NAV3 gene can be used as a potential new diagnostic marker for CRC and membrane proteins targeted by NAV3 as novel therapy targets.

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Characterization of cellular resistance mechanisms towards NAD synthesis inhibitors APO866 and CHS-828

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CHS-828 is a pyridyl cyanoguanidine, which has completed phase I clinical trials in oncology and displays potent antitumor activity against a broad selection of malignant cell types. Recently, we identified its mechanism of action as being an inhibitor of nicotinamide adenine dinucleotide (NAD) synthesis. It displays similar characteristics as the structurally distinct compound FK866 (APO866) – an inhibitor of nicotinamide phospohoribosyl transferase (Nampt) – that is currently in several phase II trials. Here, we report that NYH/CHS – a derivate of the SCLC cell line NYH with specific resistance towards both CHS-828 and APO866 – carries a triplet deletion in one copy of the Nampt gene corresponding to a deletion of Asp93. This deletion is not found in wild type NYH cells. NYH/CHS resistance towards CHS-828 remains unchanged after 60 passages of culturing without drug and the deletion persists. Furthermore, we have induced high-grade resistance towards APO866 in several cell lines including NYH/APO866 and HCT-116/APO866. Both cell lines show marked cross-resistance towards CHS-828. Interestingly, NYH/APO866 displays the same deletion as NYH/CHS suggesting that the NYH wild type cell line harbours a small subpopulation of cells with this mutation, which leaves the cells more resistant to the Nampt inhibitors. In the HCT-116/APO866 cell line a point mutation in one copy of the Nampt gene leads to a H191R substitution. This histidine is part of the binding site for APO866 but is not involved in binding of nicotinamide mononucleotide. NYH/CHS does not have increased expression of Nampt compared to wild type. However, HCT-116/APO866 display increased Nampt expression when compared to wild type cells. Further investigations of the mechanisms of acquired resistance towards APO866 and CHS-828 will be presented. In conclusion, malignant cells can gain resistance towards Nampt inhibitors, either by mutations in the Nampt gene that do not interfere with nicotinamide mononucleotide production, or by increasing the expression of Nampt. Also, it is likely that CHS-828 inhibits Nampt by binding in a manner similar to APO866.

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Discovery and characterization of a new potent orally available Cdc7 inhibitor with anti-tumor activity

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Cdc7 is serine/threonine kinase essential for the initiation of DNA replication. We have previously shown that inhibition of Cdc7 kinase by RNA interference or small molecules inhibitors (Montagnoli et al., 2004; Montagnoli et al., 2008) causes p53 independent tumor cell death, while it only causes reversible cell cycle arrest in primary fibroblasts supporting the rationale for the development of Cdc7 kinase inhibitors for cancer therapy. Here we report the discovery and the properties of a low nanomolar orally available small molecule inhibitor of Cdc7 kinase.
This compound is extremely potent in blocking proliferation and inducing apoptosis in a large panel of cancer cell lines both from solid and haematological tumors. Consistently with Cdc7 inhibition, cells show a DNA replication block, induction of apoptosis and inhibition of phosphorylation of the Mcm2 protein on a Cdc7 specific phospho-site (Montagnoli et al., 2006). This compound also shows very favourable PK parameters with low clearance, high volume of distribution and good oral bioavailability in rodent and non-rodent species. Concerning the in vivo profile, oral administration of this compound causes tumor and, occasionally, tumor regression in a variety of animal tumor models. Notably, the compound is well tolerated also after prolonged exposure. A clear modulation of biomarkers correlated with compound activity is also observed.
The excellent preclinical features make this compound a good candidate for clinical trials.

