In contrast, all neuroblastoma cell lines analyzed proved to be infectable with H-1PV, with complete viral replication yielding virus titer increase of up to 10.000-fold within 48 to 96 h after infection. Lytic infection was observed at TCIDs50 between 0.001 and 10 pfu/cell. Cell killing was independent of the status of MYCN amplification in the respective cell lines.
As with neuroblastoma cells, all medulloblastoma cell lines were efficiently infectable with H-1PV, displaying a dose-dependent cytotoxicity with TCIDs50 between 0.001 and 5 pfu/cell. Similar to the results with neuroblastoma cells, efficient viral replication could be shown in medulloblastoma cells by an increase of virus titers in a range of two log-steps within 72 to 96 h after infection.
In both, neuroblastoma and medulloblastoma cells, H-1PV induced a G2-arrest and subsequent apoptosis.
Conclusions: The data infer that application of oncolytic H1-PV may be a promising treatment option for embryonic tumours of neuroectodermal origin. This, however, has to be substantiated by a pre-clinical evaluation of the therapeutic efficacy for these tumours *in vivo*.

217 Testing of a poly(ADP-ribose) polymerase (PARP) inhibitor on human BRCA2 heterozygous cell lines

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The PARP inhibitor, AZD2281, olaparib®, is a promising targeted anti-cancer agent for patients with specific DNA-repair defects such as found in BRCA1 and BRCA2 mutation carriers. Early clinical trials suggest that this targeted drug is effective on tumour cells and well tolerated by normal tissues in mutation carriers.
Materials and Methods: Response to olaparib, was tested on three heterozygous mammary epithelial cell lines derived from carriers of a 999del5 BRCA2 founder mutation (A176, A240, A256) and one non-BRCA cell line (D492) transformed in the same way, as well as the commercially available BRCA2 deficient pancreatic cell line Capan-1 and the mammary cell line MCF7. Heterozygous cell lines were examined for BRCA2 allel loss using TaqMan qRT-PCR and copy number changes on CGH arrays (385K aCGH; NimbleGen Systems). CellTiter 96® Aqueous One Solution Cell Proliferation assay (MTS assay) was used to estimate survival and determine the maximum tolerated dose of olaparib and IC_{50} values for all cell lines. Cell death was also assessed with annexin-V and probidium iodide staining. Immunostaining for Rad51 and γH2AX was carried out to evaluate DNA double stand breaks and DNA repair.
Results from olaparib testing using the MTS assay show that the heterozygous cell-lines A176, A240 and A256 have similar IC_{50} values as both the non-BRCA cell lines, D492 and MCF-7. Whereas, Capan-1 shows increased sensitivity to the inhibitor. Annexin-V and PI staining show that the Capan-1 cell line goes through apoptosis at low dosages. Only at exposure to high dosages did the heterozygous cell lines show PI staining. Immunostaining with γH2A.X and RAD51 antibodies indicates that the Capan-1 cell-line has loss of γH2AX/RAD51 colocalization after treatment with olaparib, whereas the heterozygous BRCA2 cell-lines show colocalization after treatment.
In conclusion: Human mammary cell lines heterozygous for a BRCA2 mutation that have retained the second BRCA2 allel are not more sensitive to PARP inhibitor olaparib treatment than non-BRCA2 mammary cell line controls.

218 L-Asparaginase-loaded red blood cells: a promising therapy in solid tumours

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Tumour cells deficient in asparagine synthetase (ASNS) are unable to synthesize enough L-asparagine (L-Asn) to meet metabolic demands and, therefore, depend upon circulating L-Asn in the plasma for survival. L-asparaginase (L-aspa), which depletes L-Asn via deamination, has been used in the treatment of acute lymphoblastic leukemia (ALL) for over 40 years, since the majority of such tumours are deficient in ASNS. Patients able to tolerate more than 25 weeks of L-aspa experience significantly increased survival, but side effects often preclude that duration of use. However, in a phase II clinical trial, entrapment of L-aspa inside red blood cells (GRASPA®) has been found to strongly reduce side effects and also to improve L-aspa pharmacokinetics. Recent evidence suggests that subsets of solid tumours (head and neck, ovarian and pancreatic) are deficient in ASNS, providing rationale for testing L-aspa against such tumours.
To demonstrate the utility of L-aspa in solid tumours, we investigated:
- Expression of ASNS on human cell lines and tumour samples by immunohistochemistry and western blot
- IC50 and GI50 of L-aspa and cell viability in L-Asn deprived medium
- *In vivo* studies in subcutaneous and intraperitoneal metastasis models.

