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