Conclusion: Bone-targeted therapy consisting of one dose of strontium-89 plus alternating chemotherapy demonstrated promising activity in patients with AIPCa with an acceptable tolerability. This program continues to enroll patients.

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Tissue lysate arrays as a cell based assay for validation of signal transduction inhibitors

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Targeted cancer therapeutics directed towards molecular pathways that underlie the malignant phenotype offers a new attractive therapeutic strategy for patient management. The PI3K-AKT pathway regulates a wide spectrum of tumor-related biological processes. Deregulation of the PI3K-AKT pathway occurs in multiple tumor lineages, suggesting that this pathway is an attractive target for cancer therapy and thus a number of PI3K-AKT pathway inhibitors are currently in development. The use of functional assays could rapidly prioritize and validate lead compounds. We report, herein, tissue lysate arrays as a cell based assay for molecular screening of PI3K-AKT inhibitors. The assay is based on lysis of drug treated cells under stringent conditions followed by arraying on a solid matrix. The matrix can then be probed with pairs of antibodies identifying activation state and total amount of the protein. The assay can rapidly assess over 100 different attributes of functional proteomics, pathways and networks. Based on this technology, we demonstrate that two newly developed compounds KP-372−1 and KP86328 (by QLT/Kinetek Pharmaceutics Inc. Vancouver, Canada) effectively inhibit signaling through the PI3K-AKT cascade by different mechanisms. Both of the inhibitors, KP-372−1 and KP86328 decrease AKT kinase activity of purified enzyme. In intact cells, both drugs reduce the activation of AKT downstream targets including p70S6K and GSK3a/b, but do not affect ligand-induced MAPK activation, suggesting that these inhibitors selectively block PI3K-AKT pathway. However, KP372−1 blocks basal and EGF-induced phosphorylation of AKT, but does not interfere with EGF-induced EGFR signaling, suggesting that it targets the PI3K-AKT pathway at a level downstream of PTK receptors but upstream of AKT. In contrast to KP-372−1, KP86328 does not alter basal or EGF-induced AKT phosphorylation, indicating this compound targets AKT kinase activity in intact cells. Interestingly, both drugs activate JNK in sensitive cell line (MDA-MB-468, with loss of functional PTEN) but not in resistant cell line (MDA-MB-231, with functional PTEN). Both drugs reduce cell growth in sensitive cell lines resulting from apoptosis. A broad range of information obtained from tissue lysate arrays on multiple signaling pathways affected by targeted therapeutics allows the development of a "fingerprint" database leading to rapid assessment of on and off target activity and identification of pathway networks.

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Insulin-like growth factor-binding protein 3: single-agent and synergistic effects with paclitaxel in breast tumour models

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Insulin-like growth factors (IGFs) are peptides with potent mitogenic and antiapoptotic properties that have been implicated in the development of many types of human cancers, including those of the breast. IGF receptor-mediated signaling is modulated by IGF binding proteins (IGFBPs) which regulate IGF bioavailability. Of the six IGFBPs identified to date, IGFBP-3 is the major circulating and highest affinity carrier protein for IGFs. IGFBP-3 inhibits cell proliferation largely through sequestering circulating IGFs and preventing their interaction with IGF receptors. It also acts in the cellular environment as a potent antiproliferative agent by inducing cell cycle arrest and apoptosis independent of IGF binding. Previously we have shown that recombinant human IGFBP-3 (rhIGFBP-3) did not have a single-agent effect in estrogen-receptor $(ER)^+$ human breast cancer MCF7 xenografts, but significantly enhanced the tumour inhibitory effect of Paclitaxel. The present study was designed to examine the anti-tumour effect of rhIGFBP-3 in a $(ER)^-$ human breast cancer model, to compare the effects of rhIGFBP-3 on ER^+ and ER^- breast cancers and to investigate the mechanism underlying these effects, with the objective of determining the potential therapeutic utility of rhIGFBP-3 in the clinical setting.
MDA-MB-231 ER^- human breast tumour-bearing balb/c nude mice were treated with either Paclitaxel (17mg/kg once daily for days 1−5), rhIGFBP-3 (10mg/kg, b.i.d. on days 1−21), or the combination of the two agents. As a single agent, rhIGFBP-3 inhibited tumor growth up to 40% but failed to show synergy with Paclitaxel. rhIGFBP-3, thus, demonstrated differential effects in the ER^+ and ER^- breast tumor models. Western studies of the PI3-AKT and MAP kinase pathways confirmed that MDA-MB-231 and MCF7 cells have different signaling profiles and rhIGFBP-3 signals through different cellular pathways in the two cell lines. In MDA-MB-231 cells rhIGFBP-3 completely reverses IGF-I-induced activation of PI3-AKT signaling while having no effect on the constitutively activated MAP kinase pathways. In MCF7 cells, rhIGFBP-3 completely reverses the IGF-I-induced activation of the 42kd-MAP kinase and the IGF-I-induced additional activation of the partially autophosphorylated 44kd-MAP kinase and AKT. Ongoing work is directed towards correlating rhIGFBP-3 effects with signaling pathways of the tumour cells and translating the findings into optimal clinical protocols.