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Molecular sequelae mediating antitumor activity of G-quadruplex-interactive agent TMPyP4 in retinoblastoma cell lines

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Introduction: Guanine (G)-quadruplexes are 4-stranded DNAs with stacks of G-quartets formed by 4 Gs in a planar structure through hydrogen bonding. The formation of G-quadruplexes presented in the promoter or regulatory regions of important oncogenes, and in the single-stranded G-rich overhang of telomeres has been shown *in vitro*. G-quadruplex structures may affect essential cellular processes. In this study, we investigated the molecular mechanism of the antitumor activity of the cationic porphyrin 5, 10, 15, 20-tetra-(N-methyl-4-pyridyl)porphyrin TMPyP4 in retinoblastoma cell lines.
Material and Methods: We investigated the molecular mechanism of the antitumor activity of TMPyP4 in Y79 and WERI-Rb1 retinoblastoma cells using MTS assay, analysis of apoptotic cells, cDNA microarray, Western blotting.
Results: TMPyP4 (10–100 μM) directly inhibits telomerase activity in vitro TRAP assay, suggesting that TMPyP4 can form stable G-quadruplexes in telomere templates and interfere with telomere replication by blocking the elongation step catalyzed by telomerase. The anti-proliferative activities of TMPyP4 assessed by the MTS assay are shown in terms of IC_{50}: Y79 cells, 60 μM; WERI-Rb1 cells, 45 μM. Moreover, treatment TMPyP4 at doses of 10, 50 and 100 μM for 48 hours and 10, 20, 50 and 100 μM for 72 hours significantly inhibited the growth of Y79 cells, and treatment TMPyP4 at doses of 10, 20, 50 and 100 μM for 48 and 72 hours significantly inhibited the growth of WERI-Rb1 cells. The apoptotic cells were measured with a fluorescent marker for activated caspases, CaspACE™ FITC-VAD-FMK. Treatment TMPyP4 at doses of 0, 10, 20, 50 and 100 μM for 48 hours induced apoptosis in Y79 cells (4.4%, 13.9%, 26.4%, 60.5%, and 56.2%) and WERI-Rb1 cells (18.5%, 28.3%, 30.1%, 41.6%, and 48.2%). cDNA microarray analysis in cultured Y79 cells with 20 μM TMPyP4 for 48 hours revealed upregulation of 26 genes, and downregulation of 41 genes. Moreover, we found that TMPyP4 increased the expression of p53 protein at 4 to 24 hours in Y79 cells, but not in WERI-Rb1 cells. There was no change in $p21^{CIP1}$ protein expression in both Y79 cells and WERI-Rb1 cells. In addition, we found activation of MAPKs in both Y79 and WERI-Rb1 cells.
Conclusion: This study provides understanding the molecular mechanism of the antitumor effects of TMPyP4. G-quadruplex structure is a potential therapeutic target in retinoblastoma.

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Once weekly rIL-21 in combination with cetuximab as 1st line therpay in CRC. A dose finding safety trial

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Background: IL-21 is a class I cytokine with antitumour properties due to enhanced proliferation and effector function of CD8+ T cells and natural killer (NK) cells.
The safety and efficacy of rIL-21 is currently tested as monotherapy and in various combinations. Cetuximab is a chimeric IgG1 monoclonal antibody (mAb) used in the treatment of stage IV CRC. Preclinical data indicate enhanced antitumour activity when combining IL-21 and cetuximab.
Methods: A phase 1, multi centre, open label, safety and tolerability study of escalating doses of rIL-21 in combination with cetuximab. Both drugs were administered once weekly i.v. in: asymptomatic first line patients with stage IV CRC; PS 0−1; a life expectancy >3 months; with no requirement of immediate chemotherapy and without resectable metastases. One week after cetuximab loading dose (400 mg/m^2); escalating doses of rIL-21 were administered as bolus infusion after the maintenance dose of cetuximab (250 mg/m^2). DLTs were monitored for 7 weeks of combined treatment and patients without symptomatic progression hereafter, were offered an additional 8 weeks of combined treatment.
Objectives: To assess safety and tolerability of escalating doses of rIL-21; to determine the MTD and investigate dose-response relationship for selected biomarkers, pharmacokinetics and to assess immunogenicity.
Results: A total of 13 pts have been included (the trial is still recruiting) at 3, 10, 30 and 100 μg/kg. All patients have experienced rash (grade ⩽2). Other adverse events (AE) are fatigue and dry eyes; all grade ⩽2

