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