species have been prepared and their reactions with GMP have been studied in model experiments by means of capillary zone electrophoresis (CZE). Kinetics of these reactions have been investigated by CZE applying diode array detection (CZE-DAD), and the reaction products have been characterized by CZE coupled to a mass spectrometer via an electrospray ionization interface (CZE-ESI-MS). Formation of monoadducts and bisadducts with GMP could be demonstrated and the expected increase in GMP binding in acidic solution has been confirmed. Binding of dichlorobis(2-hydroxyethylamine)platinum(II) proceeds more than six times faster at pH 6.0 (half time 4.5 $\pm$ 0.7 h) than at pH 7.4 (half time 28.5 $\pm$ 2.1 h) in chloride-free phosphate-buffered solution. In the presence of 100 mM chloride the half times are 11.0 $\pm$ 0.3 h and 40.5 $\pm$ 3.5 h, respectively. Evaluation of the concept in human tumor cell lines cultured under normal vs. acidic conditions (with or without hypoxia) is ongoing and results will be presented.

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Enzyme-mediated insolubilization therapy

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We have developed a new strategy that aims to concentrate therapeutic radionuclides (energetic β-particle emitters, e.g. iodine-131, and alpha-particle emitters, e.g. astatine-211) within solid tumors. This approach, which we have named EMIT (Enzyme-Mediated Insolubilization Therapy), is a method for enzyme-dependent, site-specific, *in vivo* precipitation of a radioactive molecule (from a water-soluble precursor) within the extracellular space of solid tumors. The prodrug, ammonium 2-(2'-phosphoryloxyphenyl)-6-iodo-4-(3H)-quinazolinone, labeled with iodine-125 (125IPD) and its authentic compound labeled with iodine-127 (IPD) have been synthesized, purified, and characterized. The prodrug is water soluble and non-fluorescent. In the presence of alkaline phosphatase (ALP), 125IPD and IPD are hydrolyzed, respectively, to 125I-labeled 2-(2'-hydroxyphenyl)-6-iodo-4-(3H)-quinazolinone (125ID) and its 127I-labeled derivative (ID), iodinated molecules that are water insoluble and fluorescent. Fluorescence microscopy and autoradiography demonstrate that the *in vitro* incubation of 125IPD/IPD with ALP-expressing confluent/clustered tumor cells leads to the hydrolysis of the prodrug and its entrapment. Biodistribution studies in mice injected intravenously with 125IPD or 125ID show that neither compound is retained by normal tissues and organs. In addition, when the mice are initially injected subcutaneously with ALP and then intravenously with 125IPD, radioactivity is localized only in the ALP-rich regions. Finally, our results also indicate 125ID remains indefinitely within the tissues where it is produced. We believe that EMIT is a strategy that will lead to the active and specific concentration and entrapment of therapeutic radionuclides within solid tumors, the consequent protracted irradiation of tumor cells within the range of the emitted particles, and the effective therapy of solid tumors.

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Enzymatic activation of prodrugs by prostate-specific membrane antigen (PSMA)

