Fighting tubulin-targeting anticancer drug toxicity and resistance

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Abstract

Tubulin-targeting drugs, like taxanes and vinca alkaloids, are among the most effective anticancer therapeutics used in the clinic today. Specifically, anti-microtubule cancer drugs (AMCDs) have proven to be effective in the treatment of castration-resistant prostate cancer and triple-negative breast cancer. AMCDs, however, have limiting toxicities that include neutropenia and neurotoxicity, and, in addition, tumor cells can become resistant to the drugs after long-term use. Co-targeting mitotic progression/slipage with inhibition of the protein kinases WEE1 and MYT1 that regulate CDK1 kinase activity may improve AMCD efficacy, reducing the acquisition of resistance by the tumor and side effects from the drug and/or its vehicle. Other possible treatments that improve outcomes in the clinic for these two drug-resistant cancers, including new formulations of the AMCDs and pursuing different molecular targets, will be discussed.

Introduction

Anti-microtubule cancer drugs (AMCDs: taxanes, vinca alkaloids, etc.) are used in first- or second-line therapeutic protocols, alone or in combination, to treat human cancer of different histotypes. Although not all the patients immediately respond to treatment, meaning that their cancer cells have naive resistance against these drugs, most patients do respond. Unfortunately, in a large number of cases, patients that initially respond to therapy acquire resistance at later stages. Moreover, AMCD use is often limited by severe side effects that force a reduction in dosage, often dropping below the level necessary to obtain significant therapeutic effects. Nevertheless, new and detailed information on the molecular mechanisms causing resistance and collateral damage is now offering opportunities for improving AMCD therapy. Here, taking prostate and breast cancers as examples, we will review how recent mechanistic information on AMCD actions are helping to identify new therapeutic strategies and targets to overcome AMCD resistance and toxicity.

AMCD use in prostate cancer therapy

Prostate cancer is the most common malignancy diagnosed and the third cause of cancer-related death in men (Siegel et al. 2017). Advances in screening and diagnosis have allowed detection of the disease in early stages in approximately 85% of cases. Radical prostatectomy, radiation therapy and active surveillance are treatments of
choice for early, organ-confined tumors that can be cured in the majority of the cases (Kahn et al. 2014). In about 30% of cases, however, the tumor relapses in 5–10 years, often at metastatic sites. Since prostate cancer is in most cases initially androgen sensitive, for these patients and for patients with a disseminated disease at the time of diagnosis, androgen deprivation by surgical or chemical castration is the treatment of choice (Amaral et al. 2012). Androgen deprivation can be achieved surgically, by orchiectomy, or chemically by targeting the androgen receptor (AR) pathway, either desensitizing it with luteinizing hormone-releasing hormone (LHRH) agonists or blocking it by LHRH antagonists or by antagonists of the AR (Pham et al. 2016). Androgen deprivation therapy is quite effective in inducing apoptosis of hormone-dependent cancer cells, resulting in tumor regression. However, most prostate cancers develop resistance to it. As illustrated in Fig. 1, acquired resistance is mostly the result of AR gene amplification or of point mutations and expression of splice variants coding for more sensitive, less specific or constitutively active AR proteins (Wadosky & Koochekpour 2016). Recently, many new treatments have been approved for therapy of castration-resistant prostate cancer (CRPC). After two successful clinical trials (Petrylak et al. 2004, Tannock et al. 2004), the taxane docetaxel in combination with prednisone was the first-line treatment approved by the FDA for patients with good performance status and with asymptomatic, minimally symptomatic or symptomatic CRPCs (Lowrance et al. 2016). Of note, a recent clinical trial has shown that docetaxel increases overall survival when administered with androgen deprivation therapy, suggesting it can also be beneficial for patients with hormone-sensitive prostate cancers (Sweeney et al. 2015). Taxanes, together with vinca alkaloids, are AMCDs. Microtubules are major components of the cytoskeleton. In interphase, they form fibers that function as tracks for the intracellular transport of proteins, vesicles and organelles. In mitosis, cytoskeleton is dramatically reorganized and microtubules are assembled to form the mitotic spindle, required for the congression of replicated chromosomes at the metaphase equator and, later, for the segregation of sister chromatids in anaphase. Microtubules are cylindrical tubes made of $\alpha$- and $\beta$-tubulin heterodimers (for more details on microtubule physiology, the readers may refer to de Forges et al. 2012, Forth & Kapoor 2017). Taxanes bind $\beta$-tubulin and stabilize pre-existing microtubules; vinca alkaloids bind to the vinca domain at the interface between $\alpha$- and $\beta$-tubulin, preventing microtubule polymerization (Lee et al. 2015, van Vuuren et al. 2015). Thus, by affecting microtubule dynamics, AMCDs interfere with many cellular key processes including assembly of the mitotic spindle. An incomplete or abnormal mitotic spindle activates the spindle assembly checkpoint (SAC), a safeguard mechanism that delays progression into anaphase until mitotic spindle assembly completion to prevent errors in chromosome segregation and generation of aneuploid cells (Musacchio & Salmon 2007). After substantial time of mitotic arrest induced by an active SAC, cells can die in a manner called mitotic catastrophe, an oncosuppressive mechanism initiated during the M phase, requiring a prolonged mitotic arrest.

