

# COMBINED GENE DIRECTED ENZYME PRODRUG THERAPY WITH AND IMMUNOSTIMULATORY CYTOKINES (INTERLEUKINS-12 AND -18) FOR TREATING PROSTATE CANCER IN IMMUNOCOMPETENT MICE

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**Background/Purpose:** Metastatic prostate cancer (PC) is hard to treat. We have previously shown that using cytosine deaminase (CD) plus uracil phosphoribosyl transferase (UPRT) mediated gene directed enzyme prodrug therapy (CDUPRT-GDEPT) to treat mouse RM1 hormone-refractory prostate cancers (HRPC) in immunocompetent mice generated *local and distant bystander effects* with dose-dependent tumor infiltration by CD4<sup>+</sup> T cells, macrophages and NK cells<sup>1</sup>. As this suggested a potential for synergy with immunotherapy, using funding from a Department of Defense Prostate Cancer Award Alternative Funding grant (DAMD17-02-1-0107), we tested the efficacy of CDUPRT-GDEPT together with immuno-stimulatory interleukins, murine (m) interleukin (IL)12 and IL18, against RM1 cells *in vivo*.

**Methods:** Intraprostate (iprost) tumors of parental RM1 or stable transformants expressing GFP/CDUPRT (RM1CDUPRT) cells and RM1 lung pseudometastases were generated by iprost or intravenous (iv) injection respectively, in C57BL/6 mice. To assess cytokine effects, mice with iprost RM1 tumors were injected with Adenoviruses expressing mIL12 (AdmIL12) and/or mIL18 (AdmIL18) on day 5; on day 6, RM1 cells were given iv to establish lung metastases. Mice were euthanased on day 17, and prostate weight/volume, and lung colony counts assessed; tissues were assessed by immunohistochemistry and serum cytokine profiles by Luminex technology. To assess combined CDUPRT-GDEPT plus cytokine therapy, mice with iprost RM1CDUPRT tumors were injected with AdmIL12 and/or AdmIL18 iprost on day 5, then 5-Fluorocytosine injections intraperitoneally for 11 days, and RM1 cells iv on day 6. As above, mice were euthanased for analysis on day 17; additionally, mouse survival was assessed.

**Results:** Unlike mIL12 or mIL18 alone, their combination caused a significant reduction in local PC growth with clear synergy in reducing RM1 lung colonies. Serum cytokine analysis showed significant increases in Th1 IFN  $\gamma$  and IL18 and reduction in Th2 IL4 and IL10 levels in the mIL12 + mIL18 group. Combining CDUPRT-GDEPT with mIL12 + mIL18 led to further growth reduction of local PCs and lung colonies compared with individual therapies. There was a clear survival advantage with 28.7% of mice alive on day 33, despite suppression of serum cytokine levels compared with controls.

**Conclusions:** We conclude that combined CDUPRT-GDEPT with cytokine therapy provides a significant reduction in growth of local and remote deposits of HRPC RM1 tumors in mice. Despite their immunosuppressed state, combination therapy was more effective and provided survival advantage. This project has the potential to impact on PC mortality through the generation of local and anti-metastatic effects by local delivery of combined GDEPT and cytokine gene therapy. Such therapy may well improve the prognosis for PC for those thousands of men who succumb to the disease each year.

<sup>1</sup> Khatri et al, Combination of cytosine deaminase with uracil phosphoribosyl transferase leads to local and distant bystander effects against prostate-cancer in C57BL/6 mice. *J Gene Med* 2006;8:1086