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PSMA is a 100 kDa type II transmembrane glycoprotein that possesses a number of characteristics that make it a suitable target for prostate-specific therapy. PSMA expression is highly restricted to prostate tissue with strongest expression in both primary and metastatic prostate cancers and PSMA expression is upregulated upon androgen withdrawal. The extracellular domain of PSMA is accessible to agents in the extracellular peritumoral fluid thus making it possible to target prodrugs for enzymatic activation. Two discrete enzymatic functions for PSMA have been described. PSMA possesses the hydrolytic properties of an N-acetylated a-linked acidic dipeptidase (NAALADase) and is able to hydrolyze the neuropeptide N-acetyl-l-aspartyl-l-glutamate (NAAG). In addition, PSMA also functions as a pteroyl poly-g-glutamyl carboxypeptidase (folate hydrolase) and is able to progressively hydrolyze g-glutamyl linkages of both poly-g-glutamated folates and methotrexate analogs with varying length glutamate chains. In order to develop prodrugs that can be activated within prostate cancers by PSMA's enzymatic activity, a PSMA specific peptide carrier is required. In the present study a number of peptide substrates for PSMA were screened in order to identify specific and efficient substrates for the NAALADase and/or folate hydrolase activity of PSMA. A series of substrates were synthesized in which the amino acid portion consisted primarily of alpha or gamma carboxy-linked aspartic and/or glutamic acids of varying chain was coupled to the 4-N[N-2,4diamino-6-pteridinyl-methyl)-N-methylamino-benzoate](APA) portion of methotrexate. These substrates were then characterized on the basis of rates of PSMA hydrolysis and stability in human serum. Gamma-linked substrates were hydrolyzed by PSMA but were relatively unstable in human serum. Only one alpha-linked dipeptide substrate (APA-Asp-Glu) was both hydrolyzed by PSMA and stable to hydrolysis in human serum. Substrates that combined both alpha and gamma linked Asp and Glu residues were both hydrolyzed by PSMA and stable to hydrolysis in human serum. These substrates are currently being used to develop prodrugs out of the potent natural product thapsigargin (TG). TG induces proliferation independent apoptosis in all cell types. TG prodrugs that are specifically activated by PSMA represent a novel therapy that could be given to men with prostate cancer while avoiding significant systemic toxicity.

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Elaboration of synergy between the prodrugs TST220 and TST334 and conventional chemotherapeutics

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TST220 and TST334 are prodrugs designed for activation by matrix metalloproteinases 2 (MMP2) and 9 (MMP9). TST220 contains a protease-sensitive cleavage site of 23 amino acid residues whereas the recognition sequence in TST334 is 8 residues. The activated TST220 and TST334 are cytotoxics with IC_{50} prodrug/IC_{50} drug ratios, *in vitro*, of 25 and 1200 respectively. They induce cell death via a pro-apoptotic pathway and exhibit anti-tumor activities in both human xenograft and murine tumor models. Preliminary combination studies indicated a strong synergy between the prodrugs and the conventional cytotoxic drug adriamycin (doxorubicin). Moreover, the two prodrugs showed evidence of efficacy in the treatment of adriamycin-resistant tumours. In this study, drug synergy and efficacy against drug-resistant tumors was elaborated in a P388 animal model. Subcutaneous tumors were initiated in BDF1 mice and the animals then treated i.v. with 5 injections of an adriamycin/prodrug combination or saline (control) at 4-6 day intervals. In low-dose monotherapy, TST220 (15 μg/kg) and TST 334 (200 μg/kg) produced roughly 1 day delays in tumor growth. Adriamycin (at 5 mg/kg) produced a modest 3 day delay. However, the combination of either prodrug with adriamycin (i.e., at the same concentrations) produced delays in tumor growth of >17 days (combination delay a minimum estimate due to the death of the control group). TST220 and TST334 showed significant efficacy in the treatment of P388Adr, an adriamycin-resistant variant of P388. Three intravenous injections of prodrug (monotherapy) at 4 day intervals resulted in on average a 5 day delay in tumour growth. Preliminary results of synergy between TST prodrugs and other conventional agents is also reported. Molecules of TST class do not cause genetic damage nor do they cause bone marrow suppression. In proposed human trials TST prodrugs are expected to potentiate the activity of adriamycin without exacerbating side effects.

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Phase 2 study of TLK286 (GST P1-1 activated glutathione analog) in patients with non-small cell lung cancer who failed prior platinum-based regimens

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Introduction: TLK286 is a novel glutathione analog that is activated by the enzyme glutathione-S transferase P1-1 (GST P1-1). GST P1-1 is constitutively expressed in many cancers including non-small cell lung cancer (NSCLC) and is overexpressed following treatment with platinum-based regimens. Following activation of TLK286 by GST P1-1, apoptosis is induced through the stress response pathway.
Methods: Up to 55 Stage IIIB or IV NSCLC patients who had failed prior platinum-based therapy (up to two cytotoxic regimens) and may have had prior adjuvant therapy and cytostatic agents such as EGFR tyrosine kinase inhibitors were to be enrolled in this multicenter single-arm study. Patients received TLK286 at 1000 mg/m^2 once every 3 weeks until tumor progression or unacceptable toxicities. Adverse events were graded by NCI-CTC, objective tumor response was measured by RECIST, and survival was estimated by Kaplan-Meier.

