

# SUSTAINED LONG-TERM IMMUNE RESPONSES FOLLOWING COMBINATION *IN SITU* GENE THERAPY WITH RADIOTHERAPY AND HORMONAL THERAPY FOR PROSTATE CANCER

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**Objectives:** We are currently conducting clinical trials of combination adenoviral vector mediated Herpes Simplex Virus-thymidine kinase (HSV-*tk*) + valacyclovir (VCV) *in situ* gene therapy with radiotherapy (XRT) and hormonal therapy for prostate cancer. We now report sustained long-term immune responses using this approach.

**Methods:** 33 patients with PSA > 10 or Gleason > 6 or T2b-T3 were treated as follows: Gene therapy: 3 separate intraprostatic injections of AdHSV-*tk* were performed on days 0, 56, and 70. Each injection was followed by 2 weeks of VCV. XRT was delivered 2 days after the second AdHSV-*tk* injection for 7 weeks. Hormonal therapy: lupron and flutamide were initiated on day 0 and continued for 4 months. Peripheral blood lymphocytes were analyzed by fluorescent antibody cell sorting (FACS). Samples were taken at selected intervals before, during and after the treatments. 100  $\mu$ l of heparin-treated blood was incubated with dual color labeled antibody pairs: CD45/CD14, CD3/CD19, CD3/CD8, CD3/CD4, CD8/HLA-DR, CD4/HLA-DR, CD3/HLA-DR, and CD3/CD56<sup>+</sup>CD16.

**Results:** The pre-treatment mean percentages of total CD8<sup>+</sup> T cells, activated CD8<sup>+</sup> (DR<sup>+</sup>CD8<sup>+</sup>) T cells, and activated CD4<sup>+</sup> (DR<sup>+</sup>CD4<sup>+</sup>) T cells were 19.2%, 14.7%, and 7.8% respectively. Two weeks after the initial hormone and first gene therapy injection, the mean percentages of total CD8<sup>+</sup> T cells, DR<sup>+</sup>CD8<sup>+</sup> T cells, and DR<sup>+</sup>CD4<sup>+</sup> T cells were 26.6%, 37.5% (P=0.0003), and 9.7% respectively. On day 56, the mean percentages of total CD8<sup>+</sup> T cells, DR<sup>+</sup>CD8<sup>+</sup> T cells, and DR<sup>+</sup>CD4<sup>+</sup> T cells were 24.1% (P=0.0074), 25.1% (P<0.0001), and 9.4% respectively. On day 70 (two weeks after the second gene therapy injection), the mean percentages of total CD8<sup>+</sup> T cells, DR<sup>+</sup>CD8<sup>+</sup> T cells, and DR<sup>+</sup>CD4<sup>+</sup> T cells were 21.8% (P=0.0116), 20.7% (P=0.0010), and 9.8% respectively. On day 84 (two weeks after the third gene therapy injection), the mean percentages of total CD8<sup>+</sup> T cells, DR<sup>+</sup>CD8<sup>+</sup> T cells, and DR<sup>+</sup>CD4<sup>+</sup> T cells were 20.7%, 23.5% (P=0.0001), and 10.8% (P=0.0006) respectively. The mean percentages of DR<sup>+</sup>CD8<sup>+</sup> T cells at 8 months was 20.8% (P=0.0031) and of DR<sup>+</sup>CD4<sup>+</sup> T cells at 12 months was 11.2% (P=0.0015).

**Conclusions:** We present evidence showing sustained long-term (up to 8-12 months) systemic T-cell responses to 3 consecutive AdHSV-*tk* injections during combination *in situ* gene therapy, XRT and hormonal therapy for prostate cancer. These results suggest the potential for sustained activation of cell-mediated immune responses against cancer with continued treatment.