![Figure 1](http://erc.endocrinology-journals.org)

**Figure 1**
Mechanisms of resistance to androgen deprivation therapy. Three main mechanisms of resistance to androgen deprivation therapy related to androgen receptor (AR) are depicted. Other mechanisms of resistance have been described, such as androgen-independent activation of AR mediated by oncogenic signaling (see text and Wadosky & Koochekpour 2016). (A) Castration-resistant prostate cells overexpress AR because of gene amplification; (B) point mutated ARs are constitutively active; (C) AR splice variants are ligand independent. The AR splice variant 7 (ARv7), one of the most clinically prevalent, is depicted. In any case, ARs bind to DNA, leading to a deregulated expression of androgen-target genes and, in turn, to aberrant proliferation of prostate cells. AR, androgen receptor; ARE, androgen-responsive element; DHT, dihydrotestosterone.
that acts to avoid genomic instability (Vitale et al. 2011). Thus, the AMCD-induced, SAC-mediated block in mitosis, resulting in mitotic catastrophe, provides a strong mechanistic rationale for the therapeutic use of AMCDs. Besides their effects on spindle assembly in mitosis, in the case of prostate cancer, taxanes also have some other key specific effects on AR signaling as a consequence of their action on microtubule-driven intracellular trafficking. AR interactions with microtubules and dynein motor protein are crucial for AR nuclear translocation upon ligand binding. Thus, the taxane-induced microtubule stabilization inhibits the translocation of the ligand-bound AR to the nucleus, preventing androgen-induced gene transcription (Darshan et al. 2011). Moreover, taxanes induce nuclear accumulation of FOXO1, a well-known AR repressor, thus further inhibiting AR transcriptional activity (Gan et al. 2009). Finally, it has been reported that docetaxel causes the downregulation of AR expression levels in prostate cancer cell lines (Kuroda et al. 2009).

Unfortunately, resistance to AMCDs is commonly seen in a wide variety of cancer patients, significantly reducing their clinical response (Gottesman et al. 2016). Resistance to AMCDs can be native, i.e. the patients do not respond at all to the first administration of therapy or acquired during or after treatment by initially susceptible patients. For instance, about 50% of CRPC patients do not respond at all to docetaxel therapy, a sign that their tumor cells have native resistance to taxane therapy. It has been hypothesized that these patients express constitutively active AR splice variants, selected by the androgen ablation therapy and responsible for disease progression, in addition to resistance to taxanes. Indeed, nuclear accumulation and transcriptional activity of the AR splice variant 7, one of the most clinically prevalent, is not affected by taxanes (Thadani-Mulero et al. 2014, Zhang et al. 2015). Accordingly, AR splice variant 7-expressing prostate tumor xenograft mice are resistant to docetaxel treatment (Thadani-Mulero et al. 2014).

