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Selective PIN1 PPIase inhibitor, AG122005, causes a p53 independent p21/waf1/cip1 induction

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PIN1, a resident nuclear protein, has a role in the regulation of the cell cycle and may have oncogenic potential being over expressed in a wide variety of transformed cell types. PIN1 has recently been identified as a critical regulator of the tumor suppressor p53 during DNA damage response. Following its stress-induced phosphorylation by p38 MAPK, p53 forms a complex with PIN1, undergoing conformational changes and enhancing its transactivation activity towards the cyclin dependent kinase inhibitor, $p21^{waf1/cip1}$, potentially causing a transient G1 growth arrest. In order to investigate whether induction of $p21^{waf1/cip1}$ is strictly dependent on PIN1 interactions with p53, both p53 wildtype and null cell lines were tested for $p21^{waf1/cip1}$ induction using either specific PIN1 antisense oligonucleotides or exposure to AG122005, a small molecule PIN1 inhibitor (PPIase; Ki = 1.36 μM). The PIN1 antisense treatment resulted in 8 and 9-fold increases in $p21^{waf1/cip1}$ message and protein levels, respectively, in A549 cells. After 48 hours of AG122005 exposure, $p21^{waf1/cip1}$ protein levels were 4 and 5-fold induced in the p53 null cell lines, HT29 and CA46, respectively. When tested in p53 wildtype backgrounds, A549 and MCF-7 cells, $p21^{waf1/cip1}$ levels were induced to 2 and 6-fold of respective controls after exposure to AG122005. The same experiment was performed in an isogenic pair of HCT-116 cells transfected with the viral oncogene E6 which targets p53 for ubiquitinated mediated proteolysis. After 48 hours of exposure to AG122005, $p21^{waf1/cip1}$ was induced in both the p53 null-E6 background and the p53 wildtype-CMV background to 3 and 2-fold basal level, respectively. These results demonstrate that the PIN1 PPIase inhibitor, AG122005, is able to induce $p21^{waf1/cip1}$ independently of p53 status of the tumor cell. The lack of dependence on p53 for a known molecular mechanism of G1 arrest along with other demonstrated roles in cell cycle regulation and oncogenesis suggest the utility of PIN1 as a potential new drug target for treatment of human cancers.

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AICAR: a rational identified small molecule targeting Hsp90 chaperone function in cancer cells

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Background: The molecular chaperone heat shock protein 90 (Hsp90) is viewed as a "druggable" target for rational cancer therapy, due to its role at the crossroads of multiple signalling pathways associated with cell proliferation and cell viability. One Hsp90 client protein is survivin, an inhibitor of apoptosis protein (IAP) selectively overexpressed in most human tumors and involved in control of cell division and inhibition of apoptosis. In the present study, we used a structure- and dynamics-based computational design strategy to identify the non-peptidic small molecule 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR) as a structurally novel inhibitor of Hsp90.
Material and Methods: The recently described peptidic antagonist of the survivin/Hsp90 complex, shepherdin [Plescia et al., Cancer Cell, 2005 (7)], was used as a scaffold to rationally identify low molecular weight compounds that may act as structurally novel Hsp90 antagonists, and a three-dimensional pharmacophore was built to screen a database of non-peptidic structures. ELISA was used to verify the specific binding of AICAR to Hsp90. To define the cellular effects of the drug, after a 72-h exposure to AICAR (31.25–250 μM), cells of different tumor cell types were assessed for growth potential, ability to undergo apoptosis and expression/activity of several Hsp90 client proteins.
Results: Experimental tests showed that AICAR binds the Hsp90 N-domain, destabilizes multiple Hsp90 client proteins *in vivo*, including survivin, AKT, CDK6 and telomerase, and exhibits dose-dependent antiproliferative activity in multiple tumor cell lines, while not affecting proliferation of normal human fibroblasts. Moreover, AICAR induced an apoptotic response in all tumor cell lines, as a consequence of the proteolytic activation of capase-9 and capase-3.
Conclusions: Based on these results, we propose that AICAR represents a viable lead for further development of novel Hsp90 antagonists structurally different from geldanamycins.

