action have not been fully understood yet. However, the outer part of the phospholipids within the membrane of cancer cells has a slightly more negative charge than in normal cells, which is mainly due to phosphatidylserine, which can make up 3−9% of the total membrane phospholipids of their outer leaflet. In this work we have designed 3 peptides that showed potential therapeutic efficacy against a number of cancer cell types.

Methods: Based on the fact that currently a high number of tumour suppressor genes is known, including their mode of action, we designed 96 novel peptides with potential tumour suppressor activity in silico, which were then screened in vitro on U87 human glioma cells for biological activity by high throughput MTS assays. Three of these novel mimetic peptides showed considerable anti-tumour activity, whereby one peptide exhibited a particularly outstanding and durable tumour suppressor efficacy. This peptide was studied further with respect to its anti-tumour efficacy both in vitro and in vivo. To avoid proteolytic degradation, which is often the case for small proteins, the design and 3D structure of the peptide were modified without loosing biological activity. Using radioactive labelled peptide its distribution and pharmacokinetic profile was determined *in vivo*.

Results: Time lapse confocal microscopy revealed that the peptide induced cell death at a concentration >10 μg/ml within minutes after application and continued to work progressively for an exposure time of 6 h, resulting in 80 to 94% cell death at 35 μg/ml, depending on the respective cancer type and cell line tested. Both electron and atomic force microscopy revealed holes in the plasma membrane with a subsequent degradation of cell membrane components. Using different viability assays on a number of human tumour cell lines, including 5 osteosarcoma, 6 glioma and 4 breast cancer cell lines, the peptide showed severe cytolytic action at a concentration of >15 μg/ml. In contrast, a number of normal human cell lines, were significantly less sensitive to treatment. The pharmacokinetic profile of the peptide, including its half-life and systemic toxicity as studied in vivo, the IC 50 doses for the respective cell lines at various time points were determined in vitro. Based on these results, 4T1 murine breast carcinomas were initiated in BALB/c mice. At a tumour size of 1 cm^2 the mice were treated by a single-shot local bolus injection of 600 μg/100 μl of peptide. This led to a significant reduction of tumour size within 2−3 days post injection and reduced tumour re-growth in the following 4 weeks. Histological evaluation revealed severe necrosis in the tumour treatment group.

Conclusions:A new stable lytic peptide with high anti-tumour efficacy was developed that shows resistance towards proteolytic degradation. Compared to a number of normal cell lines, the peptide showed significant toxic effects on several human tumour cell types in vitro. Moreover, its pharmacological profile and distribution was delineated in vivo.

225 The complex between the beta1 integrin and hERG1 potassium channels as a new molecular target in antineoplastic therapy

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Background: hERG1 channels are voltage dependent K^+ channels often aberrantly expressed in primary human cancers. hERG1 channels exert pleiotropic effects in cancer cells, in turn regulating cell proliferation, cell motility and invasiveness or stimulating the process of neo-angiogenesis. hERG1 can induce such diverse effects in cancer cells since it triggers and modulates intracellular signaling cascades. This role depends on the formation, on the plasma membrane of tumour cells, of macromolecular complexes with the beta 1 subunit of integrin receptors. Therefore, the beta1 integrin/hERG1 complex may represent a novel molecular target in antineoplastic therapy, and its molecular characterization can represent a very useful task in designing novel antineoplastic therapies.

Materials and Methods: We have characterized the beta1 integrin/hERG1 channel complex by both immunoprecipiation experiments and Fluorescence resonance energy transfer (FRET) microscopy using fluorochrome tagged proteins (YFP-integrin and CFP-hERG1). Several mutants of either the target proteins were also produced and used.

Results: The experiments we have performed have clearly indicated that hERG channels and beta 1 integrins directly interact to form a plasma membrane complex in living HEK cells, characterized by an intermolecular distance lower than 4 nm. Intracellular epitopes of both the beta1 integrin and the hERG1 channel are apparently involved in mediating complex formation. This result, besides providing a useful confirmation of the biochemical characterization of this complex, represents an important step to design and produce molecular tools, such as bifunctional antibodies, capable of targeting, and possibly, unlocking the complex.