Mechanisms of acquired resistance to AMCDs

Dismally, also patients that initially respond to AMCDs may later develop resistance. In about 50% of CRPC patients that respond to docetaxel, acquired resistance causes disease progression after approximately 8 months of therapy (Harrington & Jones 2011).

Four main mechanisms of acquired resistance to AMCDs have been described so far (Horwitz et al. 1993, Wani & Horwitz 2014, Barbuti & Chen 2015). We will highlight particular mechanisms of resistance to AMCDs in prostate cancer; however, some of these are common to the many cancer histotypes that acquire resistance to the drugs. First, AMCD-resistant tumors can upregulate the expression of the ABC transmembrane efflux transporters (Childs et al. 1998, Ambudkar et al. 1999). The enhanced efflux leads to a reduced intracellular concentration of the drug, below the level necessary to obtain an effect. To overcome ABC transporter-mediated resistance, various approaches have been exploited, including the development of drugs that specifically inhibit ABC family members. However, up to now, most of the phase III clinical trials with such inhibitors have failed (Robert & Jarry 2003, Shukla et al. 2011, Bugde et al. 2017). Interestingly, it has been recently reported that the antiandrogens bicalutamide and enzalutamide inhibit ABCB1 efflux activity. These data suggest that combinational therapies with bicalutamide/enzalutamide and docetaxel might be effective in CRPC, independent of AR status. Indeed, preclinical studies have demonstrated that bicalutamide reverses docetaxel resistance in xenograft mice (Zhu et al. 2015). Another approach actively pursued to overcome ABC-induced resistance is the identification of chemically modified AMCDs with lower affinity for the efflux transporter. In 2010, FDA approved cabazitaxel, a second-generation taxane, for CRPC patients with disease progression on or after therapy with docetaxel-based chemotherapy (de Bono et al. 2010). Cabazitaxel differs from docetaxel in the side chain, in which methoxy groups replace hydroxyl groups, lowering affinity for the ABC transmembrane efflux transporters (Nightingale & Ryu 2012).

Tumors acquire resistance to AMCDs also by deregulating apoptotic pathways. Many AMCD-resistant tumors, including docetaxel-resistant prostate cancer cells, express low levels of the proapoptotic Bax protein and high levels of the antiapoptotic BCL-2 and BCL-XL proteins, thus generating resistance to therapy-induced apoptotic cell death (O’Neill et al. 2011). To date, only a few preclinical studies have explored the efficacy of antiapoptotic protein antagonists in restoring sensitivity in AMCD-resistant cancer cell lines with deregulated apoptosis. Disappointingly, however, a phase II clinical trial evaluating the efficacy of docetaxel in combination with LY2181308, an antisense oligonucleotide against the antiapoptotic protein survivin, failed to demonstrate enhanced antitumor activity in CRPC patients (Wiechno et al. 2014).
Mitotic slippage, SAC and mitotic duration

