1. SCIENTIFIC ABSTRACT

Pancreatic cancer is the fourth leading cause of cancer deaths in men and women in the United States. According to the American Cancer Society, approximately 31,860 new diagnoses of and 31,270 deaths from pancreatic cancer will occur in the United States in 2004 (*Jemal, 2004*). For all stages combined, the one-year survival rate from time of diagnosis is 21%, and the five-year survival rate is approximately 4% [*American Cancer Society (ACS), 2003*]. This poor prognosis is due to the absence of symptoms early in the disease, the aggressiveness of the cancer, and the lack of effective therapies. Currently, surgery is the only potentially curative intervention, but this option is available only to the 10-20% of patients who have localized disease (Stages I and II) at the time of diagnosis. Even in these patients, disease-specific survival after surgical resection is poor, with a five-year survival rate of only 17% (*ACS, 2003*). For the majority of patients with pancreatic cancer who have metastatic disease or locally advanced, unresectable disease (Stage IV), few treatments have been shown to affect survival. Pancreatic cancer is markedly resistant to chemotherapeutic regimens, used either alone or in combination therapy. Radiation therapy alone has little impact on pancreatic cancer, although the use of radiation therapy in combination with chemotherapy is one treatment option for management of the disease. Untreated, the median survival for patients with pancreatic cancer is 3-6 months (*Tamm, 2001*). In a large population of patients treated with gemcitabine (3023 patients), the median overall survival was 4.8 months; thus, for patients in whom gemcitabine is ineffective and who then experience disease progression or unacceptable toxicity, assuming administration of 1 - 2 cycles of therapy (1 - 2 months), median survival can be estimated to be approximately 3 - 4 months following failure of primary therapy *(Storniolo, 1999; Heinemann, 2002)*.

The immunotherapeutic approach to the treatment of cancer is based on the observation that human tumor cells express a variety of tumor-associated antigens (TAAs) that are not expressed or are minimally expressed in normal tissues. These antigens, which include viral tumor antigens, cellular oncogene proteins, and tissue-specific differentiation antigens, can serve as targets for the host immune system and elicit responses that result in tumor destruction. Because tumors have developed a variety of mechanisms to evade immune detection and activation, the development of effective therapeutic vaccines for cancer will hinge on the ability to effectively activate immune responses. Antigen-specific, active immunotherapy is designed to generate immune responses, particularly cell-mediated responses, against specific TAAs using vaccines that express one or more of these antigens.

Early strategies for active immunization against cancer relied on the use of whole tumor cells or tumor cell fragments as immunogens. However, the identification and isolation of genes encoding TAAs has allowed the development of recombinant or synthetic antitumor vaccines designed to elicit immune responses

to one or more antigens known to be expressed by a particular tumor type *(Tsang, 1995; Kawakami, 1994; Boon, 1994).* In collaboration with investigators at the NCI, Therion has demonstrated that clinically relevant immune responses can be achieved using recombinant pox virus vectors - viruses genetically engineered to express one or more antigens, alone or in combination with costimulatory molecules - to present TAAs to the immune system.

Immunization with live recombinant virus allows expression of TAAs and subsequent co-presentation of antigenic peptides derived from the TAAs with host histocompatibility antigens, a strategy that favors the induction of cell-mediated immune responses. Recombinant pox viruses can infect antigen-presenting cells (APCs), including dendritic cells and macrophages, resulting in efficient expression of TAAs simultaneously with costimulatory molecules required for the elicitation of T cell responses. TAAs expressed by recombinant pox viruses are presented to the immune system together with highly immunogenic virus proteins, which may act as adjuvants to enhance immune responses to the TAAs. Thus, the use of recombinant pox virus vectors for the presentation of TAAs to the immune system results in the generation of killer T cells that specifically destroy the selected tumor with little incremental toxicity.

Therion uses two different pox virus vectors for the delivery of TAAs and costimulatory molecules to cancer patients - vaccinia virus, the prototype member of the orthopoxvirus genus, and fowlpox virus, an avipox virus.

Vaccinia virus has been used for over 200 years as a vaccine for smallpox and has a well-established safety profile. The virus actively replicates in human cells, resulting in the presentation of high levels of antigen to the immune system over a period of one to two weeks, substantially increasing the potential for immune stimulation. The immune response specific to vaccinia then eliminates the virus. Phase I studies using other vaccinia-based cancer vaccines have supported the safety of this vector (see Section 1.3.1).
Fowlpox virus is a member of the genus Avipoxvirus. Productive fowlpox virus infection is restricted *in vivo* to avian species and *in vitro* to cells derived from avian species. However, fowlpox-mediated gene expression does occur in infected non-avian cells *(Taylor, 1988)* and a number of studies have shown that immunization of mammalian species by recombinant fowlpox virus can stimulate both humoral and cell-mediated immunity to the expressed transgene. Phase I studies using other fowlpox-based cancer vaccines have supported the safety of this vector (see Section 1.3.1).