Conclusions: This strategy could represent a novel targeted approach for antineoplastic therapy.

226 Therapeutic potential of targeting sphingolipid signaling pathways in various types of cancers

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Background: Sphingolipids are bioeffector molecules which control various aspects of cell growth, proliferation, apoptosis, senescence, and drug resistance. Ceramides, the central molecule of sphingolipid metabolism, are inducer of apoptosis and inhibitors of proliferation. Sphingosine-1-phosphate (S1P) and glucosyleceramide, converted from ceramides by sphingosine kinase-1 (SK1) and glucosyleceramide synthase (GCS) enzymes respectively, inhibit apoptosis, induce cell proliferation and resistance to chemotherapeutic drugs. In this compherensive study, we examined the therapeutic potential roles of bioactive sphingolipids in chronic myeloid leukemia (CML), acute myeloid leukemia (AML), breast and prostate cancer cells by itself and in combination with anticancer agents (nilotinib, dasatinib, and imatinib for CML, resveratrol, a potential agent, for CML and AML, paclitaxel, doxorubicin, tamoxifen, and cyclophosphamide for breast, and docetaxel for breast and prostate cancers).

Material and Methods: The cytotoxicity analyses of anticancer agents, ceramide analog (C8:ceramide), GCS inhibitor, and SK1 inhibitor were conducted by XTT cell proliferation assay. Apoptosis was assesed by the changes in caspase-3 enzyme activity, loss of mitochondrial membrane potential (MMP), and Annexin-V assays. siRNAs for the supression of GCS and SK1 and plasmids overexpressing ceramide synthase genes (LASS1−6) were transfected by Dharmafect and Effectene transfection kits, respectively. Expression analyses of LASS1−6, SK1, and GCS genes were determined by RT-PCR.

Results: Our study demonstrated that increasing intracellular concentrations of ceramides by C8:ceramide application or overexpression of LASS genes decreased proliferation and induced apoptosis. On the other hand, hampering the conversion of ceramides to glucosyleceramide and S1P by inhibition of GCS and SK1 by siRNA or chemical agents resulted in apoptosis and decreased proliferation of cancer cells. More importantly, there were strong synergistic increases in apoptotic effects of the anticancer drugs on the cancer cells in which endogenous ceramide levels were increased by molecular or biochemical techniques, as determined by XTT and trypan blue assays, changes in caspase-3 enzyme activity, loss of mitochondrial membrane potential, and Annexin-V staining by flow cytometry. There were dose dependent increases in expression levels of LASS genes and decreses in SK1 and GCS genes in response to stress generated by the anticancer agents in these cancer cells.

Conclusion: Our data strongly suggest the potential roles of bioactive sphingolipids by itself or in combination with other anticancer drugs for the treatment of cancers. Increasing endogenous ceramides through exogenous ceramide analogues or mimetics and decreasing prosurvival lipids, S1P and GC, can open the way of more effective treatment of cancer in addition to inhibition of drug resistance.

227 Pharmacokinetic and biodistribution studies of anti MUC1 PEGylated aptamers with potential in the targeted radiotherapy of breast cancer

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Background: MUC1 is a known tumour marker, overexpressed and aberrantly glycosylated in epithelial tumours, and breast cancer in particular. Aptamers have great potential as targeted radiopharmaceuticals for the diagnosis and imaging of disease, with short *in vivo* half life and rapid renal clearance. Their conjugation to polyethylene glycol (PEG) modifies their pharmacokinetic properties, allowing longer circulation times and improved tumour uptake. We report the labelling of PEGylated anti MUC1 aptamers with ^{99m}Tc, and their pharmacokinetic properties and biodistribution in experimental models.

