with interstitial cystitis (IC), a disease characterized by thinning or ulceration of the bladder epithelium. The peptide chain of APF has 100% homology to the 6th transmembrane domain of frizzled 8, a Wnt ligand receptor. Because increased Wnt expression and TCF-dependent promoter activation by β-catenin are associated with human urinary bladder carcinoma, and decreased expression of E-cadherin (a negative regulator of β-catenin) is associated with invasiveness of bladder carcinoma cells, Wnt signaling may play an important role in bladder cancer. Early studies indicated that purified native APF increased E-cadherin expression and decreased proliferation of bladder epithelial cells *in vitro* at high picomolar concentrations, and both native and synthetic APF were shown to inhibit normal bladder epithelial as well as bladder cancer (T24) cell proliferation *in vitro* at high picomolar concentrations. We therefore determined whether APF is also active against cell lines derived from other urologic carcinomas.
Materials and Methods: Two bladder cancer lines (TCC-SUP and SCaBER) and two kidney cancer lines (A498 and ACHN) were incubated with varying concentrations of synthetic APF or the inactive unglycosylated peptide for 48 hrs prior to determination of cell growth by live cell count and/or ^{3}H-thymidine incorporation.
Results: All four cell lines derived from bladder or kidney malignancies (TCC-SUP, SCaBER, A498 and ACHN) proved to be as sensitive to APF as normal bladder epithelial and T24 cells, having an IC_{50} in the low (0.25−2.5) nanomolar range. In comparison, none of these cell lines was inhibited by the inactive unglycosylated backbone peptide.
Conclusions: APF appears to be a potent inhibitor of both normal bladder epithelial cell and urologic cancer cell proliferation, and its ability to stimulate E-cadherin expression may also be useful for inhibiting bladder cancer invasion. Studies in progress will determine whether carcinomas from tissues other than the urinary tract are also sensitive to APF, as well as correlate its effects on cell proliferation with cellular production and localization of E-cadherin and β-catenin.

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An interleukin-6 antagonist modified for bone targeting preserves anti-myeloma biological activity

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Multiple Myeloma (MM) is a malignancy affecting terminally differentiated B-cells. In MM, malignant plasma cell clones are predominantly localized in the bone marrow microenvironment, due to adherence both to extra cellular matrix proteins and to bone marrow stroma cells (BMSCs). This interaction, between tumor cells and BMSC, triggers production of cytokines mediating growth and survival of MM cells. Interleukin-6 (IL-6) has been demonstrated to be a major growth factor in MM, as it is responsible for both autocrine and paracrine growth induction and for preventing apoptosis of human myeloma cells. We generated an IL-6 receptor antagonist, Sant7, which acts as growth inhibitor and enhances apoptosis on human MM by sequestering the IL-6 receptor alpha and preventing wild type IL-6 signalling. However, when injected in experimental animals, Sant7 has a very fast pharmacokinetic, reaching effective concentrations in the BM microenvironment only for limited periods. Recent reports demonstrated that conjugation with amino-bisphosphonate (ABP) targets systemically administered proteins to the bone of experimental animals. To target the IL-6 antagonist to BM microenvironment we tested the conjugation with ABP. Sant7-ABP conjugation required first the conversion of the amino group of ABP to thiol group using Traut's reagent. The thiolated ABP was then treated with the hetero-bifunctional linker SMCC (Sulfosuccinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate) and the resulting reaction mixture was added to the protein solution. The conjugated product was analysed by MALDI-TOF mass spectrometry (Matrix Assisted Laser Desorption Ionization-Time of Flight). Mass shift of conjugation product respect to unconjugated protein suggested 1 to 6 molecules of ABP were conjugated to Sant7, depending on the reaction conditions. The biological activity of the Sant7 preparation was tested performing growth inhibition assay on INA-6 IL-6 dependent human MM cell lines. The growth inhibition assay on INA-6 IL-6 dependent human MM cell line demonstrated that the Sant7-ABP product is biologically active, able to inhibit the activity of IL-6 induced INA-6 proliferation. Furthermore we demonstrated that the inhibition is not due to the ABP moiety. Our results suggest that the Sant7-APB conjugate can be used as effective therapeutic agent against the MM due to potential specific bone marrow targeting.