Another crucial mechanism of resistance to AMCDs is mitotic slippage. As discussed previously, cells respond to a prolonged arrest in mitosis by activating the mitotic catastrophe pathway. However, it has been suggested that the cells have to be arrested in mitosis long enough to reach a certain proapoptotic threshold (Gascoigne & Taylor 2009, Topham & Taylor 2013). Even in the presence of AMCDs, SAC and mitosis arrest are not permanent: cells can adapt to the SAC and slip out of mitosis mainly because cyclin B is slowly degraded to the point it can no longer sustain the activity of the master mitosis regulator Cdk1 (Brito & Rieder 2006). Most of the cells that slip out of mitosis progress to the G1 phase of the cell cycle without dividing, in a tetraploid state, then, either stop dividing, becoming senescent or die at later stages (Gascoigne & Taylor 2009). Nevertheless, a small fraction of cancer cells that have slipped through mitosis, especially if they are p53 null, may continue dividing, thus, resisting the treatment and generating further aneuploidy via aberrant mitosis (Gascoigne & Taylor 2009, Topham & Taylor 2013). By generating higher genomic instability rates, this process predisposes, in principle, to the acquisition of a more malignant phenotype. Thus, a fraction of AMCD-treated cells that have slipped through mitosis before the threshold necessary to induce mitotic catastrophe was reached, can indeed survive. It can, therefore, be inferred that mitotic slippage is a crucial mechanism for the development of cells resistant to AMCDs. To avoid this mechanism of AMCD resistance, it might be sufficient to block the cells in mitosis long enough to reach the proapoptotic threshold necessary to induce mitotic catastrophe. To this end, it has been suggested that stronger and longer sustained mitosis arrest could be induced by inhibition of APC/C^{Cdc20}, the ubiquitin ligase responsible for cyclin B and securin degradation and, therefore, crucial for mitosis exit (Shirayama et al. 1999). However, the two so far described APC/C^{Cdc20} inhibitors, TAME and Apcin, though promisingly efficient in blocking the exit from mitosis, are not yet available for clinical use (Zeng et al. 2010, Sackton et al. 2014). The rationale for using TAME and Apcin is that interfering with APC/C^{Cdc20}-mediated cyclin B and securin degradation enforces an AMCD-induced block to mitosis exit, thus, increasing the chance of cell death by induction of mitotic catastrophe.

Dose-limiting AMCD side effects

Of note, AMCD clinical benefits are curtailed not only by resistance but also by dose-limiting collateral damage. The most relevant AMCD side effects are neutropenia, a consequence of toxicity on hematopoietic precursor cells, and peripheral neuropathy, due to the critical role of microtubules in neuronal axoplasmic transport (Zhou & Giannakakou 2005). In addition, AMCDs are highly insoluble and the vehicles used for their administration are quite often responsible for the worsening of the side effects. For example, peripheral neurotoxicity can be greatly exacerbated by Cremophor EL (CrEL), a polyethoxylated castor oil used as vehicle for the intravenous administration of the highly hydrophobic paclitaxel (ten Tije et al. 2003, Barbuti & Chen 2015). Despite premedication with corticosteroids and histamine antagonists, AMCDs cause acute hypersensitivity reactions in about 40% of the patients characterized by respiratory distress, hypotension, angioedema, generalized urticaria and rash (Kadoyama et al. 2011). In addition, the cumulative toxicities of dexamethasone used as a premedication may contribute to treatment-related morbidity. In CRPC, docetaxel is often given in combination with prednisone; however, the use of prednisone in CRPC is highly controversial. On the one hand, prednisone, besides its common anti-inflammatory and anti-emetics effects, can be particularly beneficial to CRPC patients. Prednisone is directly cytotoxic to prostate cells and also decreases the release of the protumorigenic adrenal androgens by inhibiting the secretion of pituitary adrenocorticotropic hormone (Ndibe et al. 2015). On the other hand, it has been shown that glucocorticoid receptor is overexpressed in docetaxel-resistant prostate cells, and this upregulation is functionally relevant as resistance is reverted by receptor antagonisms (Kroon et al. 2016). Thus, it can be beneficial to find a way other than premedication with corticosteroids to limit side effects in AMCD-treated patients. To avoid AMCD-induced reactions and also to optimize delivery and distribution, many new AMCD formulations have been developed and tested; for example, albumin nanoparticles, liposomes and microspheres are currently being tested as AMCD
vehicles (Hennenfent & Govindan 2006, Yassine et al. 2016). Docetaxel-carboxymethylcellulose nanoparticles displayed enhanced antitumor activity in murine models of CRPCs (Hoang et al. 2014). This formulation is more tolerable, allowing administration of greater doses that could result in enhanced antitumor activity. Moreover, carboxymethylcellulose provides enhanced docetaxel solubilization. Intensive research has also been focused on using nanoparticle drug delivery systems to improve specific delivery of AMCDs to tumor cells. For example, micelles conjugated with the prostate-specific membrane antigen (PSMA) ligand and loaded with docetaxel are specifically targeted to prostate cancer cells that overexpress PSMA. The PSMA formulation has shown enhanced antitumor activity in preclinical studies in prostate xenograft nude mice when compared to free docetaxel. However, the data relative to the utilization of improved AMCD formulations in humans are still limited (Ganju et al. 2014), with the remarkable exception of the albumin-bound paclitaxel approved by the FDA for the treatment of breast cancers after a very successful phase III clinical trial (Gradishar et al. 2005). In addition, new drugs targeting microtubules have been identified, like epothilones and halichondrins, that may have fewer side effects compared to the classical AMCDs (Zhou & Giannakakou 2005). Epothilones are microtubule-stabilizing agents with a mechanism of action similar to that of taxanes; halichondrins suppress the growth of microtubules, inhibiting polymerization. Both ixabepilone, a semi-synthetic epothilone derivative, and eribulin mesylate, an analogue of halichondrin B, have been FDA approved for patients with metastatic breast cancer whose disease has progressed despite prior anthracycline and taxane therapy (Dybdal-Hargreaves et al. 2015, Li et al. 2017). Many other epothilone and halichondrin derivatives have shown promising antitumoral activity in preclinical studies; some of them are in clinical trials for the treatment of tumors of different histotypes and the results are eagerly awaited (Perez 2009, Cortes & Vidal 2012, Forli 2014).

