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Selective in vivo therapeutic targeting of prostate cancer with a specific double-amino-acid auxotroph of Salmonella typhimurium

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Background: As a new paradigm for bacterial therapy of cancer, we have generated a selectively tumor-targeting *Salmonella typhimurium* strain by selecting for auxotrophy for specific amino acids.
Methods: *S. typhimurium* auxotrophic strain A1 was obtained after nitrosoguanidine (NTG) mutagenesis. The interaction between the tumor cells and bacteria was visualized by dual-color fluorescence with green fluorescent protein (GFP)-expressing *S. typhimurium*-A1 growing in red fluorescent protein (RFP)-expressing PC-3 human prostate cancer cells (1).
Results: A1 invaded and induced apoptosis in PC-3 cells *in vitro*. W hen A1 was inoculated iv in PC-3 bearing nude mice, the tumor: liver bacterial ratios ranged between 500:1 to 2000:1 by day-4 after injection. The bacteria disappeared from the liver by day-10. A1 selectively grew in the PC-3 tumor and suppressed tumor growth after tail vein injection. A1 also selectively grew in the PC-3 tumor after intratumor injection. The tumor completely regressed by day-20 with no obvious adverse effect on the host. The results show that the A1 auxotroph could selectively target the PC-3 tumor *in vivo* with little or no growth in normal tissue suggesting that the A1 auxotroph selectively received nutritional supplementation in the tumor. A1 was identified as a Leu and Arg double auxotroph by analysis of growth in minimal medium supplemented with various combinations of amino acids. The results suggest that the PC-3 tumor is enriched selectively for Leu and Arg enabling the *S. typhimurium* auxotroph to selectively grow in these cells *in vitro* and *in vivo*.
Conclusions: This new "designer-bacteria" approach to tumor targeting will be extended by identifying specific auxotropic mutations for selective targeting of different tumor types. (1) Z hao, M., Yang, M., Baranov, E., Wang, X., Penman, S., Moossa, A.R., and Hoffman, R.M. Spatial-temporal imaging of bacterial infection and antibiotic response in intact animals. Proc. Natl. Acad. Sci. USA 98, 9814–9818, 2001.

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The relationship of hypoxia and erythropoetin to schwannoma protein and message expression and cellular proliferation: an opportunity for tumor cell functional and growth inhibition

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Background: Surgical and radiation based therapy of schwannomas, particularly when located intracranially or within the spine, is associated with risk that might be foregone if a medical therapy were available. Based upon an anecdotal clinical report of a vestibular schwannoma rapidly becoming symptomatic and growing after treatment with synthetic erythropoetin (EPO) a series of similar lesions were analyzed for EPO and EPO receptor (EPOR). This demonstrated positive expression by immunohistochemistry for EPO in 93% and for EPOR in 64%. Additionally, hypoxia is in important property of a variety of tumors. EPO is known to be an important hypoxia responsive element. The purpose of this work was to assess *in vitro* TM-31 human schwannoma cell's (1) EPO, EPOR and HIF1α response to hypoxia and (2) proliferation in response to EPO.
Methods: Subconfluent TM-31 cells were cultured in 1% O_2 (hypoxia) or with room air (normoxia) for 5 and 24 hours to assess EPO, EPOR, and hypoxia-inducible factor 1α (HIF1α (protein and mRNA expression. Under normoxia TM-31 cells were grown in escalating concentrations of human recombinant EPO (rhEPO) for up to 7 days.
Results: The quantity of EPO message expressed increased from 5 to 24 hours. On the other hand, a slight decrease in EPOR message was expressed over the same interval. HIF1α message in hypoxic conditions increased compared to baseline over 5 hours but was little different from baseline after 24 hours. EPOR protein demonstrated a possible increase in it's phosphorylated form expression after 24 hours of hypoxia. A marked and progressive increase in HIF1α protein expression in hypoxia from 5 to 24 hours was noted as compared to normoxia. Proliferation as assessed by an MTT assay showed 10%, 15%, and 20% increase over control circumstances with 0.5 U/ml of rhEPO after 1, 5 and 7 days respectively, all significant at $p < 0.05$. At higher concentrations (1–32 U/ml rhEPO), lesser degrees of proliferation stimulation were seen.
Conclusions: TM-31 cells accelerate poliferation in response to low doses of EPO. Expected variation in EPO/EPOR/HIF1α expression occurs with hypoxia. These *in vitro* observations suggest an opportunity to develop and assess a series of possible schwannoma therapies based on inhibition of EPO and EPOR function and alteration of responses to hypoxia.