except in one patient who experienced diarrhoea and fatigue grade 3 at 100 μg/kg. Both events were dose limiting toxicities (DLT) per protocol. Grade 3 lymphopenia has been observed within the 24 h following dosing in most of the patients dosed at ⩾30 μg/kg.
So far, 55% of the patients that have completed the first 8 weeks of treatment continued with extended treatment. The pharmacokinetic profiles of neither cetuximab nor rIL-21 seem to be affected by the combined treatment. The immune activation serum marker, soluble CD25 (sCD25), remains elevated between the weekly dosings at 30 and 100 μg/kg in a dose dependent manner.
Conclusion: The safety profile is manageable with no overlapping toxicities of concern of rIL-21 so far (3 ug/kg to 100 ug/kg) and cetuximab administered once weekly i.v.

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Toxicology and pharmacokinetics of humanized AR47A6.4.2, the first unconjugated therapeutic monoclonal antibody targeting TROP-2

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TROP-2 is a signal transduction molecule which is widely expressed in a number of human carcinomas. In the clinic, high levels of TROP-2 expression have been correlated with poor prognosis and a decrease in patient survival in human colorectal cancer as well as in head and neck carcinomas. Overexpression studies have validated Trop-2 as an oncogene, as its overexpression alone was sufficient to induce tumorigenesis in mice. The first unconjugated therapeutic antibody targeting TROP-2, AR47A6.4.2, was identified using the Arius' FunctionFIRST™ platform. The antibody demonstrated dose-dependent tumor growth inhibition in established in vivo xenograft models of pancreatic, colon, breast and prostate cancer. In vitro, studies have shown that AR47A6.4.2 treatment diminishes MAPK phosphorylation. IHC staining of human cancers demonstrated that the epitope for AR47A6.4.2 is present on the majority of human adenocarcinomas. Normal tissue binding was observed predominantly in ductal epithelium of the pancreas, liver, lung and kidney. The binding pattern was almost identical in cynomolgus monkey tissues. To further clinical development, a humanized form of the antibody was generated which had high affinity and potent anti-tumor efficacy. A dose-ranging toxicology study was carried out in cynomolgus monkeys with the humanized antibody. In the first phase, monkeys (2 per group) were given a single 1 hr infusion of 10, 25 or 80 mg/kg of huAR47A6.4.2. The animals were alert and active during the infusion and throughout the study. Clinical chemistry, coagulation, and hematology (blood cell counts or morphology) were assessed at several time-points. Upon necropsy, both of the mid-dose-treated monkeys and one of the high-dose treated monkeys had focal red spots in the large intestine that did not appear dose-dependent. A second cohort of monkeys was infused with 0, 10 and 25 mg/kg huAR47A6.4.2 every 3 days for 3 doses to confirm the findings above, and to follow up on pharmacokinetic analysis. The positive outcomes of these studies demonstrate the viability of targeting TROP-2 in a primate model at doses substantially higher than doses required for therapeutic effect in pre-clinical cancer models. These studies support the clinical development of a therapeutic monoclonal antibody targeting this novel cancer-associated target.

