Xenograft Expanded FANG Autologous Tumor Cell Vaccine Development and Manufacturing for Clinical Use

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Many tumors are of insufficient size or otherwise inaccessible for surgical ressection to allow successful autologous vaccine manufacturing. However, small accessible lesions can be obtained or small samples obtained more safely by needle biopsy. We have previously demonstrated with our TAG vector that small quantities of tumor can be expanded in immunodeficient mice and harvested for clinical tumor vaccine production (P. Kumar, et al, BioProcessing J, 8(1): 30-36, 2009; BB-IND 13401). 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 $TGF\beta_1$ and $TGF\beta_2$. In contrast, our TAG vector expressed both GM-CSF and a $TGF\beta_2$ antisense. Data from our TAG Phase I autologous vaccine clinical trial and others indicate that $TFG\beta_1$ overexpression is present in a wide range of cancers. In fact our data suggest that $TGF\beta_1$ expression may be up to tenfold higher than $TGF\beta_2$ in the more than thirty tumors we examined in that study. So while the TAG vector blocked $TGF\beta_2$ expression, there was no effect on $TGF\beta_1$ expression. The FANG expression vector is identical to the TAG vector except that the $TGF\beta_2$ antisense coding sequence has been replaced with the furin shRNA sequence. FANG plasmid DNA was GMP-S manufactured. The first clinical grade xenograft FANG vaccine was manufactured from a xenograft expanded breast cancer tumor. The tumor was expanded at the Van Andel Research Institute using their colony of athymic nude mice under conditions optimized for clinical xenograft expansion. The breast cancer tumor is ER, PR negative and Her-2/neu +++. From the time of surgery to removal of 6 F1 tumors for vaccine manufacturing was 323 days. The combined weight of the tumors was 6.2g. The cGMP manufacturing is a 2 day process that consists of tissue disaggregation into a single cell suspension, purging of murine cells, electroporation of FANG vector, overnight incubation, gamma irradiation, cryopreservation and quality control testing. The prefreeze cell viability was 90%. The transfected tumor cell GM-CSF expression was $259pg/1x10^6$ cells/ml. Nontransfected $TFG\beta_1$ expression was $860pg/1x10^6$ cells/ml, the transfected $TFG\beta_1$ expression was $142pg/1x10^6$ cells/ml and the $TGF\beta_1$ knockdown was 83%. Nontransfected $TGF\beta_2$ expression was $479pg/1x10^6$ cells/ml, the transfected $TGF\beta_2$ expression was $127pg/1x10^6$ cells/ml and the $TGF\beta_2$ knockdown was 73%. These data are consistent with prior manufacturing data for autologous FANG vaccines (BB-IND 14205) and for GMCSF and $TGF\beta_2$ data for TAG vaccines (BB-IND 13650).