Balance between desired and unwanted effects of chemotherapeutic and anti-angiogenic drugs

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Summary and future perspectives

This thesis describes ways to target endothelial cells to enhance anti-tumor effects of cytostatics with as less as possible effect to normal quiescent endothelial cells.

Anti-angiogenic therapy is a new anti-tumor treatment modality, which is currently in the phase of clinical testing. Aim of this treatment is to block angiogenesis either by e.g. endothelial cytotoxicity or inhibition of endothelial growth factors.

In *chapter 2*, "accidental anti-angiogenesis" as a new treatment modality with classic chemotherapeutic drugs is explored. A number of chemotherapeutic drugs used in the treatment for several advanced solid tumors is linked to cardiovascular toxicity. Based on their toxicity on normal endothelial cells at ultra-low concentrations seen in human umbilical vein endothelial cell (HUVEC) and murine xenograft models, chemotherapeutic drugs possess an anti-angiogenic potential towards the rapidly dividing tumor endothelial cells. Clinical trials with "metronomic dosing"-based regimens (more frequent dosing in low concentrations) to substantiate this potential are reviewed. This new modality might lead to long-term survival and higher tumor response rate.

ABT-510, a nonapeptide derived from the naturally occurring angiogenesis inhibitor thrombospondin-1, is a new anti-angiogenic agent. In preclinical tumor models, 75% of the maximal activity was achieved when plasma ABT-510 concentrations exceeded 100 ng/mL for more than 3 hours a day. We performed a phase I study, described in *chapter 3* with ABT-510 in patients with advanced solid tumors. ABT-510 was administered subcutaneously as continuous infusion 100 mg/24 hours and as bolus injections 100, 200 and 260 mg once daily and 50 and 100 mg twice daily subcutaneously in cycles of 28 days. Blood and urine samples for pharmacokinetic and -dynamic analyses were collected. Anti-tumor activity was evaluated at regular intervals. Thirty-nine patients received a total of 126 cycles, with a median number of two cycles. Administration of ABT-510 by continuous infusion was hampered by onset of painful skin infiltrates at the subcutaneous infusion site and no dose escalation was performed. In the bolus injection regimens, the most commonly observed toxicities were mild to moderate reactions at the injection site and fatigue. The maximum tolerated dose was not reached, but 260 mg once daily was defined as the maximum clinically practical dose. ABT-510 pharmacokinetics was linear across dosage ranges tested and the potential therapeutic threshold was achieved by all dose regimens. Median serum basic fibroblast growth factor (bFGF) levels showed a decrease from 10.9 before to 3.0 pg/mL 56 days after start of treatment ($p=0.05$). Prolonged stable disease, lasting more than five cycles, was observed in six patients.

The favorable toxicity profile, the linear and time-independent pharmacokinetics with biologically relevant plasma concentrations reached, the significant number of patients with prolonged stable disease and the convenient way of dosing make ABT-510 an interesting compound for further studies.

In *chapter 4*, changes in tumor metabolism and tumor perfusion as early response markers in drug development PET scanning was studied performed as part of this phase I trial with the ABT-510. Nine patients, who received ABT-510, underwent PET scans consisting of a [^{15}O]H_2O and [^{18}F]fluorodeoxyglucose (FDG) study one day before and 22 days after start of ABT-510 treatment. PET data were analyzed using region- and volume-of-interest analysis, resulting in a mean metabolic rate of glucose and perfusion index. Data were compared with CT scans assessed on day 1 and after 56 days of treatment. The average mean metabolic rate of glucose on day 1 of tumor tissue was 0.43 ± 0.23 versus 0.072 ± 0.07 μmol glucose/min. mL for background tissue. The mean change in mean metabolic rate of glucose was +3.2% ± 15% for tumor tissue and +0.1% ± 24% for background activity and the average decrease in perfusion index was -7.3% ± 13.9%. Neither changes in metabolic rate of glucose nor changes in perfusion index correlated with volume changes as assessed by CT scans on day 56. PET scanning using H_2[^{15}O]O and [^{18}F]FDG was feasible, but could not demonstrate an effect of ABT-510 on tumor perfusion or metabolism in this study.