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Aberrant expression of glycosylation in juvenile gastrointestinal stromal tumours

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Background: Most adult gastrointestinal stromal tumors (GISTs) are thought to be caused by activating mutations in the KIT or PDGFRA gene. However, many juvenile GISTs lack either mutation and are considered to develop with a different pathogenesis. To investigate the molecular characteristics of juvenile GISTs, we analyzed the proteome difference in phosphorylated protein between adult and juvenile GISTs.
Material and Methods: Eleven GIST samples (7 adult cases and 4 juvenile cases lacking either mutation) were analyzed by using the differential display. And, the specific phophorylated proteins were digested by trypsin and searched by using LC-MS/MS. Furthermore, the analysis of post translational modification was done using the enzymes; glycopeptidase F and neuraminidase.
Results: Comparative analysis of tyrosine phosphorylated protein levels showed that juvenile GISTs possessed phosphorylated KIT in spite of lacking mutation in the KIT gene. Moreover, downstream signals of KIT were also activated as in adult GISTs. Although, SDS-PAGE gels showed that there was a difference of each KIT bands between adult and juvenile GISTs, they became the same after removal of N-glycans or sialic acids. Moreover, one of the most typical enzymes, ST6Gal1, which transfers Neu5Ac residues in α2−6 linkage to Gal β1−4GlcNAc units on N-glycans, is significantly less expressed in juvenile GISTs.
Conclusion: Aberrant expression of glycosylation in juvenile GISTs is generated by post-translational modification and may play a role in the progression of juvenile GISTs.

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HP1gamma epigenetically regulates cell differentiation and exhibits potential as a therapeutic target for various types of cancers

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Heterochromatin protein 1 (HP1) is a chromosomal protein that participates in chromatin packaging and gene silencing. Three HP1 isoforms (α, β, and γ) occur in mammals, but their functional differences are still elusive. In this study, we have found that HP1γ is decreased along with adipocyte differentiation, while HP1α and β are expressed constantly in cultured preadipocyte cells. In addition, ectopic overexpression of HP1γ inhibited adipogenesis. Furthermore, we did not detect HP1γ protein in the differentiated cells in various normal human tissues. These results suggest that the loss of HP1γ is required for cell differentiation. On the other hand, the methylation level of lysine 20 (K20) on histone H4 showed a significant correlation with HP1γ expression in these preadipocyte cells and normal tissue samples. However, all of cancer tissues examined were positive for HP1γ but sometimes negative for trimethylated histone H4 K20. Thus, dissociation of the correlation between HP1γ expression and the trimethylation may reflect the malfunction of epigenetic control. Finally, suppression of HP1γ expression restrained cell growth in various cancer-derived cell lines, suggesting that HP1γ may be an effective target for gene therapy against various human cancers. Taken together, our results have demonstrated the novel function of HP1γ in the epigenetic regulation of cell differentiation and cancer development.

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Efficient LNA-mediated antagonism of the oncogenic microRNA-155 in vitro and in vivo

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Background: microRNAs (miRNAs) are ~22 nt endogenous non-coding RNAs that post-transcriptionally repress expression of protein-coding genes by base-pairing with the 3′-untranslated regions of the target mRNAs. Emerging evidence implies that animal miRNAs play important roles in the control of many biological processes, and miRNAs are both implicated in the onset and progression of cancers. microRNA-155 (miR-155) is located within exon 2 of the non-coding B cell integration cluster (Bic) gene. Overexpression of miR-155 has been reported in haematological malignancies, such as B-cell lymphomas, and when overexpressed as a transgene in B cells, miR-155 gives rise to pre-B-cell lymphomas. Hence, miR-155 is a potential therapeutic target for treatment of haematological B-cell malignancies.
Materials and Methods: Locked nucleic acid (LNA)-modified oligonucleotides have proven outstanding in miRNA recognition and detection due to their high specificity and affinity, and recent studies have also reported efficient LNA-mediated miRNA silencing in vivo.
Results: Using the monocytic-macrophage RAW264.7 cell line as an in vitro model system, we identified a potent LNA-antimiR oligonucleotide for functional antagonism of endogenous miR-155, as demonstrated by luciferase reporter assays and by western blot analysis of miR-155 targets. Furthermore, systemic delivery by intravenous injections of the LNA-antimiR into mice resulted in miR-155 antagonism in the spleen with concomitant derepression of direct miR-155 target proteins.
Conclusion: We report here efficient LNA-mediated silencing of miR-155 in vitro and in vivo.