794 POSTER

Anti-proliferative activity of mycobacterium phlei dna and mycobacterium bovis strain bacillus calmette-guerin DNA towards human bladder cancer cells

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Purpose: Live bacillus Calmette-Guérin (BCG), an attenuated form of Mycobacterium bovis, and a cell wall extract from Mycobacterium phlei (M. phlei) where mycobacterial DNA is complexed to the cell wall (MCC), have been shown to possess anti-cancer activity against bladder cancer. We have shown that M. phlei DNA present in MCC inhibits the proliferation of a wide range of cancer cells. M. phlei DNA differs significantly from BCG DNA in genomic size and base composition. In this study, we have compared the anti-proliferative activity of DNA isolated from M. phlei with DNA isolated from BCG towards human bladder cancer cell lines.

Methods: We determined the anti-proliferative of M. phlei DNA and BCG DNA using a panel of human bladder cancer cell lines (HT-1376, UMUC-3, T24 and 5637) by tetrazolium reduction. Apoptosis was monitored by the release of nuclear mitotic apparatus (NuMA), the cleavage of poly(ADP) ribose polymerase and the activation of caspase pathways.

Results: M. phlei DNA inhibited the cellular division of the four human bladder cancer cell lines tested, while DNA isolated from BCG was inactive. This inhibition was independent of p53 and/or p21 mutations. Inhibition of cell division was accompanied by the release of NuMA, the cleavage of poly(ADP) ribose polymerase and the activation of caspases, characteristics of cells undergoing apoptosis. Synthetic phosphodiester oligonucleotides derived from the genome of M. phlei were found to cause a greater induction of apoptosis than purified M. phlei DNA. Only synthetic GT-rich phosphodiester oligonucleotides (11-33 base length) were found to possess pro-apoptotic activity against bladder cancer cells. AC-rich oligonucleotide showed no activity.

Conclusion: Our data show that DNA isolated from BCG does not possess any direct activity on the cellular division of bladder cancer cell lines while M. phlei DNA has intrinsic antiproliferative and apoptosis-inducing activity. This may explain why BCG has been reported to have no direct pro-apoptotic activity in vitro on bladder cancer cells or following intravesical administration. The pro-apoptotic activity of M. phlei DNA can be reproduced by using synthetic GT-rich oligonucleotides.

795 POSTER

Increased urinary cytokines and apoptotic markers following intravesical administration of a mycobacterial cell-DNA complex (MCC) in patients with carcinoma in situ of the bladder

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Purpose: A cell wall extract from Mycobacterium phlei, where mycobacterial DNA in the form of short oligonucleotides is complexed to the cell wall (MCC), has been shown to possess activity against carcinoma in situ of the bladder. We have found that MCC is able to induce in vitro the synthesis of cytokines by immune effector cells as well as inducing apoptosis in human bladder cancer cell lines. We have therefore evaluated whether MCC was able to modulate the synthesis of cytokines and to induce apoptosis following intravesical administration in patients with carcinoma in situ (CIS) of the bladder.

Methods: Sixteen patients with CIS of the bladder who failed to respond to either Mycobacterium bovis BCG treatment or chemotherapy were enrolled in 9 centers in Australia and Canada. Each patient was treated once weekly for a period of 6 weeks with 4 mg of emulsified MCC administrated intravesically. Urine samples were collected before treatment and after 3 and 6 weeks of treatment (6 to 8 and 18 to 24 h post-treatment). Urinary IL-6, IL-8, IL-12, IL-18, soluble Fas ligand (sFasL) and nuclear mitotic apparatus protein (NuMA) were evaluated using commercial ELISA kits and standardized relative to urinary creatinine levels.

Results: An elevation of 100% over baseline (pre-treatment) levels was found after intravesical administration of MCC for IL-6 (in 75% and 80% of patients at week 3 and 6, respectively), IL-8 (in 81% of patients at week 3 and 80% at week 6), IL-12 (in 44% of patients at week 3 and 73% at week 6), IL-18 (in 33% of patients at week 3 and 57% at week 6), sFasL (in 25% of patients at week 3 and 33% at week 6) and NuMA (in 40% of patients at week 3 and 47% at week 6). The results indicate that maximal induction of the cytokines IL-12 and IL-18 by MCC occurs following 6 weeks treatment. In contrast, IL-6, IL-8, sFasL and NuMA were maximally induced at 3 weeks treatment.

Conclusion: Our results indicate that MCC is able to induce the synthesis of cytokines (IL-6, IL-8, IL-12 and IL-18) and to trigger apoptosis (sFasL and NuMA) in the bladder microenvironment confirming the initial in vitro observations. The clinical significance of these increases in immunomodulatory and apoptosis markers will be evaluated at the completion of the phase II study.

