



- [Immune Recovery And Wellness – www.irw4help.com](http://www.irw4help.com)
- [More Clinical Research – www.irw4help.com/research](http://www.irw4help.com/research)

Treatment of bladder carcinomas using recombinant BCG DNA vaccines and electroporative gene immunotherapy.

Lee CF, Chang SY, Hsieh DS, Yu DS.

Graduate Institute of Life Sciences, National Defence Medical Centre, National Defence University, Taipei, Taiwan, ROC.

Intravesical immunotherapy with live *Mycobacterium bovis* bacillus Calmette-Guerin (BCG) is the treatment of choice for superficial bladder cancers. Nevertheless, a significant proportion of patients do not respond to this therapy, and adverse effects are common. Here, we report the cloning of recombinant mycobacterial DNA vaccines and demonstrate the ability of multicomponent and multisubunit DNA vaccines to enhance Th1-polarized cytokine-mediated responses as well as effector cell responses. Splenocytes from immunized groups of mice were restimulated in vitro and examined for cytotoxicity against murine bladder tumor (MBT-2) cells. We used four combined recombinant BCG DNA vaccines (poly-rBCG) for electroporative gene immunotherapy (EPGIT) in vivo, and found that tumor growth was significantly inhibited and mouse survival was prolonged. Increased immune cell infiltration and induction of apoptosis were noted after treatment with poly-rBCG alone, with the murine interleukin-12 (mIL-12) vaccine alone, and-most significantly-with the poly-rBCG+mIL-12 vaccine combination. Electroporation of poly-rBCG+mIL-12 resulted in complete tumor eradication in seven of eight mice ($P < .01$) within 28 days. Thus, EPGIT using multicomponent multisubunit BCG is highly effective in the treatment of bladder cancer. This approach presents new possibilities for the treatment of bladder cancer using recombinant BCG DNA vaccines.

Immunotherapy for bladder cancer using recombinant bacillus Calmette-Guerin DNA vaccines and interleukin-12 DNA vaccine.

Lee CF, Chang SY, Hsieh DS, Yu DS.

Department of Surgery, Tri-Service General Hospital, National Defense Medical Bureau, Taipei, Taiwan.

PURPOSE: We investigated the efficacy of recombinant bacillus Calmette-Guerin (BCG) DNA (poly-rBCG) and murine interleukin (IL)-12 (mIL-12) vaccines in inducing T helper 1 polarized cytokines and suppressing bladder tumor growth in mice. **MATERIALS AND METHODS:** Four mycobacteria candidate genes (Ag85A, Ag85B, Mpt64 and PstS3) were cloned, fused with ESAT6 and ligated into eukaryotic expression vectors. Combined poly-rBCG and mIL-12 vaccines were transferred into a murine bladder tumor model. The efficiency of gene expression was detected using Western blotting, flow cytometry and semiquantitative reverse transcriptase-polymerase chain reaction. Systemic cytokine responses, tumor growth and cumulative survival rates were monitored. **RESULTS:** Transfected bladder cancer cells showed high in vitro and in vivo expression of the recombinant subcomponents. Mice with tumors injected with poly-rBCG plus mIL-12 produced serum interferon-gamma significantly within 21 days but no significant elevations in tumor necrosis factor-alpha, IL-2, IL-4 or IL-5 were found. On day 28 after electroporation the growth of MBT-2 implants treated with poly-rBCG, mIL-12 or poly-rBCG plus mIL-12 was significantly inhibited. The cumulative survival of mice treated with poly-rBCG plus mIL-12 was significantly higher than that of the other 3 groups. **CONCLUSIONS:** Highly immunopotent recombinant vaccines of bacillus Calmette-

Guerin DNA were produced that elicited T helper 1 immune responses with a high serum interferon-gamma level, inhibited tumor growth and prolonged the survival of tumor bearing mice. Thus, electroporation immunogene therapy using poly-rBCG plus mL-12 may be an attractive regimen for the treatment of bladder cancer.

Immunotherapy of Experimental Bladder Cancer with Recombinant BCG Expressing Interferon-gamma.