Until now, monotherapy with angiogenesis inhibitors show a limited response rate, while combination with chemotherapy may increase the potential anti-tumor activity.

In several tumor types such as cervical cancer and head and neck cancer, the addition of chemotherapy with standard radiotherapy improved response and survival rates compared to radiotherapy alone. In *chapter 5*, a study is described in women with cervical cancer treated with standard radiation therapy in combination with a standard-dose carboplatin and escalating paclitaxel doses. The aim of this study was to enhance anti-tumor efficacy of the standard radiotherapy by adding chemotherapeutic drugs with anti-angiogenic potential, namely metronomic dosed carboplatin and paclitaxel. In an animal model it was recently shown that microvasculature damage regulates tumor cell response to radiation (Garcia-Barros M et al. Science 2003; 300: 1155-1159). Eight women with cervical cancer stage IB2 – IVA were treated with standard radiation therapy in combination with standard carboplatin (area under the curve, AUC = 2, once weekly, x 6 times) and escalating doses of paclitaxel (60 mg/m^2, once weekly, x 4 times, then x 5 times and x 6 times). At the lowest dose level, four weekly paclitaxel cycles in six patients, three developed grade 3 diarrhea and one severe radiation enteritis several weeks after radiotherapy. Two patients did not achieve complete remission and underwent additive salvage hysterectomy. All patients remained free of local

recurrence, but one patient had distant metastases after 13 months with a ongoing median follow-up of 26 months (range, 11^{+} - 37^{+} months).

In conclusion, it was observed that standard pelvic radiotherapy in combination with weekly carboplatin and paclitaxel is poorly tolerated due to dose-limiting diarrhea. This is not a feasible regimen for further studies.

Chapter 6 explores another way of metronomic dosing by administrating topotecan intraperitoneally resulting in a semi-continuous infusion in stead of bolus intravenous injections. Patients with advanced ovarian cancer received carboplatin and paclitaxel intravenously in combination with escalating doses topotecan intraperitoneally as first-line treatment. The maximal tolerated dose topotecan, the pharmacokinetics of topotecan and the toxicity profile of the treatment regimen were studied. Women with advanced ovarian cancer stage IIB – IV received 6 cycles of standard doses of intravenous carboplatin and paclitaxel in combination with escalating doses intraperitoneal topotecan as first-line treatment. The projected dose steps of intraperitoneal topotecan were 10, 15, 20 and 25 mg/m^2. Topotecan pharmacokinetics in peritoneal fluid and plasma was studied during the first cycle. Tumor response was evaluated after treatment completion. Twenty-one patients entered this trial. Febrile neutropenia, thrombocytopenia requiring platelet transfusion and fatigue grade 3 were dose-limiting at topotecan dose level 25 mg/m^2 intraperitoneal. Nausea, vomiting, pain, diarrhea, alopecia and neuropathy were frequent, but not dose-limiting. Topotecan 20 mg/m^2 intraperitoneal was considered to be the maximum tolerated dose. Pharmacokinetic data were comparable to those of monotherapy intraperitoneal topotecan. The peritoneal: plasma AUC ratio for total topotecan was 46 ± 30. The 15 patients who completed treatment, had a median progression-free survival of 17^{+} months (range 4 – 39^{+}).

Combined with standard-doses of carboplatin and paclitaxel intravenously, topotecan 20 mg/m^2 intraperitoneal is the maximum tolerated dose and therefore recommended for further studies. The efficacy of this regimen should be further explored in a phase III study. Several modalities have been developed to reduce side effects of chemotherapy on normal tissues.

Amifostine is dephosphorylated in normal tissues to a free thiol with cytoprotective potential against chemotherapy-induced toxicity without reducing anti-tumor activity. In *chapter 7*, ovarian cancer patients were randomized to receive standard paclitaxel and carboplatin without or with amifostine. The aim of the randomized, multi-center phase II study was to assess the efficacy of amifostine in the prevention or reduction of drug-induced toxicity. Ninety patients with primary ovarian cancer were randomized to receive paclitaxel 175 mg/m^2 and carboplatin at an AUC = 6 for six cycles, without or preceded by amifostine

