differential modulator of chemotherapy-induced apoptosis, Cancer Res. 63 (2003) 6221–6228.
[273] S. Lafarge, V. Sylvain, M. Ferrara, Y.J. Bignon, BRCA1 leads to increased chemoresistance to microtubule-interfering agents, an effect that involves the JNK pathway, Oncogene 20 (2001) 6597–6606.
[274] P. Tassone, P. Tagliaferri, A. Perricelli, S. Blotta, B. Quaresima, M.L. Marrelli, A. Goel, V. Barbieri, F. Costanzo, C.R. Boland, S. Venura, BRCA1 expression modulates chemosensitivity of BRCA1-defective HCC1937 human breast cancer cells, Br. J. Cancer 88 (2003) 1285–1291.
[275] C. Chabalier, C. Lamare, C. Racca, M. Privat, A. Valette, F. Larminat, BRCA1 downregulation leads to premature inactivation of spindle checkpoint and confers paclitaxel resistance, Cell Cycle 5 (2006) 1001–1007.
[276] R.H. Wang, H. Yu, C.X. Deng, A requirement for breast-cancer-associated gene 1 (BRCA1) in the spindle checkpoint, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 17108–17113.
[277] L.C. Hsu, T.P. Doan, R.L. White, Identification of a gamma-tubulin-binding domain in BRCA1, Cancer Res. 61 (2001) 7713–7718.
[278] C.X. Deng, Roles of BRCA1 in centrosome duplication, Oncogene 21 (2002) 6222–6227.
[279] A.J. Levine, p53, the cellular gatekeeper for growth and division, Cell 88 (1997) 323–331.
[280] W. El-Diery, T. Tokino, V.E. Velculescu, D.B. Levy, R. Parsons, J.M. Trent, D. Lin, W.E. Mercer, K.W. Kinsler, B. Vogelstein, WAF1, a potential mediator of p53 tumour suppression, Cell 75 (1993) 817.
[281] B. Vogelstein, D. Lane, A.J. Levine, Surfing the p53 network, Nature 408 (2000) 307–310.
[282] K. Lohr, C. Moritz, A. Contente, M. Dobbelstein, p21/CDKN1A mediates negative regulation of transcription by p53, J. Biol. Chem. 29 (2003) 32507–32516.
[283] A.F. Wahl, K.L. Donaldson, C. Fairchild, F.Y. Lee, S.A. Foster, G.W. Demers, D.A. Galloway, Loss of normal p53 function confers sensitization to taxol by increasing G2/M arrest and apoptosis, Nat. Med. 2 (1996) 72–79.
[284] V. Rantanen, P. Ehgblom, M. Raitanen, S. Hietanen, M. Haarala, S. Grenman, S. Syrjanen, Mutations of TP53 do not correlate with the sensitivity to paclitaxel — a study using 27 gynaecological cancer cell lines, Eur. J. Cancer 38 (2002) 1783–1791.
[285] C.M. Woods, J. Zhu, P.A. Mc Queney, D. Bollag, E. Lazarides, Taxol-induced mitotic block triggers rapid onset of a p53-independent apoptotic pathway, Mol. Med. 5 (1995) 506–526.
[286] D. Debernardis, E.G. Sire, P. De Feudis, F. Vikhanskaya, M. Valenti, P. Russo, S. Parodi, M. D' Incalci, M. Broggini, p53 status does not affect sensitivity of human ovarian cancer cell lines to paclitaxel, Cancer Res. 57 (1997) 870–874.
[287] D. Hawkins, G. Demers, D.A. Galloway, Inactivation of p53 enhances sensitivity to multiple chemotherapeutic agents, Cancer Res. 56 (1996) 892–898.
[288] F. Vikhanskaya, S. Vignati, P. Beccaglia, C. Ottoboni, P. Russo, M. D'incalci, M. Broggini, Inactivation of p53 in a human ovarian cancer cell line increases the sensitivity to paclitaxel by inducing G2/M arrest and apoptosis, Exp. Cell Res. 241 (1998) 96–101.
[289] T. Strobel, S.K. Kraeft, L.B. Chen, S.A. Cannistra, BAX expression is associated with enhanced intracellular accumulation of paclitaxel: a novel role for BAX during chemotherapy-induced cell death, Cancer Res. 58 (1998) 4776–4781.
