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Targeting CDC25 phosphatases in cancer therapy

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CDC25 phosphatases are key regulators of cell proliferation. They dephosphorylate and activate Cyclin-Dependent Kinases (CDK)–cyclin complexes, thus allowing phosphorylation of key substrates and cell cycle progression [1]. Over the last few years, original compounds inhibiting CDC25 phosphatases both in vitro and in cultured cells have been identified and characterised [2]. For instance, BN82685 inhibits enzyme activity and cell proliferation in the hundred nanomolar range. Using cellular assays we have confirmed the target specificity of this compound toward CDC25 activity. Inhibitors of CDC25 phosphatases have also proven to be active in vivo on human tumour xenografted in mice and to be active by oral route [3]. We have also recently shown that BN82685 dependent inhibition of CDC25 phosphates activity results in microtubule dynamics alteration in interphase and impairs the correct assembly of the mitotic spindle. Furthermore, we show that combining low concentrations of both BN82685 and paclitaxel inhibits the proliferation of HT29 human colon cancer cells, suggesting that therapeutic combination of CDC25 inhibitors with microtubule targeting agents may be of valuable interest. Altogether, our data confirm that targeting of CDC25 phosphatases with small inhibitory compounds is a promising novel approach in cancer therapy.

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A phase I study of R547, a novel cyclin dependent kinase inhibitor, in patients (pts) with advanced cancer: preliminary results

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Background: Dysregulation of cyclin dependent kinases (CDKs) is common in neoplasia. R547 (**R**) is a potent, specific inhibitor of CDKs 1, 2, and 4. The safety and pharmacokinetics (PK) of R were explored in a phase I dose escalation study.
Materials and Methods: R administered as a 90 min infusion D1, D8 (21 day cycle). Inclusion criteria: ECOG PS 0–2, adequate hematologic, hepatic, and renal function. Exclusion criteria: brain metastases, neuropathy $\geqslant$ grade 2, NYHA III/IV CHF, recent MI, CVA, or current antihypertensive therapy. DLT (cycle 1) definition: $\geqslant$ gr 3 non-hematologic toxicity, febrile neutropenia, gr 4 neutropenia > 5 days, gr 4 thrombocytopenia, or dose delay $\geqslant$2 weeks due to toxicities. PK blood samples collected cycle 1, D1, D8.
Results: 32 pts have received R to date (3–6 per cohort; dose range of 8.6−195 mg/m^2). Six pts remain on study. Mean pt age 60 yrs (range 28−81), mean prior treatments 5.0 (range 0–10). Mean treatment cycles R 2.6 (range <1–6); in the 155 mg/m^2 cohort mean 5.0 (range 2–6). *Toxicities*: Principal events include nausea (50%), hypotension (39%), fatigue (36%), emesis (29%), and headache (29%). Toxicities were clinically manageable with addition of iv fluid, anti-emetic, and prn analgesic support. For 3 pts treated in the 195 mg/m^2 cohort, DLTs of gr 3 somnolence, gr 3 confusion, and gr 3 fatigue occurred. Two of these 3 were successfully retreated following dose reduction to 155 mg/m^2. *PK:* C_{max} and AUC are dose proportional over the range 8.6−195 mg/m^2. For the 155 mg/m^2 cohort, day 1 PK (n = 4) are $t_{1/2}$ = 5.5 hr, C_{max} = 4800 ng/mL (CV 36%), and AUC = 27,600 ng hr/mL (CV 32%). No significant difference in PK parameters between cycle 1, D1 and D8. The mean AUC for 155 mg/m^2 cohort exceeds exposures efficacious in R xenograft studies. *Activity*: Tumor regression in non-target lesions has been noted in 1 pt with metastatic squamous ca skin (155 mg/m^2 cohort).
Conclusions: Treatment with R is tolerable as a dose of 155 mg/m^2 on D1, D8 (21 day cycle). Treatment-related toxicities of hypotension, nausea, emesis, and headache are clinically manageable with supportive measures. PK data for R suggest linearity over the dose range 8.6−155 mg/m^2. Exposures predictive of preclinical efficacy have been achieved in the clinic. Antitumor activity has been observed in a patient with heavily pretreated squamous ca skin. Accrual continues with additional enrollment on a 2nd schedule (3 hr infusion).

