

## Scientific Abstract:

Cancer is a leading cause of death in the Western World. Although most patients die from disseminated carcinoma, a substantial number die due to complications of locally advanced disease. Furthermore, patients who die of metastases frequently also suffer the morbidity of advanced local disease.

Current cancer therapy includes surgery, chemotherapy, and radiation therapy (RT). These treatment modalities, either alone or in combination, provide high degrees of local control in early stage disease but often fail in eradicating bulky tumors. The addition of gene transfer to currently available modalities provides the potential to improve control rates for these tumors.

According to recent statistics, cutaneous melanoma accounts for approximately 4% of all cancers diagnosed in the United States. The lifetime risk for developing cutaneous melanoma has been estimated as 1 in 78 for women and 1 in 53 for men. Although treatment is generally successful if the primary tumor is excised early in the course of the disease, there is no reliable treatment for metastatic melanoma. Metastasis rates from a primary cutaneous melanoma are influenced by several factors. Metastases may involve one or more local or distant lymph nodes, present as distant skin or subcutaneous nodules, or present as lung, liver, brain, gastrointestinal or bone lesions. Five-year survival rates for patients with distant metastases from primary melanoma (AJCC stage IV) are estimated at 16.2%. Currently, treatment of distant metastases is dependent on the location and number of metastatic lesions, and may include surgical resection, immunotherapy, palliative radiation therapy, chemotherapy, hormonal therapy, or experimental treatments. There is no proven cure for metastatic melanoma. The primary goal of this study is to evaluate the anticancer activity of intratumoral injections of TNFerade™ plus radiation in patients with unresectable stage IV melanoma.

The study will consist of a single-arm, open label, in which patients with metastatic melanoma will receive intratumoral injections of TNFerade™ ( $4 \times 10^{11}$  PU) plus radiation (50 Gy) as a 4-week treatment, followed by a 36-month follow-up period. Approximately 29 patients, with metastatic melanoma (stage III or IV) who are not eligible for curative surgery and who are candidates for experimental therapy, will be enrolled. Eligible patients will include those with melanoma involving the regional lymph nodes and surrounding tissues as well as those with unresectable cutaneous, subcutaneous, nodal or soft tissue metastases. Patients with metastases outside the treatment field may be enrolled if the sites of metastases do not limit life expectancy to less than 3 months.

Patients will receive TNFerade™ by intratumoral injections at a total dose of  $4 \times 10^{11}$  PU twice weekly for the first two weeks and weekly thereafter for the next two weeks (total of 6 doses over 4 weeks). Radiation (50 Gy) will be administered concomitantly throughout the duration of TNFerade™ treatment. The index lesion(s) requiring radiation treatment will be identified in advance; this/these will be the lesion(s), which will receive TNFerade™ treatment. At each injection session, a total dose of  $4 \times 10^{11}$  PU

in up to 2 mL injection volume will be administered. An attempt should be made to treat all lesions contained within the proposed radiation field, up to a maximum of 10 lesions. If multiple lesions are to be treated, the total TNFerade™ dose will be divided among the lesions, with the total dose administered not exceeding  $4 \times 10^{11}$  PU in a volume of 2 mL at any delivery session. The TNFerade™ dose delivered to each treated tumor may be further divided into up to 10 aliquots, depending on tumor size and location. The minimal injection volume is 100  $\mu$ l per injection. During each delivery session, the same lesions treated in the first administrations will continue to be treated, unless repeated injection in a particular lesion is contraindicated by extensive necrosis/ulceration, or in the event of a complete response, where no tumor would remain for injection. When possible, the radiation should begin 4 hours or more after initiation of TNFerade™. A total of 50 Gy radiation should be delivered in 20 fractions (2.5 Gy each).

The objective of this study is to evaluate the anticancer activity of TNFerade™ plus radiation in patients with metastatic melanoma. The primary endpoint of anticancer effects of treatment will be evaluated by local tumor response rates at 2 months post-treatment. Only treated tumors will be evaluated for assessment of local response rate. For the safety assessments, standard clinical and laboratory tests, including comprehensive liver function tests and evaluation of adverse events (graded according to NCI Common Toxicity Criteria) will be employed. In addition, adenovirus neutralizing antibody titer, serum TNF alpha, and cultures for both replicating and replication-incompetent adenovirus from urine and throat swabs will be assessed. Secondary endpoints will also include further assessment of the anticancer activity of TNFerade™ by evaluating the following: time to locoregional progression and duration of local response, extent of tumor necrosis, time to overall disease progression and time to death, the frequency of conversion of unresectable tumors to resectable status, generation of a comprehensive pharmacokinetics profile and evaluation of the mechanism of action for TNFerade™.