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Fatty acid synthase is a potential therapeutic target in Micro-satellite-instable colorectal cancers

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Background: Many human epithelial cancers, particularly those with a poor prognosis express high levels of fatty acid synthase (FASN), a key metabolic enzyme linked to synthesis of membrane phospholipids in cancer cells. Over-expression of FASN is linked with activation of phosphatidylinositol-3′-kinase (PI3K)/AKT pathway. In this study, we investigated the role of FASN and its relationship with PI3K/AKT activation in a large series of colorectal carcinoma (CRC) tissues in a tissue micro array (TMA) format followed by in vitro and in vivo studies using CRC cell lines and NUDE mice.
Materials and Methods: Analysis of apoptosis and cell cycle was evaluated by flow cytometry and DNA fragmentation assays. FASN and phospho-AKT protein expression were determined by IHC and Western blotting.
Results: Correlation of FASN with various clinico-pathological parameters on 448 CRC samples was assessed. Activated AKT was found in 283/400 (68.8%) of CRC and was associated with FASN over-expression. FASN over-expression was observed in 109/403 (27.1%) and was significantly more common in Micro-satellite-instable (MSI) than Micro-satellite-stable (MSS) tumors ($p < 0.01$). In addition, our in vitro data using HCT-15, an MSI CRC cell line showed a better apoptotic response following inhibition of FASN activity as compared to Colo-320, an MSS CRC cell line. Finally, treatment of HCT-15 cell line xenografts with C-75 resulted in growth inhibition of tumors in NUDE mice via down-regulation of FASN and AKT activity.
Conclusions: These data identify FASN as a potential biomarker and a novel therapeutic target in distinct molecular subtypes of CRC.

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Obatoclax in SCLC: preclincal evaluation of a BH3 mimetic

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Lung cancer is the leading cause of cancer death with small cell lung cancer (SCLC) representing approximately 15% of cases. Despite combination chemotherapy with platinum/etoposide survival rates remain dismal warranting the development of novel therapeutic strategies. The Bcl-2 family of proteins are key regulators of apoptosis, with most apoptotic stimuli converging at the mitochondrial surface where interactions between Bcl-2 family members determine cell fate. The observation that Bcl-2 is present in 75% of SCLC clinical specimens and that over-expression of anti-apoptotic Bcl-2 family members confers resistance to chemo-radiation in vitro has promoted clinical development of Bcl-2 targeted therapies in SCLC.
Obatoclax, a novel BH3 mimetic, is thought to bind inclusively to the BH3-binding groove of anti-apoptotic Bcl-2 family proteins. Using a short-term viability assay (MTS) for suspension cells, the IC50 values for obatoclax were calculated as 0.07–1.04 μM in a panel of eight SCLC cell lines in vitro. Induction of apoptosis (PARP cleavage) in response to obatoclax was both time- and concentration-dependent. Chou-Talalay combinational index studies showed synergy over 96 hours with the clinically relevant chemotherapeutics cisplatin and etoposide. The synergy was schedule-dependent, with exposure of cells to 48 h obatoclax prior to cisplatin and etoposide resulting in greater synergy than either the reverse sequence or 96 h concomitant treatment, in all eight cell lines. Experiments determining the timing of apoptosis in relation to obatoclax sensitivity as a single agent and in combination with cytotoxics will be reported.

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Effects of PPARgamma agonists on adrenocortical carcinoma in a murine xenograft model

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Objective: The purpose of this study was to evaluate effect of rosiglitazone (RGZ), a synthetic high-affinity ligand for PPARgamma, in a mouse model of human adrenocortical carcinoma. Previous studies in vitro demonstrated that treatment of human adrenocortical cancer cell line with PPARgamma agonists reduced malignant potential (Ferruzzi P, et al. J Clin Endocrinol Metab, 2005).
Material and Methods: Tumour xenograft was obtained by subcutaneous injection of 7×10^6 H295R cells, a human adrenocortical cancer cell line, in the right flank of nude Balb/c mice. When the tumour size reached a mean of 5 mm, the animals were randomly allocated to 2 groups of 9 mice treated with: (1) RGZ 5 mg/kg in oral administration (gavage) 6 days a week; (2) water, same schedule (control group). Tumour volume was evaluated twice a week for 31 days by the formula: tumour volume = length x width2/2 and tumour response was estimated versus initial volume. Once mice were sacrificed, tumours were removed, fixed and stained with haematoxylin and eosin.
Results: We observed a significant reduction of tumour growth in the RGZ group ($p = 0.007$). At the analysis of tumour specimens, in the control group tumour presented characteristics of invasiveness, richness in small vessels and numerous mitotic figures. In the RGZ group tumour presented expanding and not infiltrating borders, there were an evident lack of vessel and numerous apoptotic bodies.
Conclusions: This study supports a role of RGZ in adrenocortical carcinoma therapy. Further investigations are underway to improve our knowledge on RGZ molecular mechanisms in tumour and to define the optimal RGZ dose for anticancer effect.

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NAV3 gene aberrations in colorectal cancer target signalling pathways associated with inflammation and the progression of cancer

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We have previously shown that chromosome 12q21 aberrations, specifically allelic loss of the neuron navigator 3 (NAV3) gene, associate with Cutaneous T-cell lymphomas. Since loss of the chromosomal region 12q is reported to associate with a poor prognosis in several cancers of epithelial origin, we looked for eventual NAV3 gene aberrations in colorectal cancers (CRC) as well as in intestinal adenomas. As a result, copy number changes, in the form of allelic loss of NAV3 but also in amplification of this gene, were found.
To mimic the in vivo gene deletion and to shed light on the function on the NAV3 gene in CRC carcinogenesis, we successfully silenced the expression on NAV3 using commercially available pooled oligonucleotides in the established colorectal cell lines CRL1539 and CRL 1541 (ATCC, Manassas, VA, USA). Post-transfection RNA-samples from different time points were processed for Agilent 4*44K microarray analysis. With thoroughly preprocessed microarray data across all samples, we identified 39 differentially expressed genes (DEGs) using fold changes as the selection criterion. These genes were further analyzed with Gene Ontology and pathway analysis tools to reveal their possible functional roles. With annotation methods we identified genes that are most likely drug targets (e.g., genes that are already associated with cancer and code membrane proteins).
Altogether, 16% of the up-regulated genes were membrane proteins and thus potential targets for antibody-based therapy. Likewise, 16% of the up-regulated genes were genes known to be associated with different types of cancer, including carcinomas of ventricle, breast, pancreas, lung and prostate as well as tumours of neural and lymphoid origin (both T- and B-cell lymphomas). In pathway analysis, two of the NAV3-regulated membrane proteins significantly target signalling pathways contributing to oncogenesis.
We conclude that NAV3 aberrations affect signalling pathways associated with cancer-related inflammation and the progression of cancer. Thus, the

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