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Heat shock protein 27 down-regulation inhibits tumor progression and enhances gemzar chemotherapy in pancreatic cancer through activation of stat-3 signaling pathway

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Background: Despites the many advances in oncology over the last few decades, almost all patients with pancreatic cancer die of the disease. Significant progress in the understanding of important molecular processes associated with the development of the progression of the disease is helping tailor more effective treatment strategies. Molecularly targeted agents are offering hope for their potential role in helping translate the improved activity of combination chemotherapy into improved survival. Heat Shock protein 27 (Hsp27) is a chaperone implicated in several physiological tumor processes. Recently, a 2'-methoxyethyl modified phosphorothioate antisense oligonucleotide (OGX-427) that is complementary to Hsp27, inhibit Hsp27 expression and enhance drug efficacy in cancer xenograft model, has been developed. Phase I clinical trial using OGX-427 in patient with localized prostate cancer and high-risk features is starting in 2006 at the Prostate Centre (Vancouver). Our aim was to characterize changes in Hsp27 during pancreatic cancer outcome and assess the effects of OGX-427 on rates of pancreatic cancer apoptosis and tumour progression.
Materials and Methods: A tissue microarray was used to measure changes in Hsp27 protein expression in 150 specimen. Effects of OGX-427 on human pancreatic cancer MiaPaCa cell proliferation and apoptosis was assessed using the MTT assay and flow cytometer.
Results: Hsp27 expression was low or absent in differentiated tumors, but increased beginning in moderately differentiated tumors to become uniformly highly expressed in metastatic samples (>90%, $p < 0.01$). OGX-427 potently inhibited Hsp27 mRNA and protein expression (>70%) in MiaPaCa cells and resulted in >2 fold increases in the apoptotic subG0-G1 fraction and 75% of proliferation inhibition. Another mechanism mediating cytoprotection in pancreatic cancer involves stabilization with increased levels of stat-3, and we demonstrate down-regulation of total stat-3 protein levels and its activated genes after treatment with OGX-427. In vitro, Hsp27 down-regulation by OGX-427 increases Gemzar chemotherapy by 30% ($p < 0.01$). In vivo testing is in progress.
Conclusion: Increases in Hsp27 chaperone expression may serve a cytoprotective role in pancreatic cancer cells through mechanisms involving stat-3 signaling pathway activation. OGX-427 seems to be very potent to inhibit pancreatic cancer in vitro and deserves further study as a potential therapeutic.

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In vivo efficacy of BI 2536, a potent and selective inhibitor of the mitotic kinase Plk1, in combination with various cytotoxic agents

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Background: Polo-like kinase 1 (Plk1), a key regulator of cell cycle progression, represents an attractive target for cancer drug development as it is highly expressed in malignant cells and serves as a prognostic marker in certain human cancer types. We have previously shown that BI 2536, a potent and selective small-molecule inhibitor of Plk1, induces mitotic arrest and apoptosis in various human cancer models *in vitro* and *in vivo*. BI 2536, the first specific Plk1 inhibitor in clinical development, has demonstrated encouraging results in phase I trials. This study was designed to examine the efficacy of BI 2536 in combination with various established chemotherapeutic agents.
Methods: The human NSCLC model NCI-H460 was used to evaluate antitumour effects of BI 2536 in combination with docetaxel and cisplatin. For combination experiments with pemetrexed the human NSCLC model Calu-6 was utilized. The human colon carcinoma model HCT 116 was chosen to assess the antitumour effects of combinations of BI 2536 with irinotecan. Nude mice bearing subcutaneously human tumor xenografts were treated intravenously with suboptimal doses of BI 2536 or the above mentioned agents alone or in combination.
Results: In the NCI-H460 model the combination of BI 2536 (50 mg/kg i.v., once weekly) and docetaxel (15 mg/kg i.v., once weekly) showed clear antitumour efficacy with a T/C value of 26% whereas the single-agent treatments were less effective (T/C values of 65% and 42%, respectively). In the same model, a combination of BI 2536 (50 mg/kg i.v., twice weekly)

