**ImmuneFx™: A Novel Immunotherapeutic Cancer Vaccine**

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While it is understood that the immune system is capable of mounting an anti-tumor response, in many instances the development of an effective response is thwarted by ineffective immune stimulation. Studies reported here used a potent priming antigen from *S. pyogenes* (Emm55) to initiate a specific anti-tumor response capable of regressing pre-existing tumors and inhibiting tumor development.

A plasmid vector containing the *emm55* gene and a G418 resistance marker was transfected into Neuro-2a, a murine neuroblastoma cell line. A stably transfected cell line expressing Emm55 was developed. This Emm55 cellular vaccine (*ImmuneFx™*) was tested as a potential cancer vaccine in an A/J syngeneic murine model. Although A/J mice are capable of mounting an immune response to Neuro-2a, without the Emm55 trigger the response was insufficient to be of therapeutic value.

In these studies, 305 (259 experimental and 46 control mice) were used. The clinical endpoints were efficacy, toxicity and dose. *ImmuneFx* was not toxic to tumor cells and did not induce unwanted side effects in the mice. Southern blot analysis did not detect *ImmuneFx* in any tissues/organs. The combined data demonstrate that *ImmuneFx* prevented tumor formation in 80% of mice treated and increased the overall survival rate of mice challenged with tumor by 81%. In tumor regression studies, mean survival was twice that of control mice, with the highest dose having a mean survival four times that of controls.

At euthanasia, tissue samples and sera were collected from all mice. Splenic cells were used in *in vitro* cytotoxic T-lymphocyte assays to assess the cell mediated immune (CMI) response to the tumor. In control mice, humoral and CMI responses were recorded against Neuro-2a. However, both humoral and CMI responses in *ImmuneFx* treated mice were significantly higher than controls.

Studies were undertaken to gain insight into the mechanism(s) responsible for tumor regression. Western blot analysis of sera from *ImmuneFx*-treated mice demonstrated an increase in tumor-specific antibody responses and an increase in the number of antigens recognized. The phenotype of the effector cells responsible for the lysis of tumor cells was also determined.

In summary, *ImmuneFx* was successful in preventing the establishment of neuroblastoma tumors, regressing pre-established tumors and improving the overall survival rate. Development of an effective immune response against neuroblastoma and successful transfection studies with human melanoma, renal cell carcinoma and kidney sarcoma cells indicate that *ImmuneFx* could provide effective immunotherapy for other malignancies.

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