454 **CHECKPOINT MODIFICATION OF EARLY T CELL ACTIVATION PROVIDES ENHANCED ANTI-TUMOR ACTIVITY AND SURVIVAL BENEFITS IN A FAST-GROWING PRECLINICAL TUMOR CHALLENGE MODEL**

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Background Checkpoint inhibitors (CPI) that target activated, impaired T cells, have revolutionized cancer treatments. However, there are limited data on CPIs that affect T cell activation. HSV-1 glycoprotein D (gD) binds to the herpes virus entry mediator (HVEM) on dendritic cells and blocks BTLA-HVEM inhibitory signaling during early $CD8^+$ T cell activation. Inhibition of BTLA signaling allows for co-stimulation through LIGHT, which binds to a different domain on HVEM, and enhances and broadens $CD8^+$ T cell responses to the target antigen(s). Here, we report the immunogenicity and efficacy of a chimpanzee adenoviral (AdC) vector expressing sequences of early (E) antigens 2, 5, 6, and 7 of HPV-16, fused into gD (AdC-gDE7652).

Methods Studies were performed in C57/Bl6 mice (n=5–10/group). Frequencies of HPV-16 specific $CD8^+$ T-cells was assessed by intracellular cytokine staining 14 days after a single intramuscular (i.m.) vaccination with AdC vectors encoding HPV-16 E7652 oncoproteins expressed with or without gD. Efficacy was tested in TC-1 challenge studies (5x10^4 to 1x10^6 cells) in mice injected with a single i.m. dose of 1x10^10 vp of AdC-gDE7652, AdC-E7652, or control antigens fused with gD 3 or 9 days after tumor cell transplantation. In some studies, mice were followed for up to 176 days. In mice with growing tumors, T cell functions (granzyme B, perforin, IFN-gamma) and exhaustion markers (PD1, TIM3, LAG3, CTLA4), in spleens and tumors were assessed.

Results The addition of gD increased HPV-16-specific $CD8^+$ T-cell frequencies approximately 10- to 15-fold. In mice vaccinated with AdC-gDE7652, 3 days post TC-1 transplantation, 25/25 (100%) cleared their tumors and remained tumor free, while all (20/20; 100%) of the control-vaccinated mice showed rapid tumor progression. When mice were vaccinated 9 days after TC-1 challenge, the addition of gD doubled the survival time over controls or AdC-E7652-vaccinated animals (60 vs 30 days); T cells within spleens and tumors of AdC-gDE7652 vaccinated animals were polyfunctional and expressed lower levels of exhaustion markers than those of AdC-E7652 or control-vaccinated mice. In 8 mice that previously cleared their tumors, all remained tumor free, upon rechallenge.

Conclusions The addition of gD, an early checkpoint modifier, that provides both checkpoint inhibition and co-stimulation of T cell activation, to an oncogenic target within a vaccine, markedly improves immunogenicity, promotes activation of polyfunctional T cells and enhances tumor clearance and survival with continued protection against rechallenge. A clinical trial evaluating a gD-based vaccine for advanced solid tumors is in development.

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