[290] A. Gualberto, K. Aldape, K. Kozakiewicz, T.D. Tisty, An oncogenic form of p53 confers a dominant, gain-of-function phenotype that disrupts spindle checkpoint control, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 5166–5171.
[291] C. Lavarino, S. Pilotti, M. Oggionni, L. Gatti, P. Perego, G. Bresciani, M.A. Pierotti, G. Scambia, G. Ferrandina, A. Fagotti, C. Mangioni, V. Lucchini, F. Vecchione, G. Bolis, G. Scarfone, F. Zunino, p53 gene status and response to platinum/paclitaxel-based chemotherapy in advanced ovarian carcinoma, J. Clin. Oncol. 18 (2000) 3936–3945.
[292] A. Gadducci, C. Cianci, S. Cosio, F. Carnino, A. Fanucchi, F. Buttitta, P.F. Conte, A.R. Genazzani, p53 status is neither a predictive nor a prognostic variable in patients with advanced ovarian cancer treated with a paclitaxel-based regime, Anticancer Res. 20 (2000) 4793–4799.
[293] M. Schmidt, A. Bachhuber, A. Victor, E. Steiner, M. Mahlke, H.A. Lehr, H. Pilch, W. Weikel, P.G. Knapstein, p53 expression and resistance against paclitaxel in patients with metastatic breast cancer, J. Cancer Res. Clin. Oncol. 129 (2003) 295–302.
[294] C. Sezgin, B. Karabulut, R. Uslu, U.A. Sanli, G. Goksel, O. Zekioglu, N. Ozdemir, E. Goker, Potential predictive factors for response to weekly paclitaxel treatment in patients with metastatic breast cancer, J. Chemother. 17 (2005) 103.

[295] K. Bhalla, Microtubule-targeted anticancer agents and apoptosis, Oncogene 22 (2003) 9075–9086.
[296] A. Strasser, L. O' Connor, V.M. Dixit, Apoptosis signaling, Annu. Rev. Biochem 69 (2000) 217–245.
[297] H.J. Brady, G. Gil-Gomez, The cell cycle and apoptosis, Results Probl. Cell Differ. 23 (1999) 127–144.
[298] D.R. Green, J.C. Reed, Mitochondria and apoptosis, Science 281 (1998) 1309–1312.
[299] N. Zamzami, G. Kroemer, The mitochondrion in apoptosis: how Pandora's box open, Nat. Rev. Mol. Cell 2 (2001) 67–71.
[300] X. Wang, The expanding role of mitochondria in apoptosis, Genes Dev. 15 (2001) 2922–2933.
[301] S.M. Srinivasula, M. Ahmad, T. Fernandes-Alnemri, E.S. Alnemri, Autoactivation of procaspase-9 by Apaf-1-mediated oligerization, Mol. Cell 1 (1998) 949–957.
[302] A.D. Schimmer, Inhibitor of apoptosis proteins: translating basic knowledge into clinical practice, Cancer Res. 64 (2004) 7183–7190.
[303] M. Irmler, M. Thome, M. Hahne, P. Schneider, K. Hofmann, V. Steiner, J.L. Bodmer, M. Schroter, K. Burns, C. Mattmann, D. Rimoldi, L.E. French, J. Tschopp, Inhibition of death receptor signals by cellular FLIP, Nature 388 (1997) 190–195.
[304] S. Hu, C. Vincenz, J. Ni, R. Gentz, V.M. Dixit, I-FLICE, a novel inhibitor of tumour necrosis factor receptor-1 and CD-95-induced apoptosis, J. Biol. Chem. 272 (1997) 17255–17257.
[305] D.B. Longley, T.R. Wilson, M. Mc Ewan, W.L. Allen, U. Mc Dermott, L. Galligan, P.G. Johnston, c-FLIP inhibits chemotherapy-induced colorectal cancer cell death, Oncogene 25 (2005) 838–848.
[306] J.M. Adams, S. Cory, The Bcl-2 protein family — arbiters of cell survival, Science 281 (1998) 1322–1326.
[307] A. Gross, J.M. Mc Donnell, S.J. Korsmeyer, BCL-2 family members and the mitochondria in apoptosis, Genes Dev. 13 (1999) 1899–1911.
[308] T.H. Wang, H.S. Wang, H. Ichijo, Microtubule-interfering agents activate c-Jun-N-terminal kinase/stress-activated protein kinase through both Ras and apoptosis signal-regulating pathways, J. Biol. Chem. 273 (1998) 4928–4936.