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Selective Aurora A inhibitors – in vitro potency, specificity and cellular mode of action

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The mitotic kinase Aurora A plays critical roles in centrosome function, bipolar spindle formation and chromosome segregation. Aberrant expression or activity of this serine/threonine protein kinase has been implicated in tumorigenesis and resistance to Taxol, making it an attractive anticancer target. To better define the inhibitory properties of our candidate aurora inhibitors, we have carried out a detailed comparison of the effects of inhibiting or knocking down Aurora A and/or Aurora B using small molecules or siRNA treatment respectively. We have identified a proprietary series of potent Aurora A inhibitors. These small molecules possess various degrees of selectivity for Aurora A versus Aurora B and are highly selective for the Aurora kinase family over a panel of more than 50 kinases. The observed cellular mode of action is consistent with selective Aurora A inhibition – monopolar spindle formation, delayed progression through mitosis, perturbation of Aurora A specific markers and apoptosis induction. Induction of p53 transcriptional activity and caspase activity is also observed. The antiproliferative activity of Aurora A selective inhibitors has been evaluated in a panel of tumour cell lines using a standard MTT cytotoxicity assay and a colony formation assay. The cellular mode of action and the PK properties of several members of the class studied in rodents (high oral bioavailability and half life) suggest the suitability of this series for further development. The rationale for the development of Aurora A specific inhibitors will also be discussed.

Cyclins and CDKs

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Pharmacodynamic effects of seliciclib (r-roscovitine, cyc202) in patients with undifferentiated nasopharyngeal cancer (NPC) using a window trial design

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Cell cycle dysregulation, characterized by inactivation of cyclin dependent kinase (CDK) inhibitors, e.g., p16 and p27, and over-expression of cyclin D1 is frequently found in undifferentiated NPC. Loss of either p16 or p27 or over-expression of cyclin D1 leads to increased Rb phosphorylation, resulting in uncontrolled cellular proliferation. Seliciclib, an orally administered inhibitor of CDKs (including 2, 7 and 9) inhibits phosphorylation of both Rb and RNA polymerase II and induces apoptosis in several human tumor cell lines including NPC cells lines through the inhibition of transcription leading to a reduction in the levels of key anti-apoptotic proteins like Mcl-1, survivin and XIAP. We evaluated the clinical, cellular and molecular effects of seliciclib in 16 patients with chemonaive NPC. Tumor biopsies and plasma were taken pre- and post-treatment (day 12) with suitable paired samples being obtained in 14 of the 16 treated patients. Of 14 evaluable patients, 7 had >25% reduction in clinically measurable cervical lymph nodes. Histologic examination of post-treatment biopsy samples showed increased tumor necrosis and immunohistochemical staining showed increased tumor cell apoptosis and decrease in Mcl-1 and cyclin D1. Tumor RNA was subjected to a low density real time PCR array consisting of a set of 384 genes related to cell cycle, apoptosis, and cell signaling. The results confirmed known biological effect of seliciclib in terms of transcriptional inhibition. Seven of 14 tumor biopsy pairs showed greater than 25% decrease in RNA levels of at least 25% of genes studied while 5 of these 7 pairs showed greater than 50% decrease in RNA levels of at least 25% of the genes examined. Biological effects attributable to seliciclib were also seen in plasma. An increase in cell death markers circulating in plasma was seen in 4/14 patients. Serum EBV copy number, a marker of disease burden in NPC, was reduced by at least 50% in 4/13 patients. In summary, seliciclib has clinical antitumor activity against NPC at a dose that is well tolerated. The clinical effect is accompanied by biological effects in tumor tissue consistent with the known mechanism of the agent. Effects observed included decreases in transcription and decreases in some cell cycle related proteins and anti-apoptotic proteins. These effects were correlated with a reduction in serum EBV copy number and an increase in plasma biomarker consistent with tumor cell death.