Summary and Discussion
Chapter 9

SUMMARY

This thesis deals with several clinical and preclinical aspects of novel microtubule-stabilizing agents in the treatment of solid tumors. Chapter 1 gives an introduction on the current position of microtubule-stabilizing agents in the treatment of malignant diseases and their possible routes of administration. In addition, an outline is given of the molecular mechanisms leading to chemotherapy-induced cell death in human cancer cells.

The first part of the thesis focuses on preclinical aspects of chemotherapeutic drugs and microtubule-stabilizing agents in particular. Despite its achievements, the effectiveness of chemotherapy is still hampered by the development of drug resistance. Chapter 2 gives an overview of the various molecular pathways leading to multidrug resistance, and a series of attempts to manipulate these pathways are discussed. These efforts are, however, limited by the co-existence of multiple, partially unidentified resistance mechanisms in human cancer. Recently, progress has been made in the field of gene sequencing and microarray technologies and these techniques could be helpful to identify genetic abnormalities in individual human tumors that could be relevant for predicting response.

In Chapter 3 we investigated the contribution of the major apoptotic pathways to the cytotoxic effects discodermolide and epothilone B, two novel microtubule-interacting agents with potent in vivo anti-tumor activity. Despite late activation of the apoptotic machinery, neither blockade of the mitochondria nor death receptor pathways did substantially reduce the cytotoxic effects of these drugs. Based on these findings, we hypothesized that the observed activation of the apoptotic machinery occurs only as a bystander effect and is not relevant for the cytotoxic effects of discodermolide and epothilone B. In support of this view, preincubation with the broad-spectrum caspase blocker zVAD-fmk, did not decrease the effects of these compounds. Our findings imply that caspase-independent routes are involved in the cytotoxic effects of discodermolide and epothilone B.

Caspase-independent cell death pathways induced by microtubule-stabilizing agents are further studied in Chapter 4. Here we present two lines of evidence indicating a central role for the lysosomal protease cathepsin B in mediating cell death by discodermolide, epothilone B and paclitaxel. First, inhibition of cathepsin B, and not of caspases or other proteases, such as cathepsin D or calpains, results in a strong protection against drug-induced cell death in several NSCLC cells. Second, these microtubule-stabilizing agents trigger disruption of lysosomes and release and activation of cathepsin B. Interestingly, inhibition of cathepsin B prevents the appearance of multinucleated cells, an early characteristic of microtubule-stabilizing agents-induced cell death, pointing to a central, proximal role for cathepsin B in this novel cell death pathway.

Chapter 5 reviews the various types of programmed cell death and their death pathways at molecular and organelle level. Not only caspases, but also calpains, cathepsins, endonucleases and other proteases can execute programmed cell death,
and they can be directed by several cellular organelles, including mitochondria, lysosomes and the endoplasmatic reticulum, which can act independently, or collaborate with each other. Although several models of caspase-independent cell death have been described, the various death routes may overlap and several characteristics may be displayed at the same time. The growing knowledge of caspase-independent cell death pathways is important for the oncology field, as they could potentially be manipulated to develop new cancer therapies.

In the second part of this thesis, we focus on clinical studies on novel microtubule-stabilizing agents. First, we describe the role of new therapeutic compounds in the treatment of non-small lung cancer in Chapter 6. No curative treatment is available for advanced stages of disease, which comprise the majority of cases and, therefore, the development of new therapeutic strategies is needed. Novel chemotherapeutic drugs such as the epothilones have demonstrated some activity in advanced non-small cell lung cancer. In addition, an increasing number of therapies targeted against critical biological abnormalities in NSCLC are being investigated in clinical trials. This approach includes inhibition of growth factors, interference with abnormal signal transduction, inhibition of angiogenesis and vaccination therapy. Promising results have thus far been obtained with some of these therapies.

Chapter 7 describes a dose-escalating phase I study on BMS-275183, an orally administered C-4 methyl carbonate analogue of paclitaxel. We studied the effects of a continuous weekly dosing regimen in 48 patients. The main DLT consisted of peripheral neuropathy whereas other grade 3 and 4 side effects comprised fatigue, diarrhea and neutropenia, and were infrequent. We identified 200 mg/m$^2$ as the MTD. The definition of this dose was not unequivocal: (re-)exploration of several intermediate dose levels was necessary to identify a safe dose. This was due to the design of the dose escalation schedule with only one patient per dose level, combined with a relatively high pharmacologic interpatient variability (53%) and strict definitions of safety which prompted dose reduction upon occurrence of grade 2 peripheral neuropathy. The observed response rate of 24% in the heavily pretreated patient group of this phase I trial indicates that BMS-275183 is a potent new taxane analog.