[309] T.-H. Wang, D.M. Popp, H.-S. Wang, M. Saitoh, J.G. Mural, D.C. Henley, H. Ichijo, J. Wimalasena, Microtubule dysfunction induced by paclitaxel initiates apoptosis through both c-Jun N-terminal kinase (JNK)-dependent and -independent pathways in ovarian cancer cells, J. Biol. Chem. 274 (1999) 8208–8216.
[310] K. Yamamoto, H. Ichijo, S.J. Korsmeyer, BCL-2 is phosphorylated and inactivated by an ASK1/Jun N-Terminal protein kinase pathway normally activated at G2/M, Mol. Cell. Biol. 19 (1999) 8469–8478.
[311] J.A. Mc Cubrey, L.S. Steelman, W.H.. Chappell, S.L. Abrams, E.W.T. Wong, F. Chang, B. Lehmann, D.M. Terrian, M. Milella, A. Tafuri, F. Stivala, M. Libra, J. Basecke, C. Evangelisti, A.M. Martelli, R.A. Franklin, Roles of the Raf/MEK/ERK pathway in cell growth, malignant transformation and drug resistance, Biochim. Biophys. Acta 1773 (2007) 1263–1284.
[312] A. Basu, G. Dubois, S. Haldar, Posttranslational modifications of Bcl-2 family members — a potential therapeutic target for human malignancy, Front. Biosci 11 (2006) 1508–1521.
[313] F. Luciano, A. Jacquel, P. Colosetti, M. Herrant, S. Cagnol, G. Pages, P. Auberger, Phosphorylation of Bim-EL by Erk1/2 on serine 69 promotes its degradation via the proteosome pathway and regulates its proapoptotic function, Oncogene 22 (2003) 6785–6793.
[314] H. Harada, B. Quearry, A. Ruiz-Vela, S.J. Korsmeyer, Survival factor-induced extracellular signal-regulated kinase phosphorylates BIM, inhibiting its association with BAX and proapoptotic activity, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 15313–15317.
[315] J. Zha, E. Harada, E. Yang, J. Jockel, S.J. Korsmeyer, Serine phosphorylation of death agonist BAD in response to survival factor results in binding to 14-3-3 not Bcl-xl, Cell 87 (1996) 589–592.
[316] T. Ito, X. Deng, B. Carr, W.S. May, Bcl-2 phosphorylation required for anti-apoptosis function, J. Biol. Chem. 272 (1997) 11671–11673.
[317] S. Haldar, A. Basu, C.M. Croce, Serine-70 is one of the critical sites for drug induced phosphorylation in cancer cells, Cancer Res. 58 (1998) 1609–1615.
[318] J.P. Mackeigan, T.S. Collins, J.P.-Y. Ting, MEK inhibition enhances paclitaxel-induced tumor apoptosis, J. Biol. Chem. 275 (2000) 38953–38956.
[319] H.M. Mc Daid, S.B. Howwitz, Selective potentiation of paclitaxel (taxol)-induced cell death by mitogen-activated protein kinase inhibition in human cancer cell lines, Mol. Pharmacol. 60 (2001) 290–301.
[320] P.M. Lorusso, A.A. Adjei, M. Varterasian, S. Gadgeel, J. Reid, D.Y. Mitchell, L. Hanson, P. Deluca, L. Bruzek, J. Piens, P. Asbury, K. Van Becelaere, R. Herrera, J. Sebolt-Leopold, M.B. Meyer, Phase I and pharmacodynamic study of the oral MEK inhibitor CI-1040 in patients with advanced malignancies, J. Clin. Oncol. 23 (2005) 5281–5293.
[321] J. Rinehart, A.A. Adjei, P.M. Lorusso, D. Waterhouse, J.R. Hecht, R.B. Natale, O. Hamid, M. Varterasian, P. Asbury, E.P. Kaldjian, S. Gulyas, D.Y. Mitchell, R. Herrera, J.S. Sebolt-Leopold, M.B. Meyer, Multicenter phase II study of the oral MEK inhibitor, CI-1040, in patients with advanced non-small-cell lung, breast, colon, and pancreatic cancer, J. Clin. Oncol. 22 (2004) 4456–4462.