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Phase II Study of Thalidomide, interleukin-2 (IL-2), and granulocyte macrophage-colony stimulating factor (GM-CSF) in patients with metastatic renal cell carcinoma (RCC)

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Background: Thalidomide's anti-RCC activity, the potential that its immunomodulatory and anti-angiogenic effects may augment the antitumor activity of IL-2, and the promising early efficacy and safety findings observed with the combination of low dose subcutaneous IL-2 plus thalidomide therapy in the treatment of patients with metastatic RCC (ASCO, 2003) form the foundation for the current study. GM-CSF increases the number and activity of macrophages, which may provide a means to enhance the immune system against tumor cells, thus improving the antitumor activity of thalidomide plus IL-2.
Methods: Eligibility includes histologic diagnosis of confirmed RCC excluding papillary, sarcomatoid, or collecting duct tumors, measurable disease, normal organ/marrow function, life expectancy $\geqslant$ 3 months, Zubrod performance status $\leqslant$ 2, no prior chemotherapy or immunotherapy, and no active CNS disease.
Thalidomide was started at 200 mg after 48 hours to 400 mg at week zero without an interruption. IL-2 at 7 mIu/m^2 and GM-CSF at 250 mcg/m^2 are given by subcutaneous injection, starting at week 1, days 1 through 5, weeks 1 through 4, with rest from IL-2 and GM-CSF at weeks 5 and 6. One cycle was 6 weeks. Response was assessed every 2 therapy cycles. 21 patients have been enrolled to date. Four patients are inevaluable for response secondary to CNS toxicity, non-compliance, or early removal from therapy. Patient characteristics: 16 male/5 female, aged 57–73 (median 64) years were included; 21 patients had confirmed RCC. All patients had metastatic disease. Sites included: lung (N=17), nodal (N=6), liver (N=6), bone (N=3), kidney (N=1), eye (N=1). Number of metastatic sites: 1 (N=15), 2(N=2), 3(N=5). Zubrod Performance Status: 0 (N=11) and PS 1 (N=10).
Results: There has been 3 complete responses, 2 partial responses, 1 stable, and 6 patients are too early. Toxicities were generally grade 1–2 and included: somnolence, constipation, rash, flu-like symptoms associated with IL-2, fluid retention associated with the combination, hypotension (which was managed with oral fluids), hypothyroidism, sinus bradycardia, and peripheral neuropathy.
Conclusion: Enrollment is ongoing, further data regarding response rate, time to progression, and toxicity will be presented.

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Bone-targeted therapy for androgen-independent prostate cancer (AIPCa)

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Background: Prostate carcinoma is linked to osteoblastic metastasis. We therefore investigated the value of bone-targeted therapy in selected patients with AIPCa. Eligibility included progressive AIPCa affecting bone, presence of worsening cancer-related symptoms, increasing prostate-specific antigen (PSA) values on 2 occasions at least 2 weeks apart, castrate serum testosterone concentrations of < 50 ug/L, disease progression after antiandrogen withdrawal, life expectancy >3 three months, Zubrod performance status of $\leqslant$ 2, and normal organ/marrow function.
Methods: Each patient received strontium-89 at 4 mCi on week 1, day 1. Each course of chemotherapy lasted for 8 weeks. Patients were treated in weeks 1, 3, and 5 with doxorubicin (20 mg per m^2 as a 24-hour intravenous infusion on the 1st day of every week) in combination with ketaconazole (400 mg orally 3 times a day daily for 7 days). In weeks 2, 4, and 6, treatment consisted of paclitaxel (100 mg/m^2 intravenously on the 1st day of every week) in combination with estramustine (280 mg orally 3 times a day for 7 days). After completion of 2 courses of chemotherapy, patients with stable or responding disease completed 2 further courses. Pts were then placed on maintenance ketaconazole until their disease progressed.
Results: 20 patients have enrolled. 14 patients have completed strontium-89 and 4 courses of chemotherapy. 6 pts are too early for evaluation. Based on a reduction in PSA concentrations of at least 50% from baseline maintained for at least 8 weeks, 18 patients responded to treatment. 12 patients had at least an 80% reduction in PSA concentrations. All patients with symptomatic bone pain reported that the pain improved during treatment. Toxic effects were assessed and included: 3 patients who developed deep venous thrombosis. 8 patients had grade 4 neutropenia. Severe thrombocytopenia grade $\geqslant$ 3 occurred in 6 paitent. Other common side effects were: edema, fatigue, pain flare, nausea, vomiting, raised transaminases, and skin reaction.

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