Recombinant vaccinia and fowlpox vectors are most effective when used in combination in prime-boost regimens. Host immune responses to vaccinia restrict its replication and thus limit its ability to continue to elicit tumor-specific immune responses after multiple vaccinations. Consequently, vaccinia-based vaccines can be used to immunize an individual only a limited number of times. However, the

immune responses to vaccinia do not inhibit fowlpox virus, which can be administered numerous times without loss of immunogenicity. Therefore, by priming with recombinant vaccinia virus and then boosting repeatedly with the corresponding recombinant fowlpox virus, maximum immune responses to the expressed tumor antigens can be obtained. This phenomenon has been demonstrated in animal models *(Hodge, 1997)* and has also been supported by results of ongoing clinical trials.

Carcinoembryonic antigen (CEA) is an 180,000 dalton glycoprotein that is over-expressed on most adenocarcinomas of the colon, rectum, stomach, and pancreas, as well as on breast cancers and non-small-cell lung cancers. It has also been identified in fetal gut and in small amounts in normal adult colonic mucosa. The CEA gene family belongs to the immunoglobulin superfamily and resides on the long arm of chromosome 19. CEA shares approximately 70% amino acid homology with non-specific cross-reacting antigen, which is found on normal granulocytes *(Hodge, 1996)*. The immunogenicity of CEA in humans has been demonstrated in several clinical trials. Foon and coworkers reported the development of humoral and T cell immunity to CEA as a result of immunization with a CEA anti-idiotype vaccine *(Foon, 1995)*. In addition, a number of clinical trials using recombinant vaccinia and/or avipox viruses expressing CEA have been conducted. These trials demonstrated for the first time that CEA, when expressed by a recombinant pox virus, can elicit or enhance human immune responses capable of recognizing and destroying tumor cells that express CEA *(Marshall, 2000; Hörig, 2000; von Mehren, 2000; Zhu, 2000; Tsang, 1995)*.

MUC-1 is a glycosylated transmembrane protein that is uniquely characterized by an extra-cellular domain that consists of a variable number of tandem repeats of 20 amino acids *(Gendler, 1990)*. Pancreatic adenocarcinomas aberrantly glycosylate as well as over-express MUC-1 *(Barrett-Boyes, 1996)*. Anti-MUC-1 immune responses, including antibodies and cytotoxic T lymphocytes (CTLs), have been demonstrated in pancreatic cancer patients *(Kotera, 1994; Graham, 1996)*. In addition, immunization with a MUC-1 peptide or a recombinant vaccinia virus expressing MUC-1 has been shown to induce MUC-1-specific immune responses in pancreatic and breast cancer patients *(Ramanathan, 2000; Scholl, 2000)*. Thus, immunization of pancreatic cancer patients with a pox virus expressing MUC-1 may boost the antitumor immunity against their cancers.

Activation of T cell immune responses requires T cell lymphocyte recognition, via the T cell receptor, of antigenic peptides presented in the context of major histocompatibility complex (MHC) molecules on APCs. In addition to this antigen-specific signal, a second, antigen-independent signal is required for T cell activation *(Hellstrom, 1993)*. This second signal is provided by the interaction of specific ligands on the T cell surface with "costimulatory" molecules expressed on APCs. The most extensively studied pathway of costimulation is that involving the interaction of the costimulatory molecule B7.1 expressed on APCs with CD28 and CTLA4 on the T cell *(Schwartz, 1992; Chen, 1992; Freeman,*

1989; Freeman, 1991). A number of additional costimulatory molecules on APCs have been identified; these include ICAM-1 and LFA-3, whose ligands are LFA-1 and CD2, respectively, on the surface of T cells *(Springer, 1990).* Recombinant vectors co-expressing three costimulatory molecules, LFA-3, ICAM-1, and B7.1, designated TRICOM, have been shown to have synergistic effects on antitumor responses in mice as compared to vectors expressing individual costimulatory molecules *(Hodge, 1999).* For example, T cell proliferation and antitumor immunity using recombinant vaccinia virus co-expressing the three costimulatory molecules were much greater than the sum of responses seen using vaccinia virus expressing individual costimulatory molecules *(Hodge, 1999).* In addition, mice immunized with a recombinant vaccinia virus co-expressing CEA and murine TRICOM exhibited greater immune responses and antitumor responses than mice immunized with a recombinant vaccinia virus co-expressing CEA and murine B7.1. Enhanced antitumor immunity was also observed in mice that were transgenic for CEA.

Granulocyte macrophage colony-stimulating factor (GM-CSF) has been shown to be an effective vaccine adjuvant because it enhances recruitment of APCs and subsequent antigen processing and presentation. Using murine tumor models, several researchers have shown that modification of tumor cells to enhance GM-CSF expression, using retroviral vectors *(Dranoff, 1993)* or vaccinia virus vectors *(McLaughlin, 1997; Kass, 2000),* results in enhanced tumor-specific immune responses capable of effecting tumor destruction. Furthermore, this immune response is effective against not only the engineered, GM-CSF-expressing tumors but also against unaltered tumor cells. Therion anticipates using GM-CSF locally, at the vaccination site, to enhance immune responses elicited by its recombinant vaccines.