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5-Methyl-indirubin: a CDK-inhibitor with activity in human tumor models in vivo

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Background: The bisindole indirubin was described more than 30 years ago as being clinically active in the treatment of human chronic myelocytic leukemia. Previously we have shown that indirubin and analogues are potent and selective inhibitors of cyclin-dependent kinases (CDKs). In systematic structure activity studies, 5-methyl-indirubin (5MI) was found to be the most potent and selective compound among approximately 20 indirubin derivatives investigated in 16 human tumor xenografts in a clonogenic assay in vitro. Here, the in vivo anti-tumor efficacy of 5MI was investigated in patient-derived tumor xenografts passaged subcutaneously in nude mice.
Materials and Methods: Tumor-bearing mice received 5MI once daily po. Treatment started when tumors had grown to diameters of 6–8 mm. Anti-tumor activity was assessed as tumor volume inhibition relative to a vehicle control group. Tolerability was analysed as mortality and body weight loss.
Results: 5MI administered daily po was highly active in the human large cell lung model LXFL 529 with a tumor inhibition to 9% of the control in the absence of mortalities and body weight loss. Dose response studies showed similar activity of 5MI in the dose range from 150−190 mg/kg/day indicating that resorption of 5-MI was probably limiting for efficacy. This was confirmed by pharmacokinetic studies since similar plasma levels were found for a range of tested dose levels. Interestingly, 5MI levels were higher in the tumor tissue compared to plasma. In subsequent experiments, a total of 28 different human tumor xenografts were tested, and anti-tumor activity (T/C<50%) was observed in 3/9 NSCLCs, 1/2 renal cancers, 2/6 mammary cancers and 1/2 prostate cancers. No activity was recorded in all 5 colon carcinomas and all 3 pancreas cancers tested as well as in 1 melanoma. The combination of 5MI with Taxol exhibited a strong synergism in the mammary model MAXF 1384. A gene signature predicting tumor sensitivity to 5MI was developed.
Conclusions: 5MI is a very promising anti-cancer agent characterized by a novel mechanism of action, good oral bioavailability, promising antitumor activity in-vivo and excellent tolerability in nude mice. An oral formulation was developed, and clinical trials are planned.

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Apoptosis and cell cycle regulating proteins in gastroenteropancreatic neuroendocrine tumors: role of p27

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Background: Gastroenteropancreatic neuroendocrine tumors represent a heterogeneous tumor entity. The growth pattern ranges from very slowly to fast growing, aggressive types of tumors. In previous analysis, we established the prognostic relevance of alterations in apoptosis- and cell cycle regulation in various malignancies. Little is known about the role of apoptosis- and cell cycle regulating proteins in this tumor entity.
Aim: Regulators of apoptosis (p53/Bax) and the G1-restriction point (p16, p21, Cyclin E, p27) were evaluated.
Patients and Methods: Tumor specimens from 89 patients with a complete 5-year follow-up were studied immunohistochemically for BAX, p16, p21, p27 and cyclin E expression and for p53 mutations by SSCP-PCR. 29 patients with localized, well-differentiated gastro-enteropancreatic neuroendocrine tumors (WDET, WHO class 1) had been curatively treated by surgical or endoscopic tumor resection. 50 patients had well-differentiated endocrine carcinomas (WDEC, WHO class 2), 10 patients were diagnosed with poorly differentiated neuroendocrine carcinomas (PDEC, WHO class 3). The functional relevance of p27 was evaluated in the human neuroendocrine cell line BON by the use of siRNA.
Results: 26/29 WDETs showed a high expression of p27, whereas all 10 PDECs displayed a low expression of p27. In the 50 patients with metastatic WDECs, 20/50 (40%) tumors had a low p27 expression. Those