and cisplatin (5 mg/kg i.p., once weekly) was more effective than either agent alone (T/C values of 10% versus 37% and 24%, respectively). Enhanced *in vivo* efficacy was also observed when combinations of BI 2536 (30 mg/kg i.v., once weekly) and irinotecan (12.5 mg/kg i.p., once weekly) were tested (T/C values of 8% for the combination versus 20% and 25% for BI 2536 and irinotecan, respectively). The effect of scheduling of combination regimens of BI 2536 and pemetrexed on *in vivo* activity was addressed in the Calu-6 NSCLC model where pemetrexed treatment (administered from d1 to d5 of each cycle at 150 mg/kg, i.p.) was combined with once weekly BI 2536 treatment (40 mg/kg, iv.) on d1 or d5 of each weekly cycle. A simultaneous start of the combination resulted in a T/C value of 26%. Administering BI 2536 at the end of each pemetrexed cycle resulted in similar antitumour activity (T/C value 19%). Single agents were significantly less active (T/C values of 66% and 39% for BI 2636 or pemetrexed, respectively).
Conclusion: Combining the targeted cell cycle inhibitor BI 2536 with various cytotoxic agents improved antitumour activity *in vivo* compared to single-agent treatments. These results lend support to further clinical studies of BI 2536 in combination with established chemotherapeutic drugs.

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A phase I safety, pharmacokinetic and pharmacodynamic study of intravenously administered PXD101 plus carboplatin or paclitaxel or both in patients with advanced solid tumors

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Background: PXD101 is a low molecular weight HDAC inhibitor of the hydroxymate class. Anti-tumour activity alone or in combination with standard chemotherapeutic agents has been demonstrated in pre-clinical models. PXD101 has been well tolerated by patients with solid and haematological malignancies in doses up to 1000 mg/m^2/d in phase I and II clinical trials. This is a Phase I study to determine the maximum tolerated dose (MTD), dose limiting toxicity (DLT), pharmacokinetics (PK) and pharmacodynamics (PD) of PXD101 administered in combination with carboplatin (C) or paclitaxel (P) or both in order to define a safe dose of the combination for a Phase II study in ovarian cancer.
Methods: Patients with histologically confirmed solid tumours, ECOG PS 0–2, $\geqslant$18 years, <3 prior chemotherapy regimens were eligible. Escalating doses of PXD101 were administered as a 30-minute IV infusion every 24 hours ($\pm$ 2 hours) for 5 days q21. C (AUC5) or P (175 mg/m^2) or both were administered 2–3 hours following PXD101 on day 3 of each cycle. Standard PK parameters were assessed for PXD101 alone, in combination with C, P or both and for C and P when administered after PXD101. Acetylation of histones H3 and H4 was performed by Western blotting of extracted histones from peripheral blood mononuclear cells (PBMC).
Results: 15 pts (median age 53 years, [range 43−66)]; 10M/5F; all ECOG PS $\leqslant$ 2) have been treated with a total of 54 cycles of PXD101 (median 2; range 1 to 6) at 4 dose levels: 1A: C and PXD101 600 mg/m^2 (5pts); 1B: P and PXD101 600 mg/m^2 (4pts); 2: C and P and PXD101 600 mg/m^2 (3pts); 3: C and P and PXD101 800 mg/m^2 (3pts). No DLT have been observed and the final dose level 4: C and P and PXD101 1000 mg/m^2 opened for inclusion in May 2006. To date, one confirmed PR in a patient with pancreatic cancer after 6 cycles and SD in 7 patients (bladder cancer 6m+, ovarian cancer 6m+, Ewing sarcoma 6m+, melanoma 5m+, cholangiocarcinoma 5m+, mesothelioma 4m+, unknown primary 2m+).
Conclusions: The novel HDAC inhibitor PXD101 is well tolerated when combined with standard dose C and P and shows activity in heavily pre-treated patients. Recruitment to a combination of C and P and full dose PXD101 at 1000 mg/m^2 continues. PK/PD and toxicity data will be presented.

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A phase I and pharmacokinetic (PK) study of CX-3543, a protein-rDNA quadruplex inhibitor, in patients (pts) with advanced solid tumors