**WEE1 and MYT1 kinase inhibitors to improve AMCD therapy**

Despite these limitations, AMCDs are still successful therapeutics for cancer treatment. Nevertheless, strategies to increase cancer cell sensitivity to AMCDs are highly anticipated, in the hope to reduce the occurrence of resistance and toxicity. Recently, by studying the mechanisms by which cancer cells adapt to an active SAC and exit mitosis despite spindle malformation, we have been working on a new way to improve AMCD efficacy that is centered on sustaining the AMCD-induced, SAC-dependent, mitotic arrest to restrain mitotic slippage until the apoptotic threshold is reached (Visconti et al. 2015a, b, 2016). As mechanistically detailed below, we propose to combine AMCD treatment with AZD1775, a drug that inhibits WEE1 kinase (Hirai et al. 2009, Matheson et al. 2016). AZD1775 is already available for clinical use; thus, this new combination therapy is readily testable in clinical trials (Hirai et al. 2009, Matheson et al. 2016).

Our suggestion is based on a novel role for WEE1 kinase at mitosis in regulating SAC adaptation (Visconti et al. 2015a, 2016). As illustrated in Fig. 2, WEE1 and MYT1 kinases are known to inhibit the cyclin B/CDK1 (CDK1)
complex, the major mitosis-promoting kinase, to control the G2-M transition and the onset of mitosis. In interphase, WEE1 and MYT1 keep CDK1 inactive by phosphorylating the inhibitory thr-14 and tyr-15 CDK1 residues (Parker & Piwnica-Worms 1992, Booher et al. 1997). At mitosis onset, CDK1 inhibitory phosphorylations are reversed by the phosphatase CDC25C (Hoffmann et al. 1993). However, as shown in Fig. 3, we have proved that WEE1 also contributes to the control of mitosis exit. Indeed, CDK1 undergoes transient WEE1-dependent tyr-15 phosphorylation at the end of mitosis (D’Angiolella et al. 2007, Visconti et al. 2012), and this is dependent on the phosphatase FCP1 that dephosphorylates and activates the WEE1 kinase activity at mitosis exit (Visconti et al. 2012, 2013). More recently, we found that the FCP1-WEE1 pathway also has a crucial role in the mechanisms by which cells adapt to the SAC. Indeed, we found that FCP1-WEE1 pathway becomes progressively activated during SAC-dependent mitosis arrest induced by AMCDs, and this progressively lowers CDK1 activity to a point in which the mitotic state cannot be maintained, leading cells to exit mitosis despite incompletely assembled spindles (Visconti et al. 2015a,b, 2016). Thus, we hypothesized that inhibiting WEE1 would enforce arrest in mitosis, synergizing with AMCDs in provoking mitotic catastrophe. Indeed, we have demonstrated that targeting FCP1 or WEE1 by siRNAs delayed SAC adaptation and mitotic slippage in taxane-treated cancer cell lines. Moreover, remarkably, we have shown that, in vinca alkaloid-treated primary leukemic blasts isolated from bone marrow specimens of lymphoblastic leukemia patients, the addition of AZD1775 significantly prolonged mitosis, resulting in reduced viability (Visconti et al. 2015a). Thus, AZD1775, by delaying mitotic slippage, limits the occurrence of AMCD resistance in cell cultures, a promising starting point to further tests in preclinical and clinical settings.

