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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.

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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