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Background: The rate of ribosomal RNA (rRNA) biosynthesis and resultant ribosome assembly determine the proliferative state of cells, and this process is highly increased in cancer cells due to genetic alterations that deregulate the signaling pathways that control rRNA biogenesis. CX-3543 directly inhibits aberrant rRNA biogenesis in cancer cells by disrupting an essential protein-rDNA quadruplex complex that is over-expressed in cancer cells, thereby selectively triggering rapid and massive apoptotic cell death in tumor cells but not normal cells. Preclinical studies with CX-3543 demonstrated potency in suppressing xenograft tumor growth with a broad therapeutic window, and no drug resistance has been observed *in vitro* to date.
Material and Methods: CX-3543 is administered by an IV infusion each day for 5 consecutive days, repeated on a 3-week cycle, to pts with advanced cancer. This evaluation was designed to determine the maximum tolerated dose (MTD), dose limiting toxicities (DLT) and PK profile of this schedule.
Results: 21 pts were enrolled (13M/8F), median age 68 (range 44−84) and tumor types: colorectal (5), prostate (4), neuroendocrine (2), lung (2), head & neck (2), and others (6). All pts had received prior systemic therapy, with a median of 4 (range 1−7) previous regimens. CX-3543 doses in mg/m^2 (no. pts/cohort) evaluated were: 10(3), 20(4), 40(3), 80(3), and 160(8). Although nine grade 3 adverse events have been reported, none are deemed related to CX-3543. Common mild to moderate toxicities included fatigue, anorexia, nausea, and stomatitis, but there is no evidence they are related to the presence or dose level of CX-3543. There has been no significant myelotoxicity or alopecia. Two pts experienced transient mild cough and chest tightness at 160 mg/m^2 that resolved spontaneously upon completion of the infusion, and no EKG or oximetry changes occurred. The protocol was amended to extend the infusion from 1h to 2 h, which has been very well tolerated. Three pts have had stable disease $\geqslant$ 4 months (neuroendocrine, colorectal and prostate). PK parameters demonstrate linearity between dose cohorts, with a $t_{1/2}$ of approximately 10 h following the first dose. Extending the infusion to 2 h at the 160 mg/m^2 dose level decreased the C_{max} as expected, but AUC remained linear.
Conclusions: To date, CX-3543 has been well tolerated and has predictable PKs. The MTD remains to be defined and further patient enrollment is ongoing.

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Pharmacokinetic and pharmacodynamic effects of MN-029, a novel vascular disrupting agent (VDA), in patients (pts) with advanced solid tumors

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Background: MN-029 (denibulin hydrochloride) is a novel VDA that binds reversibly to the colchicine-binding site on tubulin and inhibits microtubule assembly, resulting in disruption of the cytoskeleton of tumor endothelial cells (EC). Disruption of the tumor EC cytoskeleton ultimately leads to a temporary reduction in tumor blood flow. Changes in tumor blood flow can be used as a surrogate marker of biological activity in the clinic.
Material and Methods: MN-029 was administered IV as a 10−40 min infusion at 3-wk intervals in pts with advanced cancer. The study followed an accelerated titration design, with intrapatient dose escalation. Pharmacodynamic effects on tumor blood flow were evaluated using dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI).
Results: 34 pts (17M/17F) were enrolled of median age 56 (range 35−76) and the following tumor types – colorectal (7), renal (6), carcinoid (4), hepatocellular (3), ovarian (2), melanoma (2), soft tissue sarcoma (3), others (7). A total of 150 cycles of MN-029 were given, median 3/pt (range 1–26), over 10 dose levels (4, 8, 16, 24, 36, 54, 80, 120, 180 and 225 mg/m^2). Escalation proceeded until an initial dose-limiting toxicity (DLT) was observed in 1 pt in the 180 mg/m^2 cohort, consisting of a reversible episode (3 hours post-dose) of acute coronary ischemia (without sequelae and with preservation of myocardial function) probably due to coronary vasospasm. Therefore, this cohort was expanded to 6 pts, with no further DLTs observed. 2 DLTs occurred at 225 mg/m^2 (transient ischemic attack and grade 3 transaminitis), thus ending escalation. Common mild to moderate toxicities included nausea, vomiting, fatigue and diarrhea. There was no significant myelotoxicity, stomatitis or alopecia. Nine pts had stable disease after 3 cycles and five pts had prolonged ($\geqslant$ 6 months) stable disease (carcinoid [2], melanoma [2] and pancreatic [1]); the carcinoid tumor pts have had stable disease for >26 cycles and >23 cycles, respectively. Pharmacokinetic data generally indicated dose-related increases in C_{max} and AUC values, although substantial inter-patient variability was observed. Tumor blood flow reduction assessed by DCE-MRI was recorded at 120, 180 and 225 mg/m^2, but not at 80 mg/m^2.