

Scientific Abstract

Phase II Study Examining the Biological Efficacy of Intratumoral INGN 241 (Ad-Mda7) Administration in Patients with In Transit Melanoma

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This Phase II study is designed to examine the spectrum of biologic and immunologic activities of INGN 241 when administered as an intratumoral injection into melanoma in transit lesions. INGN 241 is an adenoviral vector carrying the *mda7* cDNA. MDA-7 is a novel tumor suppressor molecule with cytokine properties. The INGN 241 gene transfer construct has been previously used in human subjects in an ongoing open label Phase I study and has been well tolerated to date. The primary objectives of this study are to determine if INGN 241, injected into a melanoma in transit lesion, can induce apoptosis in regional uninjected lesions and initiate positive systemic immune activation. Secondary objectives include examination of melanoma-specific T cell immunity and of clinical response.

Eligible patients will have melanoma in transit disease with at least three regional lesions, one of which must measure 10 mm in one diameter. The dose of INGN 241 is fixed at 2×10^{12} vp in a volume of 2 ml. Prior to the first injection, one lesion will be biopsied for baseline studies. INGN 241 will then be administered as an intratumoral injection into a second lesion on days 1, 8, and 15. On day 17, samples of the injected tumor and of an uninjected lesion proximal to the injected tumor will be taken. These day 17 samples will be obtained either in the form of complete resection of all disease in the case of patients with resectable disease; or as biopsies, if all disease cannot be removed. Patients with unresectable disease may receive up to six courses of treatment. One course is defined as three weekly injections, followed by one week of rest.

The primary objectives of this study are to determine if intratumoral INGN 241 can exert regional and systemic biologic activity. We will examine the ability of INGN 241, injected into an in transit lesion, to exert antiproliferative and pro-apoptotic effects in an uninjected regional lesion. Alteration in proliferation will be assessed by immunostaining of biopsy material for Ki-67. The induction of apoptosis will be determined by TUNEL. The number and phenotype of infiltrating lymphocytes will be analyzed by immunostaining with antibody to CD3, CD4, CD8, and CD20. To assess the induction of systemic immune activation, levels of IL-6, IL-10, IFN- γ , TNF- α , IL-12, IL-1- β , and GM-CSF will be quantitated by ELISA in serum samples taken at various timepoints throughout the course of treatment. The secondary objective of specific immunity will be examined using the patient's pretreatment tumor biopsy and peripheral blood mononuclear cells isolated from blood samples obtained on day 17, and at the completion of cycles 2 and 3. Specific immunity will be examined by T cell proliferation and IFN- γ production in response to autologous tumor. The final secondary objectives of toxicity and response will be assessed by standard clinical methods.

The statistical design of this study is based on an 8% increment in apoptotic cells in an uninjected lesion (e.g., 2% apoptosis rate at baseline compared to 10% on day 17). Anticipated accrual is 25 subjects, with an early stopping rule in place.