Results: 52 patients (31 M, 21 F) median age 60 (range 40-77), median ECOG performance status 1 (range 0-1), prior chemotherapy regimens median 2 (range 1-4) have received 152+ treatments (mean 2.9 cycles, range 1-16+), with dose reductions required in 3% of doses. All patients were resistant to platinum and taxanes, 24% resistant to second line docetaxel, and 55% having failed additional 3rd line salvage therapy including gemcitabine (27%), and EGFR inhibitors (20%). No Grade 4 events were reported. Grade 3 events were infrequent. No myelosuppression, thrombocytopenia or cumulative toxicity was seen. Possibly drug-related toxicities were mild (Grade 1-2) fatigue (38%), nausea (38%), and vomiting (22%). At the interim analysis, 41/52 patients were evaluable for efficacy. Disease stabilization was seen in 21/41 (51%). The median duration of stable disease exceeds 39 weeks. Median survival for both 2nd and 3rd and 4th line patients exceeds 10 months, and requires further patient follow-up to reach median survival. The longest duration of TLK286 therapy was one year. Survival at one year requires further patient follow-up.
Conclusions: TLK286 is well tolerated in this heavily pretreated advanced NSCLC population. Efficacy in this heavily treated population that includes 55% 3rd and 4th line patients is encouraging. Median survival exceeds 10 months and has not yet been reached. Future studies of TLK286 in advanced NSCLC are warranted.

Tubulin interacting agents

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The seco-taxane IDN5390 is able to circumvent paclitaxel resistance in drug-resistant cells with overexpression of class III beta-tubulin

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A primary mechanism of drug resistance to taxanes is the overexpression of class III beta-tubulin isotype. The activity of newly developed taxanes has been assessed against a panel of human cancer cell lines showing inherent or acquired drug resistance and overexpression of such isotype and the seco-taxane IDN5390 has been selected. Levels of beta-tubulin isotypes have been determined by RT-PCR in cells treated with paclitaxel, IDN5390 and with their combination. In wt cells, paclitaxel raised the levels of class III beta-tubulin isotype, whereas IDN5390 induced the opposite effect, and combination of both compounds prevented paclitaxel-dependent class III overexpression. In paclitaxel-resistant cells showing high levels of class III beta-tubulin, paclitaxel treatment did not modulate further class III beta-tubulin, while IDN5390 alone or in combination diminished the expression of the class III isotype. Other beta-tubulin isotypes were unaffected by drug treatments. Starting from these findings, we tested the presence of a potential synergism between paclitaxel and IDN5390. Results indicated a synergism, particularly in class III overexpressing cells. Finally, the synergism has been confirmed in paclitaxel-resistant xenografts transplanted in nude mice: a significant activity was noticed in xenografts treated with combination of paclitaxel and IDN5390 (TWI 52%, LCK to 0.8), whereas as single agents paclitaxel and IDN5390 were devoid of relevant effects (TWI of 29 % LCK of 0.2 and TWI 36% and LCK of 0.4 for paclitaxel and IDN5390, respectively). At the end of the study, we assessed the class III beta-tubulin expression in the xenografts and we found that, in keeping with "*in vitro*" findings, paclitaxel induced the overexpression of class III, while IDN5390 did not and, when combined with paclitaxel, it prevented the class III overexpression. Our data indicates that IDN5390 is able to circumvent paclitaxel-resistance in cellular models with overexpression of class III beta-tubulin and that the combination between seco-analogues and paclitaxel could represent a novel strategy to overcome MDR-independent taxane resistance.