Four pancreatic and 4 ovarian cell lines were studied, as well as MOLT-4, a lymphoblastic leukemia cell line used as a negative ASNS expressing control.
In several patient tumour samples, the healthy tissues highly expressed ASNS in opposite to cancerous tissues which weakly expressed ASNS. With cell lines, ASNS expression was variable regardless of the detection method used, but only MOLT-4 was totally negative.
L-aspa was efficient *in vitro* in all cell lines studied (IC50 between 0.1 and 0.6 IU/mL). In addition, we confirmed that cytotoxicity was essentially due to L-Asn deprivation from the medium.
In vivo, a pancreatic model in mice displayed sensitivity to GRASPA in combination or not with gemcitabine. However, this sensitivity was dependent on the treatment schedule.
The results indicate that L-Asn depletion can be an effective strategy for treating solid tumours. GRASPA®, a better tolerated L-aspa formulation is under evaluation in a phase I clinical trial for pancreatic cancers and a biomarker assay for ASNS expression is being developed for patient stratification.

219 Cytotoxicity and cell death signaling in stem cell like AML cells

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Background: Acute myeloid leukemia (AML) is a result of uncontrolled proliferation of white blood cells in the myeloid line of cell differentiation in the bone marrow. High dose chemotherapy is the current treatment option but a large proportion of the patients relapse within 1−2 years and the 5-year survival threshold is low, particularly in elderly patients. Thus, new effective therapeutic drugs are highly desirable. We evaluate the cytotoxic potential of two novel treatments, gemtuzumab ozogamicin (GO), a monoclonal antibody linked to a toxin causing DNA double strand breaks and J1, an alkalyting prodrug of melphalan, in AML cell lines of different maturation stages. The aim of this study was to understand if and how these novel agents may work in different AML maturation stages with comparison to the conventional therapies daunorubicin or cytarabine.
Material and Methods: Two AML cell lines with different maturation stages were used. The partly differentiated HL60 cells were compared to the immature Kasumi-1 cells, both expressing high levels of CD33 on their surface and both positive for CD13. The cytoxic responses of the agents were compared using MTT cell viability assay. Activation of the proapoptotic proteins caspase 3 and Bax were determined using activation specific antibodies and was evaluated using FACS analysis.
Results: Treating the AML cells with GO, J1 daunorubicin or cytarabine revealed significant differences in their cytotoxic potential in HL60 and Kasumi-1 cells. Thus, daunorubicin (100 nM) and cytarabine (1 μM) were found to cause 50% growth inhibition at 48 h post drug addition in HL60 cells whereas in Kasumi-1 cells no more than 20% growth inhibition was observed. This was consistent with a lack of Bax and caspase-3 activation in Kasumi-1 cells. Comparison of GO sensitivity revealed a 50% growth inhibition in HL60 cells after 48 h of treatment with 100 ng/ml GO while no inhibition was observed in Kasumi-1 cells. This was not caused by different expression of the target CD33 on the cells, but may be explained by less efficient activation of caspase-3. A comparison of J1-induced cytotoxicity in HL60 and Kasumi 1 cells revealed a 50% growth inhibition after 48 h treatment with 0.5 μM J1 in HL60 cells whereas the Kasumi-1 cells needed 5 μM to achieve similar growth reduction. Analysis of apoptotic signaling is ongoing.
Conclusions: We demonstrate that the maturation state of the AML cells influences their responsiveness to both novel and conventional chemotherapeutics. The immature Kasumi-1 cells were more resistant to all drugs tested and only J1 was able to induce a proper cytotoxic response. Our data suggest that the capacity to trigger apoptotic signaling may explain why the drugs have a diverse cytotoxic potential in different maturation stages of AML. Further analyses of Kasumi-1 cells may reveal novel therapeutic targets that might have profound therapeutic implications for AML.

220 Identification of resistance mechanisms to EGFR inhibitors in non-small cell lung cancer cells

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Non-small cell lung cancer (NSCLC) patients are routinely treated with small molecule EGFR tyrosine kinase inhibitors, such as erlotinib and gefitinib. These inhibitors compete reversibly with ATP to bind the intracellular catalytic domain