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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.