Arnold J, De Boer EC, O'Donnell MA, Bohle A, Brandau S.

Research Center Borstel, Division of Immunotherapy, Borstel, Germany; dagger Academic Medical Center, University of Amsterdam, Department of Urology, Amsterdam, The Netherlands; double dagger University of Iowa, Department of Urology, Iowa City, IA, USA and section sign Helios Agnes Karll Hospital, Bad Schwartau, Germany.

SUMMARY: One of the most potent immunotherapies presently used is the application of Bacillus Calmette Guerin (BCG) to prevent recurrences of superficial bladder cancer. Despite its successful use, nonresponders and certain side effects remain a major obstacle. Therefore, current studies aim at developing recombinant BCG (rBCG) strains to further improve the effectiveness of the therapy. In BCG-treated patients a strong local induction of Th1-like cytokines was observed. For this reason rBCG-strains secreting Th1-like cytokines might be potentially useful agents to improve this type of immunotherapy. Because we previously demonstrated the essential role of IFNgamma in BCG-induced antitumor responses, in this study a rBCG strain secreting murine IFNgamma (rBCG-IFNgamma) was generated and tested for its immunostimulatory capacity in several in vitro and in vivo test systems. In vitro rBCG-IFNgamma specifically up-regulated expression of MHC class I molecules on a murine bladder cancer cell line (MB49), compared to the rBCG control strain (transfected with an empty vector). In a murine model of experimental bladder cancer, intravesical instillation of rBCG-IFNgamma resulted in an enhanced recruitment of CD4+ T-cells into the bladder and further induced the local expression of IL-2 and IL-4 cytokines (mRNA) compared to control rBCG. With a low-dose treatment regimen for murine orthotopic bladder cancer, rBCG-IFNgamma significantly prolonged survival, whereas the therapeutic effect of wild-type control BCG did not reach statistical significance. We conclude that this recombinant BCG strain has enhanced immunostimulatory potential and might offer new opportunities in the treatment of bladder cancer.

WT1 peptide vaccination combined with BCG-CWS is more efficient for tumor eradication than WT1 peptide vaccination alone.

Nakajima H, Kawasaki K, Oka Y, Tsuboi A, Kawakami M, Ikegame K, Hoshida Y, Fujiki F, Nakano A, Masuda T, Wu F, Taniguchi Y, Yoshihara S, Elisseeva OA, Oji Y, Ogawa H, Azuma I, Kawase I, Aozasa K, Sugiyama H.

Department of Functional Diagnostic Science, Osaka University Graduate School of Medicine, 1-7 Yamada-Oka, Suita City, 565-0871, Osaka, Japan.

A Wilms' tumor gene WT1 is expressed at high levels not only in most types of leukemia but also in various types of solid tumors, including lung and breast cancer. WT1 protein has been reported to serve as a target antigen for tumor-specific immunotherapy both in vitro in human systems and in vivo in murine models. We have shown that mice immunized with WT1 peptide or WT1 cDNA could reject a challenge from WT1-expressing tumor cells (a "prophylactic" model). However, it was not examined whether WT1 peptide vaccination had the potency to reject tumor cells in a "therapeutic" setting. In the present study, we demonstrated for the first time that WT1 peptide vaccination combined with Mycobacterium bovis bacillus Calmette-Guerin cell wall skeleton (BCG-CWS) was more effective for eradication of WT1-expressing tumor cells that had been implanted into mice before vaccination (a "therapeutic" model) compared with WT1 peptide vaccination alone. An intradermal injection of BCG-CWS into mice, followed by that of WT1 peptide at the same site on the next day, generated WT1-specific cytotoxic T lymphocytes (CTLs) and led to rejection of WT1-expressing leukemia or lung cancer cells. These results showed that BCG-CWS, which was well known to enhance innate immunity, could enhance WT1-specific immune responses (acquired immunity) in combination with WT1 peptide vaccination. Therefore, WT1 peptide vaccination combined with BCG-CWS may be applied to cancer immunotherapy in clinical settings.

Acknowledging NCBI for the use of this information. Copyright NCBI 2004.

