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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

20 patients had a statistically significant worse prognosis (median survival of 68 versus 113 months, p = 0.037). All other investigated proteins were not of prognostic significance in this subgroup of patients. Downregulation of the p27 protein in the human neuroendocrine cell line BON resulted in an increased phosphorylation of the RB protein as well as an increase of cells in the S-Phase and G2/M Phase of the cell cycle.
Discussion: The loss of p27 seems to play a critical role in the progression of gastro-enteropancreatic neuroendocrine tumors. The analysis of p27 expression identifies subgroups in metastatic disease with less favorable prognosis (p27 low expression). The underlying mechanism may be due to increased cell cycle progression in those tumors. We propose that the determination of p27 expression could be used to individualize therapeutic strategies in this tumor entity in the future.

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Lenalidomide and CC-4047 inhibit the proliferation of Namalwa cancer cells while expanding CD34+ progenitor cells. New insights on the combination therapy with HDAC inhibitors for hematological cancers

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Clinical studies involving patients with Myelodysplastic Syndrome and Multiple Myeloma have demonstrated the efficacy of lenalidomide (CC-5013) by reducing and often eliminating malignant cells while restoring bone marrow function. To better understand these clinical observations, we investigated and compared the effects of lenalidomide and its analog CC-4047, on the proliferation of two different hematopoietic cell models: the Namalwa cancer cell line and CD34+ progenitor cells. We found that both compounds have anti-proliferative effect on Namalwa cells and pro-proliferative effect on CD34+ cells, while p21WAF-1 expression was upregulated in both cell models. In Namalwa cells, we determined that the upregulation of p21WAF-1 correlates well with the inhibition of CDK2, CDK4 and CDK6 activity leading to pRb hypophosphorylation and cell cycle arrest. In contrast, in normal CD34+ progenitor cells, despite upregulated p21WAF-1 expression, we observed an increase of the cell division rate, leading to the enhancement of CD34+ expansion. Finally, we found that CC-4047 and lenalidomide have synergistic effects with two different HDAC inhibitors (Valproic acid and Trichostatin A) in both increasing the apoptosis of Namalwa cells and enhancing CD34+ cell expansion. Taken together, our results indicate that lenalidomide and CC-4047 have opposite effects in tumor cells versus normal progenitor cells and could explain, at least in part, the reduction of malignant cells and the restoration of the bone marrow observed in patients undergoing lenalidomide treatment. Moreover, this study provides new insights on the cellular pathways affected by lenalidomide and CC-4047, and proposes new potential clinical uses such as bone marrow regeneration. Finally, our in vitro experiments showing the efficacy of the combination of CC-4047 and lenalidomide with Valproic acid and Trichostatin A suggest that HDAC inhibitors might be ideal candidates for combination therapy by elevating the therapeutic index to treat hematological malignancies.

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AT7519, a selective small molecule inhibitor of cyclin dependent kinases: pharmacodynamic biomarker activity in a Phase I study

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A series of Cyclin Dependent Kinase (CDK) inhibitors was developed using Astex's fragment based medicinal chemistry approach, linked to high throughput X-ray Crystallography. A compound from this series, designated AT7519, is currently in early phase clinical development. The use of pharmacodynamic biomarkers of compound activity has become increasingly important with the advent of novel, molecularly targeted therapies, to aid determination of the minimum biologically effective dose. To this end a series of pre-clinical studies was performed to validate the biomarker assays for application in the clinical development of AT7519. We describe here the biomarker studies that are being utilised as exploratory end points in a Phase I solid tumour trial with AT7519. Pre- and post-dose skin punch biopsies were taken and the activity of the compound monitored by assessing inhibition of the proliferation markers Ki67 and Proliferating Cell Nuclear Antigen (PCNA) and the CDK substrates phospho-nucleophosmin (pNPM) and phospho-retinoblastoma (pRb). In addition the induction of tumour apoptosis was monitored in patient serum samples using a cytokeratin cleavage ELISA.
Data generated from the early cohorts on study are presented here, demonstrating that the assays developed are applicable to the clinical setting.

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Human papilloma virus integration begins in the tonsillar crypt and leads to the alteration of p16, EGFR, and c-myc during tumor formation

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Purpose: The prevalence of human papillomavirus (HPV) infection is high in the oropharyngeal mucosal regions, of which the tonsil is the most commonly affected. There may be a link between HPV and the pathogenesis of TC, because of common anatomical characteristics between cervical and tonsillar cancer (TC).
Experimental Design: We aimed to clarify whether HPV directly affects the oncogenesis and biologic behavior of TC by making a comparison between infection prevalence, physical status and viral loading numbers, and clinicopathologic prognostic factors. To compare HPV-related molecules between TC and tonsillitis (CFT), p16, survivin, HIF-1 overexpression (p = 0.022).
Conclusions: HPV-16 integration could be directly related to tonsillar carcinogenesis initially in tonsillar crypts followed by cell cycle aberration, such as p16 overexpression related to the G1-S phase and amplification of c-myc oncogene.

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Characterization of alvocidib (flavopiridol)-mediated inhibition of CDK enzyme activity and the down-regulation of gene transcription

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Drugs directed against cyclin-dependent kinases (CDKs) have been proposed as anti-cancer agents. The sanofi-aventis compound alvocidib (flavopiridol) was the first CDK inhibitor administered in man and has shown promise in Phase I against fludarabine-refractory chronic lymphocytic leukemia (CLL) [1]. The leading hypothesis for the molecular mechanism of alvocidib in CLL is that alvocidib inhibits CDK9-mediated transcription of pro-survival factors such as *MCL1* [2], resulting in apoptosis of the target B–cells. Here, we present a detailed *in vitro* characterization of alvocidib-mediated inhibition of CDK enzymatic activity and the down-regulation of gene transcription.
Alvocidib potency against several CDKs was evaluated using an enzymatic end-point assay based on ^{33}P incorporation. We show that alvocidib is a pan-CDK inhibitor with nM activity on all CDKs tested (Table 1). By far the strongest effect was observed on CDK9/T1 (IC50=2 nM), which promotes transcript elongation by phosphorylating RNA polymerase II. To further characterize CDK9/T1 inhibition by alvocidib, we used a continuous *in vitro* kinase assay, which allows the measurement of initial reaction velocities. We found that CDK9/T1 catalysis proceeds by a sequential random mechanism. The Km and Kd values for substrate and ATP will be reported and contrasted with published values for other CDK/cyclin complexes. Alvocidib inhibits CDK9/T1 in an ATP competitive manner and acts as a tight binding inhibitor (Ki=1.3 nM).

Table 1:

	IC50, nM	Ki, nM
CDK1/B1	10	
CDK2/A2	20	
CDK2/E	220	100
CDK4/D1	35	
CDK5/p25	430	
CDK7/H	150	
CDK9/T1	2	1.3

In order to investigate the consequences of alvocidib-mediated CDK9 inhibition on transcription, we monitored *de novo* mRNA synthesis in HCT116 cells by ^{3}H-uridine incorporation. We found that alvocidib abolished *de novo* mRNA synthesis within 3 hrs with an IC50 of 69 nM. These results were further supported by microarray and RT-PCR expression analysis. Six hours of 190 nM alvocidib treatment of HCT116 cells down-regulated 3,275 genes (98.7% of all drug responsive transcripts, including *MCL1*), and up-regulated only 44 genes. RT-PCR of *MCL1* mRNA levels 6 hours after drug exposure demonstrated an IC50 for