[322] J.A. Fresno Vara, E. Casado, J. De Castro, P. Cejas, C. Belda-Iniesta, M. Gonzalez-Baron, PI3K/Akt signaling pathway and cancer, Cancer Treat. Rev. 30 (2004) 193–204.
[323] I. Vivanco, C.L. Sawyers, The phosphatidylinositol 3-kinase-Akt pathway in human cancer, Nat. Rev. Cancer 2 (2002) 489–501.
[324] F. Chang, J.T. Lee, P.M. Navolanic, L.S. Steelman, J.G. Shelton, W.L. Blalock, R.A. Franklin, J.A. Mc Cubrey, Involvement of the P13K/Akt pathway in cell cycle progression, apoptosis, and neoplastic transformation is a target for cancer chemotherapy, Leukaemia 17 (2003) 590–603.
[325] A.S. Clark, K. West, S. Streicher, P.A. Dennis, Constitutive and inducible Akt activity promotes resistance to chemotherapy, trastuzumab, or tamoxifen in breast cancer cells, Mol. Cancer Ther. 1 (2002) 707–717.
[326] C. Page, H.J. Lin, Y. Jin, V.P. Castle, G. Nunez, M. Hunang, J. Lin, Overexpression of Akt/AKT can modulate chemotherapy-induced apoptosis, Anticancer Res. 20 (2000) 407–416.
[327] L. Hu, J. Hofmann, Y. Lu, G.B. Mills, R.B. Jaffe, Inhibition of phosphatidylinositol 3′-kinase increases efficacy of paclitaxel in in vitro and in vivo ovarian cancer models, Cancer Res. 62 (2002) 1087–1092.
[328] T. Shingu, K. Yamada, N. Hara, K. Moritake, H. Osago, M. Terashima, T. Uemura, T. Yamasaki, M. Tsuchiya, Synergistic augmentation of anti-microtubule agent-induced cytotoxicity by a phosphoinositide 3-kinase inhibitor in human malignant glioma cells, Cancer Res. 63 (2003) 4044–4047.
[329] J.Q. Cheng, C.W. Lindsley, G.Z. Cheng, H. Yang, S.V. Nicosia, The Akt/PKB pathway: molecular target for cancer drug discovery, Oncogene 24 (2005) 7482–7492.
[330] D.M. Nguyen, G.A. Chen, R. Reddy, W. Tsai, W.D. Schrump, G. Cole, D.S. Schrump, Potentiation of paclitaxel cytotoxicity in lung and esophageal cancer cells by pharmacologic inhibition of the phosphoinositide 3-kinase/protein kinase B (Akt)-mediated signaling pathway, J. Thoracic Cardiovasc. Surg 127 (2004) 365–375.
[331] K.M. Yamada, M. Araki, PTEN: modulator of cell signaling, growth, migration and apoptosis, J. Cell Sci. 114 (2001) 2375–2382.
[332] Y. Lu, Y.Z. Lin, R. La Pushin, B. Cuevas, X. Fang, S.X. Yu, M.A. Davies, H. Khan, T. Furui, M. Mao, R. Zinner, M.C. Hung, P. Steck, K. Siminovitch, G.B. Mills, The PTEN/MMAC1/TEP tumor suppressor gene decreases cell growth and induces apoptosis and anoikis in breast cancer cells, Oncogene 18 (1999) 7034–7045.
[333] S.S. Ng, M.S. Tsao, T. Nicklee, D.W. Hedley, Wortmannin inhibits pkb/akt phosphorylation and promotes gemcitabine antitumour activity in orthotopic human pancreatic cancer xenografts in immunodeficient mice, Clin. Cancer Res. 7 (2001) 3269–3275.
[334] T. Fujita, H. Doihara, K. Kawasaki, D. Takabatake, H. Takahashi, K. Washio, K. Tsukuda, Y. Ogasawara, N. Shimizu, PTEN activity could be a predictive marker of trastuzumab efficacy in the treatment of ErB2-overexpressing breast cancer, Br. J. Cancer 94 (2006) 247–252.
[335] N. Pathan, C. Aime-Sempe, A. Basu, S. Haldar, J.C. Reed, Microtubule targeting drugs induce Bcl2 phosphorylation and association with Pin1, Neoplasia 3 (2001) 550–559.