Prostate cancer

796 POSTER

Confirmation of a low α/β ratio of 1.5 Gy for prostate tumours

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Purpose: In 1999 when Brenner & Hall published a low value of 1.5 Gy for prostate tumors, there was much doubt about whether this could be true, including a different result claimed for a model which included hetereogeneity of radiosensitivity and α/β values

Methods: We recently reviewed 18 published studies which reported 5 year bNED following radiation treatment only with external beam radiotherapy (EBRT) or permanent implants using I-125 or Pd-103. We concentrated on intermediate risk patients (initial PSA 10–20 ng/ml) and analysed the results using LQ modelling.

Results: A graphical method gave α/β values of 1.7 Gy, with a range overlapping 1.5 Gy. A more precise Direct Analysis (maximum likelihood) method yielded **1.49 Gy with 95% CI of 1.25–1.76 Gy.** In addition a half-time of sublethal damage repair for prostate tumors was calculated from the data, this being **1.9 hours (95% CI 1.42–2.86 h).** (Heterogeneity modelling, which threw doubt on Brenner & Hall's 1999 estimate, has problems of methodology which require further investigation (King & Fowler, letter in press.)) Preliminary clinical support for a very low α/β value comes also from Martinez et al, who give EBRT of 45 Gy plus an HDR boost of two insertions ranging from 6–10 Gy per insertion. The improvement is not consistent with large α/β.

Conclusion: There now seems no doubt that α/β is low in prostate tumours. More clinical trials employing Hypofractionated EBRT, or large-dose HDR brachytherapy, appear to be warranted.

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A Phase I/II dose finding trial of the intravenous injection of CV787, a prostate specific antigen-dependent cytolytic adenovirus in patients with advanced hormone refractory prostate cancer (HRPC)

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Calydon CV787 is a genetically engineered adenovirus with the PSA and Probasin promoter and enhancer elements inserted upstream of the viral E1B and E1A genes, respectively. Control of viral replication by these two prostate-specific regulatory elements has demonstrated high specificity and potency against prostate cancer in preclinical xenograft models. To date, 19 patients with progressive hormone refractory prostate cancer have been enrolled in this ongoing phase I/II trial. A single intravenous injection of CV787 was administered per patient as part of a cohort dose-escalation design. Cohorts of 3 patients each were treated with escalating viral doses at approximately half log intervals, beginning at a dose level of 1 x 1010 viral particles to the current dose level of 3 x 1012 particles. Transient (< 24 hrs) grade 3 fatigue occurred in a single patient treated at 3 x 1012 particles; no other grade 3 or higher toxicities have been observed. Self-limited grade 1*2 fever was seen in 4 patients, and 2 patients had transient grade 2 hypotension, all at doses > 6 x 1011. Pharmacokinetic studies indicate peak virus levels within the first hour after injection with a rapid clearance from the blood; a secondary viral peak indicative of in vivo viral replication is seen in most patients beginning about 3 days after administration. Stable PSA or small declines were observed in 8 patients; however, no declines of > 50% have been noted to date. These data indicate that systemic administration of CV787 is safe, with an acceptable toxicity profile, and that adequate levels of viremia are achieved. Whether larger declines in

PSA will be achieved at higher dose levels is not known. However, the modest effect on PSA observed to date suggests some biologic activity. Once MTD is reached, phase II trials of CV787 alone and in combination are planned.

798 POSTER

Baseline quality of life measured with the EORTC QLQ-C30 helps to select a subset of 'good prognosis' metastatic hormone refractory prostate cancer patients

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Introduction and Objectives: Patients with metastatic hormone refractory prostate cancer (HRPC) are often viewed as an homogeneous group with median overall survival lasting about one year. Few prognostic factors have been identified so far. Quality of life (QL) measurements have rarely been considered as potential prognostic factors in this disease. Using data from 494 metastatic HRPC patients randomized in three EORTC trials (30903, 30921, 30944), we attempted to form three prognostic groups using clinical and biochemical parameters together with the results from baseline QL assessment.

Material and Methods: The 15 scales from the EORTC QLQ-C 30 (version 1.0) and ten baseline clinical and biochemical variables were considered. Univariate and multivariate Cox proportional hazard models stratified for trial and treatment were used. The 0.01 statistical significance level was used.

Results: Insomnia, dyspnoea and appetite loss, age, bone scan result, performance status (WHO PS) and hemoglobin level were independent predictors of survival in the multivariate analysis. Based on these 7 factors, a prognostic index was computed: PI=0 2 (if dyspnoea 67-100) 1 (if insomnia 33) 2 (if insomnia 67-100) 1 (if appetite loss 33) 2 (if appetite loss 67-100) 1 (if age 66-75) 2 (if age >75) 1 (if 5-15 hot spots) 2 (if >15/superscan) 2 (if WHO PS >1) 2 (if Hemoglobin WHO grade >0). The patients could then be classified into three groups: good prognosis (PI 0-3: 29%) with 18.7 months median survival, intermediate prognosis (PI 4-6: 39%) with 11.9 months median survival and poor prognosis (PI >6: 32%) with a median survival of 6.0 months.