In Chapter 8 a second phase I study with BMS-275183 is presented. In this trial we investigated whether twice weekly administration would improve the tolerability of BMS-275183 and reduce its neuropathic side effects. A total of 38 patients were enrolled in this trial. The MTD was 100 mg/m$^2$ and DLTs consisted of (a combination of) neutropenia, neuropathy and diarrhea. BMS-275183 had a lower incidence of neuropathic side effects compared to the weekly treatment regimen. The response rate of 13% in the heavily pretreated patient group of this trial confirms our previous finding that BMS-275183 may become a valuable contribution to the treatment of NSCLC and prostate carcinoma. This study further indicates that BMS-275183 is preferably given in a twice weekly schedule.
DISCUSSION AND PERSPECTIVES

Despite the achievements of both basic and clinical research in the past decades, cancer still is a dominant cause of death in civilized countries. Research at the present time focuses on molecular abnormalities that can lead to the development of malignant diseases and their frequent failure to respond to anti-cancer therapy, in order to develop new therapeutic strategies that improve the survival of cancer patients. Programmed cell death is crucial in the regulation of homeostasis in almost all organs and tissues and deregulation of this process can lead to the development of cancer and may cause resistance to treatment. In recent years it has become evident that the classic dichotomy of apoptosis versus necrosis is a simplification of the highly sophisticated processes of programmed cell death which guard the organism against unwanted and potentially harmful cells. Not only caspases, but also other proteases play a role in the execution of programmed cell death.

Our work described in Chapter 3 and 4 of this thesis indicates that cell death induced by the microtubule-stabilizing agents paclitaxel, epothilone B and discodermolide in NSCLC cells is not primarily exerted by caspases, but by the lysosomal protease cathepsin B. This finding is not unprecedented and adds up to the growing evidence of the contribution of lysosomal proteases to programmed cell death processes. Cathepsin B has been described to be involved in bile salt-induced hepatocyte apoptosis, regulation of immunosuppression and neuronal apoptosis (1). In addition, cathepsin D mediates cell death induced by oxidative stress and staurosporine (2,3) and is involved in bleomycin-induced pulmonary fibrosis (4). Interestingly, lysosomal proteases have been described to act both upstream, downstream and independently of the classical apoptotic machinery (1). It seems that lysosomal proteases trigger cell death not via a single specific pathway, but rather via multiple pathways that may overlap with the traditional mediators of apoptosis. The molecular identity of the mediators and the necessity of activation of caspase-dependent pathways remain to be elucidated in many cases and may vary depending on the type of cell and the applied death stimulus.

It is stating the obvious that successful treatment of cancer with chemotherapy is largely dependent on its ability to trigger cell death in tumor cells. As discussed in Chapter 2, clinical studies investigating factors predictive of chemosensitivity have failed to show an unequivocal relationship between expression of pro- or anti-apoptotic genes in solid tumors and the development of drug resistance. Some pitfalls associated with the methodology used in the clinical studies, such as low sample size, detection method, use of frozen versus fixed tumor tissue and cut-off points for positivity, may contribute to these conflicting results. Apart from methodological issues, paradoxical data may also be due to inactivation, inhibition or loss of the downstream apoptotic machinery in apoptosis-resistant solid tumor cells. As aggressive cancers may not further depend on apoptotic proteins after losing functional apoptotic pathways, the expression of these proteins may become irrelevant for cell death.
In this light, our finding that caspase-independent death pathways are responsible for cell death induced by microtubule-stabilizing agents is very interesting. Despite the enormous importance of the discovery of apoptosis as a cell death program indispensable for embryogenesis and protection against unbridled cell growth, the apoptosis-necrosis paradigm is too simple to encompass the wide spectrum of possibilities we have to eliminate faulty and potentially harmful cells. The evolutionary advantage of the existence of multiple death pathways is obvious: it protects the organism against the development of malignant diseases as many burdens have to be overcome before a cell becomes a tumor cell. This may explain the relative rarity of cancer, in respect to the huge number of cell divisions and mutations during a human life. The growing knowledge of caspase-independent cell death pathways is important for the oncology field, as they could potentially be manipulated to develop new cancer therapies.