[336] M.V. Blagosklonny, P. Giannakakou, W.S. El-Deiry, D.G.I. Kingston, P.I. Higgs, L. Neckers, T. Fozo, Raf-1/Bcl-2 phosphorylation: a step from microtubule damage to cell death, Cancer Res. 57 (1997) 130–135.

[337] L. Asnaghi, A. Calastretti, A. Bevilacqua, I. D' Agnano, G. Gatti, G. Canti, D. Delia, S. Capaccioli, A. Nicolin, Bcl-2 phosphorylation and apoptosis activated by damaged microtubules require mTOR and are regulated by Akt, Oncogene 23 (2004) 5781–5791.
[338] D.J. Rodi, R.W. Janes, H.J. Sanganee, R.A. Holton, B.A. Wallace, L. Makawski, Screening of a library of phage-displayed peptides identifies human bcl-2 as a taxol-binding protein, J. Mol. Biol. (1999) 197–203.
[339] J.H. Wu, G. Batist, L.O. Zamir, A model for the interaction of paclitaxel with the Bcl-2 loop domain: a chemical approach to induce conformation-dependent phosphorylation, Anticancer Drug Des. 15 (2000) 441–446.
[340] M. Shitashige, M. Toi, T. Yano, M. Shibata, T. Matsuo, F. Shibasaki, Dissociation of Bax from a Bcl-2/Bax heterodimer triggered by phosphorylation of serine 70 of Bcl-2, J. Biochem. (Tokyo) 130 (2001) 741–748.
[341] H. Ueno, E. Kondo, R. Yamamoto-Honda, K. Tobe, T. Nakamoto, K. Sasaki, K. Mitani, A. Furusaka, T. Tanaka, Y. Tsujimoto, T. Kadowaki, H. Hirai, Association of insulin receptor substrate proteins with Bcl-2 and their effects on its phosphorylation and antiapoptotic function, Mol. Biol. Cell 11 (2000) 735–746.
[342] D.C. Altieri, Validating survivin as a cancer therapeutic target, Nat. Rev. Cancer 3 (2003) 46–54.
[343] S.P. Wheatley, I.A. Mc Neish, Survivin: a protein with dual roles in mitosis and apoptosis, Int. Rev. Cytol. 247 (2005) 35–88.
[344] F. Li, G. Ambrosini, E.Y. Chu, J. Plescia, S. Tognin, P.C. Marchisio, D.C. Altieri, Control of apoptosis and mitotic spindle checkpoint by survivin, Nature 396 (1998) 580–584.
[345] S. Stewart, G. Fang, Destruction box-dependent degradation of aurora B is mediated by the anaphase-promoting complex/cyclosome and Cdh1, Cancer Res. 65 (2005) 8730–8735.
[346] M. Zaffaroni, M. Pennati, G. Colella, P. Perego, R. Supino, L. Gatti, S. Pilotti, F. Zunino, M.G. Daidone, Expression of the anti-apoptotic gene survivin correlates with taxol resistance in human ovarian cancer, Cell Mol. Life Sci. 59 (2002) 1406–1412.
[347] Y. Fujie, H. Yamamoto, C.Y. Ngan, A. Takagi, T. Hayashi, R. Suzuki, K. Ezumi, I. Takemasa, M. Ikeda, M. Sekimoto, N. Matsuura, M. Monden, Oxaliplatin, a potent inhibitor of survivin, enhances paclitaxel-induced apoptosis and mitotic catastrophe in colon cancer cells, Jpn. J. Clin. Oncol. 35 (2005) 453–463.
[348] J. Kato, Y. Kuwabara, M. Mitani, N. Shinoda, A. Sato, T. Toyama, A. Mitsui, T. Nishiwaki, S. Moriyama, J. Kudo, Y. Fujii, Expression of survivin in esophageal cancer: correlation with the prognosis and response to chemotherapy, Int. J. Cancer 95 (2001) 92–95.
[349] A. Lin, M. Karin, NF-kappaB in cancer: a marked target, Semin. Cancer Biol 13 (2003) 107–114.
[350] S.E. Chuang, P.Y. Yeh, Y.S. Lu, G.M. Lai, C.M. Liao, M. Gao, A.L. Cheng, Basal levels and patterns of anticancer drug-induced activation of nuclear factor-kappaB (NF-kappaB), and its attenuation by tamoxifen, dexamethasone and curcumin in carcinoma cells, Biochem. Pharmacol. 63 (2002) 1709–1716.
