

FANG Autologous Tumor Cell Vaccine Development and Manufacturing

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Gene modified cell-based cancer vaccines have demonstrated durable responses in selected patients. We have developed the FANG expression vector which we believe, when transfected into tumor cells, will evoke an enhanced immune recognition /stimulation versus our previous TAG vaccine vector. The FANG nonviral vector system expresses both GM-CSF and a proprietary bifunctional shRNA to furin. Preclinical data demonstrated that blocking furin protein expression in turn blocked the activation of both TGF β ₁ and TGF β ₂. In contrast, our TAG vector expressed both GM-CSF and a TGF β ₂ antisense. Data from our TAG Phase I autologous vaccine clinical trial and others indicate that TGF β ₁ overexpression is present in a wide range of cancers. In fact our data suggest that TGF β ₁ tends to be about tenfold higher than TGF β ₂ expression in the more than thirty tumors we examined in that study. So while the TAG vector blocked TGF β ₂ expression, there was no effect on TGF β ₁ expression. The FANG expression vector is identical to the TAG expression vector except that the TGF β ₂ antisense coding sequence has been replaced with the furin shRNA sequence. FANG plasmid DNA was GMP-S manufactured. We generated 2 nonclinical and 8 clinical vaccines under cGMP as part of our IND submission data (4 melanoma, 3 colorectal, 1 gall bladder, 1 NSCLC and 1 breast cancer). All vaccine manufacturing processes met specifications (no contamination or failure to meet final dose or quality requirements). Average cell viability is 91.5 \pm 5.3%, median 93.5% and range 78-96% (values taken on Day 2 of manufacturing). Average GM-CSF expression is 657 \pm 550pg/1x10⁶ cells/ml, median 602pg and range 80-1870pg. The mean pretransfection TGF β ₁ is 1241 \pm 1115pg/1x10⁶ cells/ml, median 1039pg. The mean posttransfection TGF β ₁ is 211 \pm 421pg/1x10⁶ cells/ml, median 20.1pg. The average percent knockdown of TGF β ₁ was 89 \pm 20%, median 97% and range 36-100%. The mean pretransfection TGF β ₂ is 293 \pm 189pg/1x10⁶ cells/ml, median 257pg. The mean posttransfection TGF β ₂ is 9.1 \pm 12pg/1x10⁶ cells/ml, median 4pg. The average percent knockdown of TGF β ₂ was 94 \pm 12%, median 99% and range 60-100%. These data indicate that the GMCSF expression is consistent with the TAG vaccine values as is the TGF β ₂ knockdown. In contrast, FANG vaccines have reduced the TGF β ₁ expression almost tenfold. The outcome of the clinical studies will determine whether this added reduction has a significant added clinical impact. Gradalis has received IND approval from FDA (BB-IND 14205). The FANG Phase I clinical trial is now open.