Methods: Aptamers against the MUC1 protein core, amino modified on the 5′ end and thiol modified on the 3′ end, have been conjugated to maleimide functionalised PEG and PolyPEG of various molecular weights and structures, using immobilised tris[2-carboxyethyl]phosphine hydrochloride as reducing agent at pH 4. The conjugates were analysed and isolated by anion exchange HPLC and gel electrophoresis, and their affinity verified using a Fluorescence Intercalator Displacement assay. The MAG2 ligand was attached to the aptamer using peptide coupling reactions between the amino modification on the aptamer and the carboxylic group on the ligand. Labelling of the PEGylated aptamers with ^{99m}Tc took place using tin

chloride as reducing agent. Radiolabelled conjugates were separated from free ^{99m}Tc using microcon filters. Pharmacokinetic and biodistribution studies were performed in immunocompromised mice bearing MCF-7 breast tumours. The difference between pegylated and non-pegylated aptamers was assessed in Wistar rats.

Results: The PEGylation of the aptamer using various PEGs was achieved in high yield without a significant effect on the aptamer binding to MUC1, for PEGs up to 40KDa. The aptamer conjugated to PEG and MAG2 demonstrated interesting pharmacokinetic and biodistribution properties. The PEGylated aptamers had improved circulation time, making them more suitable for therapeutic applications, whilst they maintained their ability to bind to MUC1. Furthermore, the presence of the PEG minimised the effect of chelators on the pharmacokinetic properties and biodistribution of the aptamer.

Conclusions: PEGylated MUC-1 aptamers of varying molecular weights have been produced successfully, maintaining their ability to bind to their target *in vitro* and *in vivo*. The bi-functionalised aptamer, conjugated on the amino modified 5′ end to MAG2, a strong ligand for ^{99m}Tc, and on the thiol modified 3′ end to different PEGs has been found to have improved pharmacokinetic properties and the ability to localise in the tumour and remain in the system longer, when compared to non-PEGylated aptamers, and counteract some of the effect the chelators have on the *in vivo* aptamer properties. These results can lead to the development of aptamers as a novel targeted radiotherapy for breast cancer.

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228 Renal cancer histone acetylation and protein ubiquitination enhanced synergistically by bortezomib and suberoylanilide hydroxamic acid

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Background: There is no curative systemic therapy for advanced renal cancer. Inhibiting histone deacetylase (HDAC) and proteasome activity acts against malignacies cooperatively, but this treatment strategy has not been tested against renal cancer. In the present study we combined the HDAC inhibitor suberoylanilide hydroxamic acid (SAHA) with the protease inhibitor bortezomib and found that they inhibited renal cancer growth by enhancing histone acetylation and protein ubiquitination synergistically.

Material and Methods: The viability and clonogenicity of renal cancer cells (769-P, A498, ACHN, Caki-1) treated with SAHA (1−5 μM) and/or bortezomib (5–100 nM) were assessed by MTS assay and colony formation assay, and the in vivo efficacy of the combination was evaluated using a murine subcutaneous tumour model. The expression of acetylated histone, phosphorylated retinoblastoma protein (Rb), cyclin D1, cyclin-dependent kinase (CDK) 4 and HDACs 1, 2, 3, and 6 was assessed using western blot analysis. Apoptosis was assayed using flow cytometry and detecting active caspase 3 and cleaved poly (ADP-ribose) polymerase (PARP). Protein ubiquitination was evaluated by western blot analysis.

Results: The combination of SAHA and bortezomib induced apoptosis and inhibited cancer cell proliferation and colony formation synergistically. In murine subcutaneous tumour models using Caki-1 cells, SAHA and bortezomib in combination inhibited tumour cell growth significantly more than did each agent alone. SAHA alone induced histone acetylation and Rb dephosphorylation, and bortezomib enhanced this acetylation and dephosphorylation by inhibiting the expression of HDACs 3 and 6, cyclin D1 and CDK4. Bortezomib alone increased protein ubiquitination in a dose-dependent fashion, and SAHA enhanced this protein ubiquitination.

Conclusions: SAHA in combination with bortezomib inhibited the proliferation of renal cancer cells in vitro and in vivo, and the effectiveness of the combination is due to their enhancing histone acetylation and protein ubiquitination synergistically.