[351] A.S. Baldwin, Control of oncogenesis and cancer therapy resistance by the transcriptional factor NF-kappaB, J. Clin. Invest. 107 (2001) 241–246.
[352] Y. Huang, W. Fan, IkK kinase activation is involved in regulation of paclitaxel-induced apoptosis in human tumour cell lines, Mol. Pharmacol. 61 (2002) 105–113.
[353] S. Mabuchi, M. Ohmichi, Y. Nishio, T. Hayasaka, A. Kimura, T. Ohta, J. Kawagoe, K. Takahashi, N. Yada-Hashimoto, H. Seino-Noda, M. Sakata, T. Motoyama, H. Kurachi, J.R. Testa, K. Tasaka, Y. Murata, Inhibition of inhibitor of nuclear factor-kappaB phosphorylation increases the efficacy of paclitaxel in in vitro and in vivo ovarian cancer models, Clin. Cancer Res. 10 (2004) 7645–7654.
[354] Q.G. Dong, G.M. Sclabas, S. Fujioka, C. Schmidt, B. Peng, T. Wu, M.S. Tsao, D.B. Evans, J.L. Abbruzzese, T.J. Mc Donnell, P.J. Chiao, The function of multiple IkB:NF-kB complexes in the resistance of cancer cells to taxol-induced apoptosis, Oncogene 21 (2002) 6510–6519.
[355] S. Goble, H.D. Bear, Emerging role of taxanes in adjuvant and neoadjuvant therapy for breast cancer: the potential and the questions, Surg. Clin. North Am. 83 (2003) 943–971.
[356] A.U. Buzdar, S.E. Singletary, V. Valero, D.J. Booser, N.K. Ibrahim, Z. Rahman, R.L. Theriault, R. Walters, E. Rivera, T.L. Smith, F.A. Holmes, E. Hoy, D.K. Frye, N. Manuel, S.-W. Kau, M.D. Mcneese, E. Strom, E. Thomas, K. Hunt, F. Ames, D. Berry, G.N. Hortobagyi, Evaluation of paclitaxel in adjuvant chemotherapy for patients with operable breast cancer: preliminary data of a prospective randomized trial, Clin. Cancer Res. 8 (2002) 1073–1079.
[357] D.F. Hayes, A. Thor, L. Dressler, D. Weaver, G. Broadwater, L. Goldstein, S. Martino, J. Ingle, I.C. Henderson, D. Berry, HER2 predicts benefit from adjuvant paclitaxel after AC in node-positive breast cancer: CALGB 9344, J. Clin. Oncol. 24 (2006) abstract 510.
[358] T. Sorlie, C.M. Perou, R. Tibshirani, T. Aas, S. Geisler, H. Johnsen, T. Hastie, M.B. Eisen, M. Van De Rijn, S.S. Jeffrey, T. Thorsen, H. Quist, J.C. Matese, P.O. Brown, D. Botstein, P.E. Lonning, A.-L. Borresen-Dale, Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 10869–10874.
[359] M. West, C. Blanchette, H. Dressman, E. Huang, S. Ishida, R. Spang, H. Zuzan, J.A. Olson Jr., J.R. Marks, J.R. Nevins, Predicting the clinical status of human breast cancer by using gene expression profiles, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 11462–11467.
[360] L.J. Van't Veer, H. Dai, M.J. Van De Vijver, Y.D. He, A.A. Hart, M. Mao, H.L. Peterese, K. Van Der Kooy, M.J. Marton, A.T. Witteveen, G.J. Schreiber, R.M. Kerkhoven, C. Roberts, P.S. Linsley, R. Bernards, S.H. Friend, Gene expression profiling predicts clinical outcome of breast cancer, Nature 415 (2002) 530–536.
[361] E.P. Mamounas, J. Bryant, B.C. Lembersky, Paclitaxel (T) Following Doxorubicin/Cyclophosphamide (AC) as Adjuvant Chemotherapy for Node-Positive Breast Cancer: Results From NSA B-28, Proc. Am. Soc. Clin. Oncol., vol. 22, 2003, abstract 12.
[362] K.S. Albain, S.J. Green, P.M. Ravdin, Adjuvant Chemohormonal Therapy for Primary Breast Cancer Should be Sequential Instead of Concurrent: Initial Results From Intergroup Trial 0100 (SWOG-8814), Proc. Am. Society Clin. Oncol., 2002, abstract 143.
