

Translational phase I study in advanced cancer patients of dual-function autologous (FANG) vaccine incorporating bifunctional shRNA^{furin} and GMCSF transgene expression.

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Previous separate trials with GMCSF gene transduced vaccines and a TGFβ2 antisense gene vaccine have established safety and demonstrated clinical benefit of transgene mediated immune stimulation and antisense mediated inhibition of an endogenous immune suppressor, respectively, and we have subsequently confirmed the same in a combined vector (TAG vaccine) phase I study. In addition to an additive interaction, TGF-β inhibits GMCSF induced maturation of bone marrow derived dendritic cells as well as expression of MHC class II and co-stimulatory molecules. A potential limitation of TAG vaccine, however, is the restricted specificity for TGF-β2, given that all three known isoforms of TGF-β ligand (TGF-β1-3) are ubiquitously produced in human cancers. Overexpression of two or more TGF-β isoforms has been documented in multiple tumor types and up to a 10-fold higher level of TGF-β1 may be produced by human colorectal, lung cancer and melanoma cells (data from TAG manufacturing quality control testing). Insofar as proteolytic cleavage is required for TGF-β isoform activation for which furin is the primary proconvertase, we constructed a furin targeted bifunctional shRNA (bi-shRNA^{furin}) to effect TGF-β1, 2 isoform knockdown. The bi-shRNA^{furin} consists of a miR-30a scaffold with two stem-loop structures; one with complementary guide and passenger strands and the other with two bp mismatches at positions 11 and 12 of the passenger strand designed to maximize target inhibition via mRNA cleavage, translational repression and p-body sequestration. Electroporation of GMP FANG plasmid into autologous tumor cells from 9 patients demonstrated significantly elevated levels of GM-CSF (80-1870 pg/ml at day 4 of culture; median, 739 pg/ml). All 9 patients demonstrated >50% reductions of TGF-β2, and 6 of 7 patients with >100 pg of endogenous TGF-β1 production demonstrated >50% reduction of this cytokine. Six of seven IRB approved phase I protocol eligible patients (melanoma (2), colorectal (2), breast, NSCLC, and gallbladder) will be treated in cohort 1 (of 3), receiving 1.0×10^7 cells/intradermal injection, monthly, for a minimum of 5 months for up to 12 months. Subsequently, cohorts 2 (1.0×10^7 cells/injection) and 3 (2.5×10^7 cells/injection), the dose depending on available material post harvesting and processing, will accrue through a total of 20 patients, Preliminary results will be presented at the meeting.