As discussed before, AMCD benefits are curtailed not only by resistance but also by toxic side effects. To limit side effects, AMCD doses should be lowered, which increases the risk of losing their desired effects. In this respect, we predict that AZD1775, synergizing with the AMCDs in inducing mitotic arrest, will allow a substantial AMCD dosage reduction to decrease side effects without losing overall efficacy in mitotic catastrophe induction.

**Use of MYT1 kinase inhibitor to expand cancer treatment options**

CDK1 kinase activity is regulated by both WEE1 and MYT1-induced inhibitory phosphorylation. In line with the role we have demonstrated for WEE1 in regulating mitotic exit, it has been recently shown that in neural stem cells, siRNA-induced MYT1 downregulation synergizes with AZD1775 in causing cell death as a result of a large increase in the mitotic transit time (Toledo et al. 2015). Interestingly, unlike in neural non-neoplastic stem cells, in patient-derived glioblastoma stem-like cells, WEE1 and MYT1 do not act redundantly to inhibit CDK1 activity, most likely because of EGFR and AKT1 oncogenic signaling. In these cells, even siRNA-induced downregulation of MYT1 on its own is sufficient to increase mitotic transit time and death (Toledo et al. 2015). These data, together with our evidence, prove that the inhibition of WEE1 and MYT1 can force mitotic arrest and induce cell death. Moreover, the evidence that the sole inhibition of MYT1 is more effective in glioblastoma stem-like cells than in...
non-neoplastic control stem cells is particularly intriguing. We have preliminary results showing that MYT1 is required for mitosis exit as siRNA-induced MYT1 downregulation blocks mitosis exit in HeLa cervical carcinoma cells. Thus, MYT1 also could be an advantageous target to increase AMCD-dependent mitotic catastrophe in cancer cells. So far, however, systematic development of specific MYT1 inhibitors has been hampered by lack of peptide substrates suitable for activity-based screening. Accordingly, GGL1, a glycolipid isolated from marine algae and reported to be a potent and selective inhibitor of the human MYT1 kinase, has failed to pass more specific functional assays (Göllner et al. 2009, Rohe et al. 2015a). The recent identification of MYT1 peptide substrates, using peptide microarrays, will hopefully allow the isolation of MYT1 inhibitors for testing in preclinical settings (Rohe et al. 2015b). MYT1 inhibitors could also be tested in combination with AMCDs. We predict that MYT1 inhibitors would cooperate with AMCDs in inducing prolonged mitosis arrest and, in turn, increasing mitotic catastrophe analogously to what happens with AZD1775. The identification of specific MYT1 inhibitors could also allow the testing of a new combinatorial therapy with AZD1775 itself.

Recent hypotheses on the lack of long-term curative effects of current chemotherapeutic treatments are that they mostly target more differentiated cancer cells in tumors, but spare cancer stem cell compartments. Indeed, CRPC cells that survive docetaxel treatment show stem-like characteristics with a marked upregulation of the Notch and Hedgehog signaling pathways (Domingo-Domenech et al. 2012). Thus, the strategy of targeting WEE1 and/or MYT1 to sustain CDK1 activity in combination with AMCDs could be particularly beneficial since it appears to target cancer stem cells with greater sensitivity as shown for glioblastoma (Toledo et al. 2015).