[363] M.L. Citron, D.A. Berry, C. Cirrincione, C. Hudis, E.P. Winer, W.J. Gradishar, N.E. Davidson, S. Martino, R. Livingston, J.N. Ingle, E.A. Perez, J. Carpenter, D. Hurd, J.F. Holland, B.L. Smith, C.I. Sartor, E.H. Leung, J. Abrams, R.L. Schilsky, H.B. Muss, L. Norton, Randomized trial of dose-dense versus conventionally scheduled and sequential versus concurrent combination chemotherapy as postoperative adjuvant treatment of node-positive primary breast cancer: First Report of Intergroup Trial C9741/Cancer and Leukemia Group B Trial 9741, J. Clin. Oncol. 21 (2003) 1431–1439.
[364] C. Hudis, M. Citron, Five-year follow-up of INT C9741: dose-dense chemotherapy is safe and effective, Breast Cancer Res. Treat. 89 (2005).
[365] M.L. Citron, D.A. Berry, C. Cirrincione, Dose-Dense (DD) AC Followed by Paclitaxel is Associated With More Frequent Moderate Anaemia Compared to Sequential (s) and/or Less DD Teatment: Udate by CALGB on Breast Cancer Intergroup Tial C9741 with ECOG, SWOG, and NCCTG, Proc. Am. Soc. Clin. Oncol., 24, 2005, Abstract 620.
[366] D.A. Berry, C. Cirrincione, I.C. Henderson, Effects of improvements in chemotherapy on disease-free and overall survival of estrogen receptor negative, node-positive breast cancer: 20-year experience of the CALGB and U.S. Breast Intergroup, Breast Cancer Res. Treat. 88 (2004) S17.
[367] H.D. Bear, S. Anderson, The effect on tumor response of adding sequential preoperative docetaxel to preoperative doxorubicin and cyclophosphamide: preliminary results from National Surgical Adjuvant Breast and Bowel Project Protocol B-27, J. Clin. Oncol. 21 (2003) 4165–4174.
[368] I.C. Smith, S.D. Heys, Neoadjuvant chemotherapy in breast cancer: significantly enhanced response with docetaxel, J. Clin. Oncol. 20 (2002) 1456–1466.
[369] H. Charfare, S. Limongelli, A.D. Purushotham, Neoadjuvant chemotherapy in breast cancer, Br. J. Surg. 92 (2005) 14–23.
[370] M. Marty, F. Cognetti, D. Maraninchi, R. Snyder, L. Mauriac, M. Tubiana-Hulin, S. Chan, D. Grimes, A. Anton, A. Lluch, J. Kennedy, K. O' Byrne, P. Conte, M. Green, C. Ward, K. Mayne, J.-M. Extra, Randomized phase II trial of the efficacy and safety of trastuzumab combined

with docetaxel in patients with human epidermal growth factor receptor 2-positive metastatic breast cancer administered as first-line treatment: the M77001 study group, J. Clin. Oncol. 23 (2005) 4265–4274.
[371] E.H. Romond, E.A. Perez, J. Bryant, V.J. Suman, C.E. Geyer Jr., N.E. Davidson, E. Tan-Chiu, S. Martino, S. Paik, P.A. Kaufman, S.M. Swain, T.M. Pisansky, L. Fehrenbacher, L.A. Kutteh, V.G. Vogel, D.W. Visscher, G. Yothers, R.B. Jenkins, A.M. Brown, S.R. Dakhil, E.P. Mamounas, W.L. Lingle, P.M. Klein, J.N. Ingle, N. Wolmark, Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer, N. Engl. J. Med. 353 (2005) 1673–1684.
[372] D. Slamon, W. Eiermann, N. Robert, T. Pienkowski, M. Martin, M. Pawlicki, A. Chan, M. Smylie, M. Liu, C. Falkson, T. Pinter, T. Fornander, T. Shiftan, V. Valero, G. Von Minckwitz, J. Mackey, I. Tabah-Fisch, M. Buyse, M.A. Lindsay, A. Riva, V. Bee, M. Pegram, M. Press, J. Crown, Phase III randomized trial comparing doxorubicin and cyclophosphamide followed by docetaxel (AC-T) with doxorubicin and cyclophosphamide followed by docetaxel and trastuzumab (TCH) with docetaxel, carboplatin and trastuzumab (TCH) in HER2 positive early breast cancer patients: BCIRG 006 study, Breast Cancer Res. Treat. (Suppl 1) (2005) S5.
