

1. **The scientific abstract.** We hypothesize that administration of melanoma antigen peptide-loaded, autologous matured dendritic cells (DC), which have been gene-modified *ex vivo* by infection with a replication-deficient adenoviral (Ad) vector expressing the CCL-21 gene, can be used to stimulate specific and therapeutic anti-tumor immunity in subjects with advanced melanoma. Our hypothesis is founded on extensive preliminary data in relevant *in vitro* and *in vivo* models of solid tumors, including melanoma (1-6). The AdCCL-21 (Lot L0604006) being used for DC-transduction has been manufactured in accordance with Current Good Manufacturing Practices (cGMP) by BDP/SALC-Frederick, Inc, and a complete manufacturing report is included as an attachment to this Appendix. A large body of work performed by the PI and associates at the H. Lee Moffitt Cancer Center demonstrate that human DC can be generated *ex vivo* from peripheral blood monocytes in the presence of GM-CSF and IL-4 (7), employed clinically as a vaccine strategy in advanced cancer patients, and that replication-deficient Ad vectors can be utilized for transduction of DC without functionally compromising their antigen presenting capacity (8-10). The anti-tumor efficacy of CCL-21 gene modified DC has been demonstrated in animal models of solid tumors (5, 6). Each component that comprises the final product (denoted Ad-CCL-21-DC) is required to generate the optimal response for anti-tumor efficacy (5, 6). The observed anti-tumor effects are mediated by the recruitment of endogenous host DC and T cells to the vaccination site with subsequent melanoma peptide-priming of specific effector T cells (1-6). Simulated clinical protocols that use primary peripheral blood mononuclear cells (PBMC)-derived DC in a manner analogous to that being proposed within this clinical trial have validated the transduction efficiency and helped develop the quality control assessments of the final product (4,7). To isolate and generate the autologous DC, the H. Lee Moffitt Cancer Center has a dedicated FDA-compliant cGMP suite (>2,000 sq. ft.) specifically designed for cellular and gene transfer studies. The facility employs a full time staff of a director, manager, institutional compliance officers and technicians, who monitor or technically generate cell-based products for all immunotherapy-based clinical trials prospectively, with both local and federal oversight. Based on a rationale that is strongly supported by laboratory data, and the availability of an experienced clinical translation team with access to highly specialized and dedicated infrastructure, we propose to test the safety, immunologic activity, and potential efficacy of Ad-CCL-21-DC within a Phase I trial in subjects with advanced, chemotherapy resistant melanoma.