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Absence of vascular regrowth at 96hrs in response to the vascular-targeting agent ZD6126 demonstrated by dynamic-contrast enhanced (DCE) MRI

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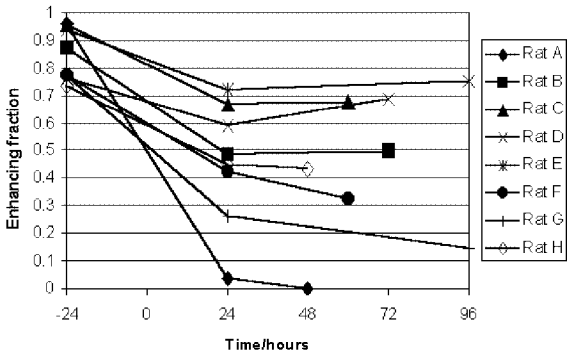
Tumour neovasculature is structurally distinct from normal vasculature and is therefore an attractive therapeutic target. The novel vascular-targeting agent ZD6126 is metabolised to the tubulin-binding agent ZD6126 phenol *in vivo*. Its action leads to the selective disruption of the cytoskeleton of newly divided endothelial cells, occlusion of tumour blood vessels and haemorrhagic tumour necrosis. We have previously shown the antivascular effect of 50 mg/kg ZD6126 on rat GH3 prolactinomas to be profound 24 h after administration [1]. This was consistent with the induction of massive central tumour necrosis with a residual viable rim of tumour cells, a common feature of the response to this agent. Tumour regrowth has been previously shown to occur from this viable rim after treatment with ZD6126 [2]. In this study we used DCE-MRI to assess regrowth of the tumour tissue up to 96 h post-treatment with ZD6126. GH3 prolactinomas were grown in the flanks of 8 Wistar Furth rats. DCE-MRI data were obtained 24 h pre-treatment using a 4.7T Varian Unity Inova. MRI was repeated 24 h post-treatment with 50 mg/kg ZD6126, followed by a final scan at 48, 60, 72 or 96 h post-treatment. Multislice dynamic data were obtained using a spin-echo sequence (TR = 120, TE = 10) for 10 min post gadopentetate injection. The gadopentetate concentration was calculated voxelwise and integrated over the first 10 images to give an IAUC. Tumour data were normalised to the median IAUC of muscle. Tumour IAUC values greater than the muscle median were defined as highly-enhancing. After the final scan tumours were excised and scored for necrosis. Post-treatment, all tumours showed a significant reduction (between 20-80%) in highly-enhancing voxels. Images of tumour IAUC implied that ZD6126 reduced the IAUC close to zero in certain areas, typically in the centre of the tumour. The fraction of highly-enhancing voxels at the final time point (48 - 96 h) remained similar to that at 24 h post-treatment for all tumours, independent of the time elapsed to the final time point.



Analysis of tumour necrosis supported this finding, indicating that, notably, there was no significant tumour regrowth up to 96 h post ZD6126 treatment in this model.
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IDN-5390, an orally active, antiangiogenic taxoid with low toxicity, ideally suited for metronomic dosing

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Our goal is the development of new semisynthetic taxoids that both overcome Pgp-based multidrug resistance and are less toxic to the host. Use of metronomic dosing (i.e., long-term, low dosing) to lower host toxicity, may be feasible with a taxoid that targets both tumor and growth of tumor vasculature. Previous studies demonstrated that IDN-5390 actively inhibits endothelial cell migration, suggesting antiangiogenic specificity (Taraboletti et al., Cancer Res. 8: 1182, 2002). However, in these studies both MCF7 human breast tumor cells and human umbilical vein endothelial cells (HUVEC) were growth inhibited by IDN-5390 *in vitro* at similar concentrations (IC_{50} 15nM). In addition, IDN-5390 was found to be less potent than paclitaxel (2nM) and the taxoid IDN-5109 (0.4nM) selected for its ability to overcome Pgp multidrug resistance. Interestingly, IDN-5390 had a lower fold resistance (121x) than paclitaxel (647x) in Pgp positive multidrug resistant MCF7/Adr cells but higher than that (45x) for IDN-5109. *In vivo*, IDN-5390