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Interleukin-12 inhibits AKT phosphorylation and upregulates cleavage and subcellular translocation of EGFP-bid within murine neuroblastoma tumors

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The prognosis of patients with advanced neuroblastoma remains poor overall despite existing therapeutic modalities. Further, several studies have now shown that neuroblastomas may possess specific molecular features that confer a resistance to apoptosis, and could ultimately contribute to the difficulty in treating these tumors. These include defects in proapoptotic gene expression and/or activity as well as constitutive overexpression of critical prosurvival factors. These clinicopathologic features have fueled intense effort to define critical mechanisms that regulate the death of neuroblastoma tumors, as well as the investigation of novel approaches for treatment. We show here that systemic administration of IL-12, a central immunoregulatory cytokine, mediates dramatic antitumor activity against even well-established orthotopic intraadrenal TBJ murine neuroblastoma tumors. Further, IL-12 induces ultrastructural changes consistent with tumor and endothelial cell apoptosis, and upregulates the expression of propapoptotic genes including FAS/FAS-L, TRAIL, TNF-RI and caspase-8 within the tumor microenvironment. Notably, although endothelial cells (EOMA) express FAS and are highly-sensitive to FAS-mediated killing, TBJ (as well as Neuro-2a) neuroblastoma cells are intrinsically-resistant to apoptosis mediated by FAS/FAS-L, TRAIL/TRAIL-R or IFN-gamma+TNF-alpha in vitro. Pretreatment of TBJ or Neuro-2a with cycloheximide sensitizes these cells to undergo receptor-mediated apoptosis in vitro, suggesting that they may overexpress a labile antiapoptotic protein. We subsequently found that compared to the normal murine adrenal gland, both TBJ and Neuro-2a overexpress phosphorylated AKT, a key anti-apoptotic, prosurvival molecule. Treatment with inhibitors of the PI3K (LY294002)/AKT (SH5)pathway can also sensitize these cells to undergo apoptosis in vitro, suggesting a protective role for AKT. We report here that administration of IL-12 can potently inhibit AKT phosphorylation within TBJ tumors. Further, downregulation of this important prosurvival pathway by IL-12 occurs in conjunction with activation and subcellular translocation of BID, an important proapoptotic target shown previously to be inhibited by activated AKT. These observations provide novel insight into mechanisms that may contribute to IL-12 mediated tumor regression, and suggest that IL-12 may possess unique therapeutic activity against tumors such as neuroblastoma that overexpress activated AKT.

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Phase I trial of low dose interferon-alpha (IFN), thalidomide with gemcitabine and capecitabine in patients with progressive metastatic renal cell carcinoma (RCC)

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Background: Limited options are available in metastatic RCC. Both IFN and thalidomide combination is active in RCC because of the anti-angiogenic properties of each agent at low doses. Gemcitabine/capecitabine combination demonstrated activity in metastatic RCC pts following immunotherapy failure. Enhanced activity with a combination of biology plus chemotherapy has been previously reported.
Methods: We are conducting a phase I trial to determine the maximum tolerated dose of the combination. Eligibility included confirmed RCC, all histologic sub-types are eligible, measurable disease, normal organ/marrow function, Zubrod PS $\leqslant$ 2, life expectancy $\geqslant$ 3 months, any prior chemotherapy or immunotherapy, and no active CNS disease.
One cycle was 3 weeks. 12 patients (9 males/3 females), median age 55 years (range 42−67 years), Zubrod PS 0 (N=1), 1 (N=7), 2 (N=4). 2 patients had no prior therapy, 1 patient had one prior therapy, 4 patients had two