229 Effects of CYP2C19 genotype on tamoxifen and estrogen metabolism

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Background: Cytochrome P450 2C19 (CYP2C19) is an important drug-metabolizing enzyme with variability determined by genetic polymorphism. The CYP2C19*2 and *3 variant alleles result in reduced enzymatic activity. In contrast, CYP2C19*17 is associated with ultrarapid CYP2C19 activity and identifies patients likely to benefit from tamoxifen treatment [4] and may lead to decreased estrogen levels and therefore reduces breast cancer risk [3]. Since tamoxifen and estrogens are also partly metabolized by these enzymes, these enzymes may influence tamoxifen and estrogen metabolism which may in turn affect treatment outcome. In the present study we examined the influence of CYP2C19 genotype on serum concentrations of estrogens as well as the levels of tamoxifen and its metabolites.

Methods: Tamoxifen and its metabolites were measured by using liquid chromatography-tandem mass spectrometry in samples from 90 post-menopausal breast cancer patients during steady state tamoxifen treatment [2]. Estrogen levels were determined using a sensitive radioimmunoassay [1].

Results: We observed negative associations between increased CYP2C19-predicted enzymatic activity and the serum concentrations of tamoxifen metabolites and estrogens. Lower serum levels of tamoxifen metabolites N-dedimethyltamoxifen (NDDtam) and tamoxifen-N-oxide (tamNox) ($p = 0.043$ and $p = 0.031$, respectively) and estrogens estrone (E1) and estradiol (E2) ($p = 0.008$ and $p = 0.045$, respectively) were observed in subjects carrying CYP2C19*17 allele when compared with patients being heterozygous or homozygous for the CYP2C19*2 and *3 alleles.

Subjects carrying CYP2C19*17 allele encoding for ultrarapid enzyme activity showed a significant higher 4-hydroxytamoxifen (4OHtam) to tamoxifen ratio ($p = 0.008$) and lower tamNox to tamoxifen ($p = 0.03$) and NDDtam to N-demethyltamoxifen (NDtam) ($p = 0.027$) compared with subjects heterozygous and homozygous for the variant allele CYP2C19*2 and wild type allele. No difference was observed between CYP2C19 genotypes and metabolic ratio of 4-hydroxy-N-demethyltaoxifen (4OHNDtam) to NDtam.

Conclusion: The observed benefit of tamoxifen treatment in patients carrying CYPC19*17 can be explained by increased hydroxylation of the potent hydroxylated metabolite 4OHtam and reduced circulating estrogen levels.

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230 Glutathione modulation reverses the growth-promoting effect of growth factors, improving the SN-38 antitumour response in WiDr colon cancer cells

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Background: Several growth factors (GF) are involved in the paracrine growth mechanisms of colon cancer cells and have therefore been identified for their potential to modulate the sensitivity of tumour cells to chemotherapeutic agents, such as SN-38. Since glutathione (GSH) plays an important role in the growth-promoting effect of GFs and it is also involved in the protection against cellular injury caused by various anticancer agents, modulating cellular susceptibility to chemotherapy, manipulation of GSH levels might yield a therapeutic gain for chemotherapy in the presence of GFs.

Materials and Methods: The effect of GSH modulation on SN-38 activity on the WiDr colon cancer cell line was studied. Cell proliferation and GSH content were assessed. Cells were exposed to the GSH modulators, L-buthionine-SR-sulfoximine (BSO) or L-2-oxothiazolidine-4-carboxylate (OTZ), before treatment with SN-38 in the presence of hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF) or epidermal growth factor (EGF).

Results: Exposure to GFs significantly increased GSH levels and induced a pro-tumour effect, producing a near 20% reduction in SN-38 antitumour activity after 48 h of incubation. Treatment with OTZ and BSO abrogated the growth-promoting effects of GFs. Moreover, the addition of OTZ to SN-38 resulted in a synergistic effect at 24 h and produced a nearly 70% increase in the cytotoxic activity of SN-38 in the presence of GF at 72 h. Similarly, the combination of BSO and SN-38 produced a significantly greater antitumour effect than SN-38 alone, leading to an approximately 50% increase in the cytotoxic activity of SN-38 in the presence of GF at 72 h.