A Phase I Study of Ad5-GUCY2C-PADRE in Stage I and II Colon Cancer Patients

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A Phase I Study of Ad5-GUCY2C-PADRE in Stage I and II Colon Cancer Patients

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Abstract
Background
Ad5-GUCY2C-PADRE is a replication-deficient human adenovirus 5 recombinant adenovirus (Ad5) vector encoding guanylate cyclase C (GUCY2C) fused to the Poly-E (PADRE). GUCY2C, a paracrine hormone receptor producing the second messenger cyclic GMP (cGMP), is selectively expressed by intestinal epithelial cells and a subset of hypothalamic neurons, but not other tissues. Importantly, GUCY2C is up-regulated in nearly all primary and metastatic human colorectal tumors. Preclinical tests in mice demonstrated selective tolerance of GUCY2C-specific CD4 T cells, but not CD8 T cells, recognizing inclusion of the wongame CD4 T helper cell epitope PADRE to maximize GUCY2C-specific CD8 T-cell responses and tumor efficacy, without autoimmunity.

Methods and Patients
This is an open-label, single arm "proof-of-concept" study evaluating a single dose level of Ad5-GUCY2C-PADRE as a vaccine for surgically-treated, node-negative colon cancer subjects (NCT01972737). Patients received a single intramuscular administration of Ad5-GUCY2C-PADRE. Safety and immunomonitoring were examined at 8, 16, and 24 weeks following vaccination.

Primary objectives were to determine the safety, tolerability, and efficacy of Ad5-GUCY2C-PADRE and to determine whether Ad5-GUCY2C-PADRE induces GUCY2C-specific immune responses. Results from the planned interim analysis following enrollment of 15 subjects.

Results
The vaccine was well tolerated, producing only mild adverse events (AEs). Short-lived injection site pain/swelling, body aches, and chills were the most common observed AEs and occurred in 30-40% of subjects. GUCY2C-specific antibodies and T-cell responses were observed in a subset of subjects. Consistent with preclinical mouse data, T-cell responses were composed of CD8+, but not CD4+ T cells. Importantly, GUCY2C-specific responses occurred only in subjects with low Ad5 neutralizing antibody (NAbs) titer at the time of vaccination, suggesting that pre-existing Ad5 immunity limits Ad5-GUCY2C-PADRE immunogenicity.

Conclusions
Interim analysis of 10 subjects receiving Ad5-GUCY2C-PADRE demonstrates proof-of-concept that GUCY2C is immunogenic in humans and that Ad5-GUCY2C-directed vaccination is safe. Moreover, the presence of GUCY2C-specific antibody and CD8+ T-cell, but not CD4+ T-cell responses is consistent with selective CD4+ T-cell tolerance observed in mouse models. These data establish GUCY2C as a safe and immunogenic target for immunotherapy in cancer patients.

Subject Demographics, AE and Immune Response

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Ad5 Neutralizing Abs Limit GUCY2C Responses in Mice

A) Prior exposure to Ad5 virus produces Ad5-specific neutralizing antibodies (NAbs) in mice, quantified as the dilution of serum that produces 50% inhibition of GFP expression by Ad5-GFP transduced A549 cells in vitro. B) GUCY2C-specific T-cell responses are eliminated in mice with Ad5 NAb titers of <1/1000. T-cell responses were quantified by IFNγ ELISpot. ** P < 0.01, T-test.

Ad5 Neutralizing Abs Limit GUCY2C Responses in Humans

A) Quantification of Ad5 NAb's in pre-vaccination blood samples revealed Ad5 NAb titters of <1/100 in 50% of subjects (Ad5 NAb Low) and >1/1000 in the remaining subjects (Ad5 NAb High). Quantification of GUCY2C-specific (B) and PADRE-specific (C) T-cell responses in subjects separated by Ad5 NAb titers revealed Ad5 Nabs as a barrier to Ad5-GUCY2C-PADRE vaccination in colorectal cancer patients. T-cell responses were quantified by IFNγ ELISpot.

Conclusions
• GUCY2C-directed vaccination is safe in colorectal cancer patients.
• GUCY2C is immunogenic in humans.
• Responses are consistent with selective CD4+ T-cell tolerance observed in mouse models.
• Together, these data establish GUCY2C as a safe and immunogenic target for immunotherapy in cancer patients.

Acknowledgments

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