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.

273 POSTER

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.

274 POSTER

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.

275 POSTER

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.

276 POSTER

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

prior therapies, and 5 patients had more than two prior therapies. Median number of metastatic sites were 2 (range 1–5). Six patients were entered at dose level 0 and 6 patient entered at dose level 1 (Table).
Results: 9 of 12 patients have completed 12 weeks of therapy. Two partial responses have been observed. Six patients had stable disease. One patient has had progressive disease. The remaining patients are too early for evaluation. Non-hematologic toxicity was generally well tolerated. Hematologic toxicity at dose level 0 consisted of 3 patients with Grade 3/4 neutropenia and 1 patient with Grade 3 thrombocytopenia. Hematologic toxicity at dose level 1 consisted of 2 patients with Grade 2/3 neutropenia.
Conclusion: The completion of our phase I experience will determine an MTD.

Study design:

Protocol stage	Dose level	No. of patients	Interferon dosage	Thalidomide dosage	Capecitabine dosage (days 1–14, Wks 1+2)	Gemcitabine dose levels (Day 1, Wk 1 and Day 8, Wk 2)
1	−1	3	1 mlu bid	200 to 400 mg	1000 mg/m^2	200
1	0	3	1 mlu bid	200 to 400 mg	1000 mg/m^2	400

277 POSTER

Analysis of c-kit expression in small cell lung cancer patients and its clinical implications

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Background: c-Kit is a growth factor receptor with tyrosine kinase activity that play an important role in the biology of cancer and its expression has been documented in several malignancies. The aim of this study was to determine the incidence and role of CD117 overexpression as a predictive or prognostic marker in patients with small cell lung cancer (SCLC).
Patients and Methods: We performed a retrospective study with 51 patients diagnosed of SCLC between January 2000 to July 2003. Relevant clinical patient information was obtained from extensive chart review (age at diagnosis, performance status – PS, weight loss, tumor stage and response to therapy). c-kit expression was analysed in paraffin-embedded tumor tissues immunohistochemically with commercial antibodies and we obtain successful results in 40 patients (39 males and 1 female) with a mean age of 62.4 years.
Results: Positive c-kit expression was observed in 32.5% patients. At the time of diagnosis 27 patients presented extended disease (ED) while 13 patients limited disease (LD). c-kit expression was observed in 46.2% in LD and 25.9% in EE although this difference was not significant (p=0.21). Patients with a PS between 0–2 represented 85.7% of the c-kit positive group and 90% in the c-kit negative one. All patients were formers o actual smokers. Weight loss was present in 52.3% of the patients at diagnosis.
36 patients received as a median 4 cycles of chemotherapy as first-line treatment (78.6% vs 83.3% in c-kit positive and negative group). The most used schedule was platinum-salts + etopiside and the most common second-line agent was topotecan. Radiotherapy of the primary tumor was administered in 42.9% of the patients in the c-kit positive group and in 30% in the negative one.
Comparing the c-kit positive group vs the negative one, was observed Complete Response in 30.8% vs 7.4% of the patients while Partial Response in 15.4% vs 11.1% (with 7.7% vs 11.1% of patients who achieved PR>80%). Stable Disease was observed in 15.4% vs 14.8% and Disease Progression in 30.8% vs 51.9% of the patients. In patients with c-kit expression, the median survival was 16.01 months vs 7.6 months in the c-kit-negative population (p=0.093).
Conclusions: c-Kit is expressed in one third of the patients with SCLC. Our findings do not suggest a significant association between c-kit expression and survival. However more studies are needed to define its possible prognostic value.

Monoclonal antibodies and targeted toxins/nuclides

278 POSTER

Immunoassay and mass spectrometry analysis of specific EGFr phospho-tyrosines; effects of panitumumab (ABX: EGF), a fully humanized anti-EGFr monoclonal antibody, and the kinase inhibitor AG1478

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Background: There is a clinical need to verify and stratify patient response rate with bio- and surrogate markers. Over expression of the epidermal growth factor receptor (EGFr/ErbB1/HER1) and its ligands have been correlated with aggressiveness and poor prognosis in various tumors such as colon, breast and prostate cancer and treatment with antagonists of this receptor have been beneficial to some patients. We have previously shown, using mass spectrometry, seven ligand induced EGFr phosphorylation sites (J Am Soc Mass Spectrom. **14**:1022–1031) and quantified their phosphate content upon ligand (EGF, TGFα (and inhibitor (AG1478) addition. We have extended these results to include inhibition by panitumumab, a high affinity (KD=5×10^{-11}) fully human monoclonal antibody to EGFr. In addition, we report the development of an immunoassay platform to measure specific phosphotyrosines of the EGFr.
Material and Methods: Proliferating cell cultures of A-431, SK-MES, H1299, H2126, or xenografts (A431 or SK-MES) were pretreated with inhibitors before addition of ligand (EGF or TGFα (. Thereafter cells or xenograft tissue were immunoassayed using a BioVeris (Igen) platform. For LC tandem mass spectrometry, EGFr was immunoprecipitated and isolated by SDS-PAGE before analysis.
Results: The phosphate content of eight sites (T669, Y992, Y1045, Y1068, Y1086, S1142, Y1148, and Y1173) was measured by mass spectrometry upon ligand addition. Some sites exhibit large dynamic ranges (Y1045, Y1068, Y1086, Y1173) in their phosphate content, while other sites reach a plateau (Y992, S1142, Y1148). Decreases in phosphate content were seen with the addition of the EGFr inhibitor panitumumab similar to what was reported for the kinase inhibitor AG1478. The measurement of the phosphate content at several Tyr residues was adapted to a BioVeris immunoassay platform. Using this high through-put methodology we were able to detect increases in phosphate content with ligand addition and decreases in phosphate content upon inhibitor (panitumumab, AG1478) treatment of cells and xenograft tumors. An immunoassay using only monoclonal antibodies was also developed measuring phosphate content at Y1068.
Conclusion: We have identified specific Tyr residues that may serve as potential markers of the EGFr responsiveness to panitumumab administration. Phosphorylation induced by ligands or a decrease in phosphorylation by inhibitors (panitumumab, AG1478) can be monitored by measuring phosphate content at these ligand induced sites using a high through-put immunoassay. These experiments will allow us to investigate more precisely, the mechanism of action of anti-EGFr therapy in patients.

279 POSTER

Correlation of acne rash and tumor response with cetuximab monotherapy in patients with colorectal cancer refractory to both irinotecan and oxaliplatin

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Background: Cetuximab (Erbitux™), an IgG1 monoclonal antibody, has demonstrated activity in patients with epidermal growth factor receptor (EGFR)-expressing colorectal cancer (CRC) both as a single agent and in combination with irinotecan. Cetuximab binds specifically to the EGFR preventing homo- and heterodimerization and signal transduction. This large phase II study was designed to explore the activity of cetuximab in patients with metastatic CRC, with no clear treatment alternative.
Methods: Patients with metastatic EGFR+ CRC were eligible to enroll in this study if they have failed at least two prior chemotherapy regimens, containing irinotecan, oxaliplatin and a fluoropyrimidine. Patients were to receive cetuximab monotherapy at an initial dose of 400 mg/m^2, followed