740 mg/m² all intravenously administered. Toxicity was scored according to NCI-CTC criteria. The mean percentual decrease of hemoglobin, leukocyte and platelet values per cycle showed no difference between both arms. No neurotoxicity was observed in 40% of the cycles without amifostine versus 49% of the cycles with amifostine; neurotoxicity grade 1 occurred in 45% versus 48%, grade 2 in 12% versus 2% and grade 3 in 2% versus 1% of all cycles (overall $p < 0.001$). Nausea grade 2 was found in 2% versus 6% ($p= 0.007$) and vomiting grade 2 in 1% of cycles paclitaxel plus carboplatin versus 8% of cycles paclitaxel plus carboplatin and amifostine ($p < 0.001$). Other non-hematological toxicities were mild and not different in both arms. The amifostine infusion was temporarily halted in five patients in 10 cycles due to hypotension, but was not reduced or discontinued for this reason. Quality of life questionnaires at treatment completion showed no difference in subjective, neurotoxicity-related symptoms such as paresthesias, weakness or fatigue. At a median follow-up of 24 months, the median progression-free survival for carboplatin-paclitaxel and carboplatin-paclitaxel with amifostine is 22 versus 16 months (n.s.). In a pooled-analysis of the data of three randomized studies in 188 patients receiving carboplatin/ paclitaxel, the addition of amifostine diminished the risk of developing grade 2-3 neurotoxicity (Odds Ratio 0.3, 95% confidence interval [0.15-0.63]).

In conclusion, amifostine showed limited activity in diminishing neurotoxicity, but no activity in preventing myelotoxicity after treatment with paclitaxel and carboplatin. There was no need for discontinuation or reduction of amifostine due to toxicity.

Recently increasingly data became available on long-term cardiovascular toxicity observed in male germ cell cancer patients. Understandable, due to low patient numbers, little is known about long-term effects of women who are treated with cisplatin-containing regimens. Therefore, cardiovascular toxicity in women treated with cisplatin-based chemotherapeutic schemes for ovarian tumors is explored in *chapter 8*. Twenty-one patients who were less than 40 years of age at the time of treatment and were at least three years disease-free after salpingo-oophorectomy followed by cisplatin-based chemotherapy were entered in the study. A history was taking and physical examination performed. Whole blood cell counts and liver and kidney function were studied. Additionally, blood samples collected after an overnight fast were analyzed for serum lipid, magnesium, endothelial and inflammatory marker proteins, thyroid-stimulating hormone and free thyroxin, insulin, glucose and platinum levels, while creatinine and albumin excretion was determined in a 24-hour urine collection. Hypercholesterolaemia was present in 62%, obesity in 24%, hypertension in 14%, and microalbuminuria in 24% of the patients. Compared to the background population, microalbuminuria was more frequent in the long-term cancer survivors (23.8 vs. 3.2 %; $p <$

0.05). Insulin resistance was found in 14% of patients. In all patients, circulating platinum levels were detected in serum and urine. Serum platinum levels correlated with follow-up duration (r= -0.56; p= 0.009) and renal function (r= -0.61; p=0.003).

These results illustrate that also in female cancer survivors treated with cisplatin-based chemotherapy signs of vascular toxicity may develop.

Conclusions and future perspectives

Angiogenesis as defined by Folkman in 1971 is an intrinsic part of the tumor growth and capacity of a tumor to metastasize. Still several issues remain to be explored: 1) the biology of angiogenic process and full characterization of tumor associated endothelial cells need to be completed for identification of new drug targets; 2) new preclinical models are required to predict accurately endothelial damage and clinical activity; 3) surrogate markers need to be identified to characterize prognostic and predictive factors for anti-tumor efficacy; 4) new combination treatments of chemotherapy with specific anti-angiogenic agents need to be explored. By combining different agents, specific problems emerge: 1) the optimal biologic active dose to inhibit angiogenesis is usually less than the maximum tolerated dose, new metronomic schedules need to be studied; 2) synergy in anti-tumor efficacy after addition of such an agent to conventional cytostatics could be accompanied by a increased toxicity such as an increase in risk for thromboembolic events. Combining these low-dose cytostatic schedules with a currently considered less toxic naturally occurring angiogenesis inhibitor such as TSP-1 might circumvent the toxicity problem. The development of this new way of treatment is still at its early stage; its potential value has yet to be proven.