Conclusion: QL dimensions of insomnia, dyspnoea and appetite loss add independent prognostic information over clinical and biochemical factors such as age, performance status, hemoglobin and bone scan results for predicting the survival of HRPC patients. They enable the definition of three groups of HRPC patients with median duration of survival from 6.0 months (poor prognosis) to 18.7 months (good prognosis). Twenty-nine percent of the patients fall in the 'good prognosis' group.

799 POSTER

Androgen suppression of advanced prostate cancer: intermittent or continuous therapy?

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Purpose: In up to 80% of advanced prostate cancer patients, the condition is improved by endocrine androgen ablative therapy. However, over time androgen independence occurs, leading to progression of the cancer. In the last few years a new therapeutic concept of hormonal treatment has emerged based on the observation that when androgen-dependent Shionogi Carcinoma in mice are intermittently exposed to androgen withdrawal, apoptotic regression of the tumour is induced. While continuous androgen suppression (CAS) can lead to a loss of libido and sexual function, intermittent androgen suppression (IAS) may improve the quality of life of patients in the intervals between hormone treatments. Data from pilot studies suggest that the cyclical effects of such treatment modality could be monitored by measuring prostate specific antigen (PSA) concentration. To obtain more data on IAS an international, prospective, randomised clinical trial was initiated in 1998. The primary objective of this study was evaluation of time to clinical tumour progression and/or PSA escape (defined as PSA concentrations over 50 ng/ml). The major secondary objective was evaluation of patients' quality of life.

Methods: Proven advanced prostate cancer patients showing normalisation of PSA (below 4 ng/ml) after 6 months of maximal androgen blockade using the combination of buserelin depot and nilutamide, became eligible for randomisation to either IAS or CAS. In total 193 patients have been randomised, 155 classified as T2-4NxM1 and 38 classified as T2-4N1-3M0. The first patient was entered in March 1998 and the follow-up of all patients enrolled ends in August 2001.

Results: Interim analyses suggest that the patients enrolled are performing well. On average, patients randomised to intermittent therapy had to restart therapy at month 14. The safety profile evaluated to date demonstrated good tolerability. At time of analysis, a total of 66 patients were withdrawn, 25 patients in the IAS group, 41 patients in the CAS group. Reasons for withdrawal were mainly clinical progression; other reasons were patient's wish or death from prostate cancer or another cause.

Conclusion: This international, prospective, randomised trial suggests that IAS is a feasible alternative to CAS.

800 POSTER

A designer inhibitor of a novel protein-kinase causes regression of human hormone refractory prostate cancer xenografts in nude mice

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A Src-related kinase, previously considered to be restricted to hemapoietic cells, was found to be expressed in the normal prostate gland. Moreover, its level is markedly elevated in prostate cancer, as determined by immmunohistochemistry and in-situ hybridization utilizing a probe specific to that kinase. The prostate gland in knockout mice for that kinase, resembled the morphology found in castrated animals. Therefore we set out to test a designer compound, directed against this kinase, for the treatment of Hormone Refractory Prostate Cancer (HRPC).

Our comparative analysis of the kinase domain revealed a distinct structural motif allegedly involved in substrate-binding. Short peptides (7 aa) derived from this region specifically inhibit substrate-phosphorylation by that kinase and abrogate the proliferation of HRPC cells (PC3 and DU-145) in-vitro at sub-micromolar range. Following structural optimization, a lead compound, KRX-123, was formulated for i.v. injection and tested for its in-vivo efficacy against established DU-145 tumors in nude mice (around 400 sq.mm at the initiation of treatment).

Once a week injection of 2.5 to 10 mg/kg of KRX-123 caused complete inhibition of tumor growth within a month, while in the control group, the tumor doubled in size during the same period of time (n=6 in each group). In the high-dose group, approximately two-third of treated animals had non-palpable remnant, by week 12. Pre-Clinical studies in rats and dogs showed that KRX-123 is non-toxic at that concentration range.

In conclusion, a promising drug candidate has been discovered for prostate cancer. A phase I/II clinical trial, testing KRX-123 in patients suffering from HRPC, is scheduled to begin in 2001.

801 POSTER

BCL-2 antisense (G3139) plus docetaxel for treatment of progressive androgen-independent prostate cancer

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Purpose: G3139 (Genta, Inc., Berkeley Heights, NJ) is an 18 mer phosphorothioate antisense oligonucleotide that targets the mRNA of BCL2, which encodes an antiapoptotic protein that is expressed in androgen-independent prostate cancer. BCL2 has also been implicated in resistence to treatment. Preclinical data have shown that G3139 has synergistic antitumor effects when used in combination with docetaxel (Taxotere®, Aventis Pharmaceuticals, Parsippany, NJ) in xenograft models. The purpose of this trial was to define the dose of G3139 with docetaxel for the treatment of patients with progressive androgen-independent prostate cancer.

Methods: Patients (pts) were treated with escalating doses of G3139 of 5, 7, and 9 mg/kg/day in cohorts of 3-6. G3139 was delivered as a five-day continuous intravenous infusion, every 3 weeks, for a maximum of