The novel microtubule-stabilizing agent epothilone B likely represents a potent new anti-cancer drug. It not only triggers alternative death pathways in tumor cells, but it is also no substrate for the P-glycoprotein pump which is an important cause of clinical drug-resistance against the conventional taxanes and other chemotherapeutic drugs. The preclinical data suggest that epothilones may be superior to paclitaxel and docetaxel. In phase I and II clinical trials, this drug appears to have considerable anti-tumor activity with acceptable levels of toxicity (5, 6). The hints of anti-tumor activity in patients with taxane-resistant tumors, warrant further investigation to draw conclusions on the ability of epothilones to overcome the problem of cross-resistance. In addition, it remains to be investigated in randomized clinical trials whether epothilone B meets its expectations and is superior to paclitaxel or docetaxel in for instance NSCLC, prostate or breast cancer patients.

The successful introduction of capecitabine as an alternative for intravenous fluorouracil exemplifies that oral delivery of chemotherapeutic drugs is an attractive approach to enlarge the possibilities and convenience of current dosing regimens. The results of the first two clinical trials with the oral taxane analogue BMS-275183, presented in Chapter 7 and 8 of this thesis, suggest that this compound may become a valuable contribution to the therapeutic arsenal of NSCLC and prostate carcinoma. However, the relatively high incidence of neuropathic toxicity found in our first trial is of concern because of the potential irreversibility and impact for daily living of this side effect. The exact mechanism of neuropathy induced by microtubule-stabilizing agents is unknown, but because the survival and function of neurons require proteins to be transported along axons from a neuron’s body to it’s distal synapses, treatment with microtubule-stabilizing agents may interrupt this active transport leading to the typical symptoms. For unknown reasons, paclitaxel triggers more neuropathic side effects than docetaxel, and it appears that BMS-275183 is even more neurotoxic. The incidence observed in our trial appeared to be higher than reported for other taxanes, although results are difficult to compare because of low patient numbers in our phase I study (6). In addition, the time to onset of neuropathy was remarkably shorter in our trial than reported for other taxanes. Whereas for paclitaxel and
docetaxel the development of neuropathy generally depends on the cumulative dose and occurs on an average after 3 to 4 cycles (i.e. 9–12 weeks) (6), we observed a more rapid development with a median time to onset of one month in our study with weekly administration. Co-administration of neuroprotective agents could be helpful to prevent these side effects, but to date randomized controlled clinical trials with such agents show conflicting results and no effective drug has emerged yet. For paclitaxel it is known that duration of the time of total paclitaxel above a pharmacological threshold level is an independent risk factor for the development of polyneuropathy. This may also hold true for BMS-275183, as adjustment of the dosing regimen from once weekly to twice weekly decreased the incidence and severity of neuropathic side effects considerably.

A major concern of the clinical application of BMS-275183 is its high pharmacological interpatient variability. We observed variabilities of 53% at the recommended phase II dose of the weekly treatment schedule to 93% at the MTD of the twice weekly dosing regimen. This is particularly relevant as patients with a high exposure to BMS-275183 were more prone to develop severe side effects. Despite our efforts, we were not able to predict which patients were to experience a high drug exposure. In our studies, all toxicities were at least partially reversible. However, in other trials with weekly administration of BMS-275183, fatal side effects have been observed in several patients (mainly sepsis due to pancytopenia) and this treatment regimen has been abandoned (unpublished data). Although the incidence and severity of severe side effects was considerably lower in our study with a twice weekly treatment regimen, the observed high interpatient variability warrants caution. Care should be taken to delay and reduce dosing upon the first signs of development of severe toxicity, in order to give the drug safely to those patients with an unexpected high exposure to the drug. Safety as well as efficacy results from phase II trials exploiting the twice weekly dosing schedule are eagerly awaited and they will determine the future of this interesting new drug.

In conclusion, the major aim in the treatment of cancer patients is to develop more effective therapies and treatment regimens that target the tumor more specifically and prevent or overcome the problem of drug resistance. Advances in the understanding of the molecular and biological basis of malignant diseases suggest optimism, but also underline the enormous complexity of human cancer and the many barriers that still have to be taken. Continuous investigations and investments will hopefully change the fate of many cancer patients in the coming decades.
REFERENCES