[373] A. Goldhirsch, R.D. Gelber, A.S. Coates, B. Thurlimann, H.-J. Senn, Panel Members, Meeting highlights: international expert consensus on the primary therapy of early breast cancer 2005, Ann. Oncol. 16 (2005) 1569–1583.
[374] G. Szakacs, J.K. Paterson, J.A. Ludwig, C. Booth-Genthe, M.M. Gottesman, Targeting multidrug resistance in cancer, Nat. Rev. Drug Discov. 5 (2006) 219–234.
[375] D.A. Dart, S.M. Picksley, P.A. Cooper, J.A. Double, M.C. Bibby, The role of p53 in the chemotherapeutic responses to cisplatin, doxorubicin and 5-fluorouracil treatment, Int. J. Oncol. 24 (2004) 115–125.
[376] S. Fan, W.S. El-Deiry, I. Bae, J. Freeman, D. Jondle, K. Bhatia, A.J. Fornace Jr., I. Magrath, K.W. Kohn, P.M. O' Connor, p53 gene mutations are associated with decreased sensitivity of human lymphoma cells to DNA damaging agents, Cancer Res. 54 (1994) 5824–5830.
[377] A. Paradiso, G. Simone, S. Petroni, B. Leone, C. Vallejo, J. Lacava, A. Romero, M. Machiavelli, M. De Lena, C.J. Allegra, P.G. Johnston, Thymidylate synthase and p53 primary tumour expression as predictive factors for advanced colorectal cancer patients, Br. J. Cancer 82 (2000) 560–567.
[378] C.A. Schmitt, C.T. Rosenthal, S.W. Lowe, Genetic analysis of chemoresistance in primary murine lymphomas, Nat. Med. 6 (2000) 1029–1035.
[379] R.I. Yarden, S. Pardo-Reoyo, M. Sgagias, K.H. Cowan, L.C. Brody, BRCA1 regulates the G2/M checkpoint by activating Chk1 kinase upon DNA damage, Nat. Genet. 30 (2002) 285–289.
[380] E. Tothova, M. Fricova, N. Stecova, A. Kafkova, A. Elbertova, High expression of Bcl-2 protein in acute myeloid leukaemia cells is associated with poor response to chemotherapy, Neoplasma 49 (2002) 141–144.
[381] M. Colleoni, E. Orvieto, F. Nole, L. Orlando, I. Minchella, G. Viale, G. Peruzzotti, C. Robertson, C. Noberasco, V. Galimberti, V. Sacchini, P. Veronesi, S. Zurrida, R. Orecchia, A. Goldhirsch, Prediction of response to primary chemotherapy for operable breast cancer, Eur. J. Cancer 35 (1999) 574–579.
[382] R. Rouzier, C.M. Oerou, W.F. Symmans, N. Ibrahim, M. Cristofanilli, K. Anderson, K.R. Hess, J. Stec, M. Ayers, P. Wagner, P. Morandi, C. Fan, I. Rabiul, J.S. Ross, G.N. Hortobagyi, L. Pusztai, Breast cancer molecular subtypes respond differently to preoperative chemotherapy, Hum. Cancer Biol. 16 (2005) 5678–5685.
[383] D. Sampath, L.M. Greenberger, C. Beyer, M. Hari, H. Liu, M. Baxter, S. Yang, C. Rios, C. Discafani, Preclinical pharmacologic evaluation of MST-997, an orally active taxane with superior *in vitro* and *in vivo* efficacy in paclitaxel- and docetaxel-resistant tumor models, Clin. Cancer Res 12 (2006) 3459–3469.
[384] E. Galletti, M. Magnani, M.L. Renzulli, M. Botta, Paclitaxel and docetaxel resistance: molecular mechanisms and development of new generation taxanes, Chem. Med. Chem. (2007) 1–24.
[385] J.A. Low, S.B. Wedam, J.J. Lee, A.W. Berman, A. Brufsky, S.X. Yang, M.S. Poruchyusky, S.M. Steinberg, N. Manna, T. Fojo, Phase II clinical trial of ixabepilone (BMS-247550), and epothilone B analog, in metastatic and locally advanced breast cancer, J. Clin. Oncol. 23 (2005) 2726–2734.