AMCD use in triple-negative breast cancer therapy and its improvement by WEE1 inhibition

The combination of AMCDs with WEE1 and/or MYT1 inhibitors can be relevant not only for the treatment of prostate cancer but also for all cancers in which AMCDs are commonly used. Moreover, this combination can be particularly beneficial for the treatment of the human cancers that have not benefited from the recent advances in target therapy, as in the case of the triple-negative breast cancers (TNBCs).

Breast cancer, the most frequent tumor worldwide and the second most prevalent cause of death in women, is a highly heterogeneous group of pathologies (Siegel et al. 2017). Using traditional immunohistochemistry techniques, breast cancer is classified into three subtypes with different biological behaviors requiring different therapeutic approaches: hormone (progesterone and estrogen)-receptor (PR and ER)-positive, human epidermal growth factor receptor 2 (HER2)-positive, and TNBCs. The recent advances in molecular profiling techniques have enabled the identification of at least seven different subtypes of breast cancers, with clinical histories and therapeutic responses still under intensive investigation (Sørlie et al. 2001, Van’t Vee et al. 2002). Thus, at the moment, the therapeutic flowchart is still based on the classification in the three classical, main subtypes. In general, surgical and radiation treatments are similar, but drug treatment is different. Endocrine therapy is specifically used for the treatment of PR- and ER-positive breast cancers. The prognosis of HER2-positive breast cancers has been radically improved by trastuzumab, a specific monoclonal antibody against HER2, and its derivative ado-trastuzumab emtansine (Amiri-Kordestani et al. 2014). At present, however, there are no specific guidelines for the treatment of TNBCs but only generic recommendations put forward by medical oncology societies (Mustacchi & De Laurentiis 2015). Usually, the taxanes paclitaxel and docetaxel are used for first-line treatment of TNBCs, alone or in combination with other antineoplastic drugs, frequently anthracyclines and gemcitabine (Mustacchi & De Laurentiis 2015). Taxanes are used in neoadjuvant, adjuvant and metastatic settings. For neoadjuvant therapy, taxanes are indicated, alone or in combination, for reducing the size and the extension of locally advanced tumors or for early breast cancers not suitable for primary conservative surgery. Also in post-operative, adjuvant settings, taxanes are efficient for the treatment of TNBCs. Recently, a phase III clinical trial has shown that in adjuvant TNBC therapy, in combination with cyclophosphamide and doxorubicin, the new generation microtubule-targeting drug ixabepilone is equally effective but less toxic than docetaxel (Yardley et al. 2015). Conventional taxanes are still the treatment of choice for metastatic breast cancers as they improve overall survival and delay time to progression. However, prolonged treatment with taxanes, as required in metastatic settings, is unrealistic because of cumulative toxicity and development of resistance. Moreover, tumors in metastatic patients with a short
disease-free interval cannot be rechallenged with taxanes if already used in adjuvant therapy, as it is inferred that the relapsed tumor will have acquired resistance (Isakoff 2010). Although resistance and side effects limit their use, taxanes are, however, still the major therapeutic in use. Disappointingly, relatively few important therapeutic advances have been made since taxane introduction over 20 years ago. Thus, the prognosis of TNBCs is significantly poorer than that for PR-, ER- and HER2-positive breast cancers. Finding effective therapeutic approaches for TNBCs is impelling as they develop earlier in life, especially in premenopausal women and are more aggressive (Chacón & Costanzo 2010). The lack of known specific molecular targets has led to extensive research to find possible vulnerabilities in TNBC. The biologic drugs already evaluated or under active research and that have shown antitumor activity in TNBC include angiogenesis inhibitors, PARP1 inhibitors, immune checkpoint inhibitors and AR antagonists, the latter used for the specific treatment of the luminal androgen receptor TNBC subtype that expresses high levels of AR (Lehmann et al. 2015, Cerrato et al. 2016, Hartkopf et al. 2016). As a different approach, chemically modified taxanes have been, and are still, extensively being tested in clinical trials, alone or in combination with other chemotherapeutics. Until now, there is evidence suggesting that nanoparticles of albumin-bound paclitaxel, allowing achievement of higher intratumoral concentrations of the drug, are more effective and less toxic than conventional paclitaxel (Gradishar et al. 2005, Mustacchi & De Laurentis 2015). Again, we believe that, for improving taxane efficacy in TNBC therapy, another strategy warranted, that of combination with AZD1775. Currently, there is only one ongoing phase II clinical trial testing AZD1775 for the treatment of TNBC but in combination with the DNA-damaging drug cisplatin. AZD1775 has been combined with cisplatin because of the role of WEE1 in regulating the G2-M transition and the G2-M checkpoint. Most human cancers rely on the G2-M checkpoint to detect and repair damaged DNA before entering mitosis. Therefore, tumor cells treated with AZD1775 lose the ability to arrest the cell cycle at G2 in response to cisplatin-induced DNA damage and are forced to enter an aberrant and, thus, lethal mitosis (Visconti et al. 2016). Given the key role we have demonstrated for WEE1 in regulating mitosis exit, combining AZD1775 with taxanes has an equally strong rationale and can be an effective strategy for treatment of TNBC. We anticipate that, following the same strategy, also a MYT1 inhibitor could add efficacy to the standard therapy with taxanes in TNBC treatment.

Conclusions

Recent studies have described the molecular mechanisms that limit AMCD therapeutic efficacy. Many strategies have been proposed to target these mechanisms, and these strategies are currently being tested in preclinical studies and in clinical trials.

To overcome efflux-mediated resistance, various approaches have been exploited, mostly aiming at enhancing the intracellular AMCD concentration. Indeed, great effort has been spent in finding new AMCD chemical modifications or vehicle-bound formulations to lower drug affinity for the efflux transporters or to optimize delivery, respectively. In addition, new microtubule-targeting agents have been tested in the hope to find new drugs able to bypass tumor cell resistance and retain the antiproliferative capacity of classical AMCDs but with fewer side effects. In some cases, these approaches have been highly successful. Cabazitaxel, a chemically modified, second-generation taxane, has been approved for prostate cancer therapy, and albumin-bound paclitaxel (nab-paclitaxel, ABI-007) and ixabepilone are in use for breast cancer treatment.

To restore the sensitivity to the apoptotic pathways, often deregulated in AMCD-resistant cells, preclinical studies have explored the efficacy of antiapoptotic protein antagonists. However, the promising results obtained in preclinical settings have not to date been confirmed in clinical trials.

Mitotic slippage, another key mechanism of resistance to AMCDs, has been proposed to be a key target to improve therapeutic efficacy (Huang et al. 2009). Indeed, APC/C\(^{Cdc20}\) inhibitors can synergize with AMCDs in inducing a more persistent mitotic arrest and more efficiently cause cancer cell death (Zeng et al. 2010, Sackton et al. 2014). The two so far described APC/C\(^{Cdc20}\) inhibitors, TAME and Apcin, appear promising molecules as they efficiently block mitosis exit in preclinical studies, but are not yet available for clinical use (Zeng et al. 2010, Sackton et al. 2014). To prevent mitotic slippage, we have proposed a different strategy: inhibiting WEE1, a crucial kinase in the slippage mechanism (Visconti et al. 2015a), in combination with AMCDs. Given the clinical availability of a WEE1 inhibitor, it would be worth establishing clinical trials in which the WEE1 inhibitor is combined with AMCD-based therapy. Such a therapeutic combination would be
particularly important in those clinical settings in which AMCDs are frequently used, as in CRPC and TNBC.

Declaration of interest
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