

**PHASE I/II TRIAL OF ANTIGEN-SPECIFIC IMMUNOTHERAPY IN MUC-1 POSITIVE PATIENTS WITH ADVANCED NON-SMALL CELL LUNG CANCER USING VACCINIA-VIRUS-MUC1-IL2 (TG 1031)**

**1. Introduction**

TG 1031, an attenuated recombinant vaccinia virus containing sequences coding for the human MUC-1, and Interleukin 2 (IL-2) genes, has been designed for use in human clinical applications, especially in oncology. The vector, vaccinia virus, has been used extensively to vaccinate against smallpox and the virus is known to be highly immunogenic. The second component of the product, MUC1, is a mucin producing gene which is overexpressed in a variety of adenocarcinomas. The third component, the IL2 gene, produces local generation of interleukin 2 and stimulates cytotoxic T lymphocyte production. This virus produces an antitumor effect, as observed in many experiments on animals. TG 1031 has been tested in a phase I clinical trial, at Institute Curie (Paris, France), in metastatic breast cancer which is discussed in section 1.4 below, and is currently being tested in phase II trials in breast and prostate cancer.

**1.1. Product Rationale**

**1.1.1. Specific tumor antigen MUC-1**

The MUC-1 protein is a highly glycosylated mucin (MW > 200 kD), normally found at the apical surface of mucin-secreting epithelial cells in many types of tissue, including the breast, prostate, lungs, pancreas, stomach, ovaries, fallopian tubes and intestine [Peat, 1992]. The function of mucin is to lubricate and protect epithelial cells from the harsh environment of the lumen.

The onset of cancer in secretory epithelial cells can be accompanied by excess expression of MUC-1 in the tumor cells [Hareuveni, 1990] ; [Ho, 1993] ; [Layton, 1990]. Tumor MUC-1 protein is much less glycosylated than normal MUC-1 protein, revealing new peptide and carbohydrate epitopes [Burchell, 1987] ; [Devine, 1990] such as that recognized specifically by the H23 [Keydar, 1989] or the SM3 [Burchell, 1987] murine monoclonal antibody cultured from the ATCC HB8630 hybridoma.

**1.1.2. Interleukin 2 (IL-2)**

TG1031 contains a second gene which codes for human interleukin 2 (IL-2). This cytokine may have an important function as an adjuvant in the immune response, as IL-2 has been shown to be an essential factor in cell-mediated and humoral immune response [Kaplan, 1992].

**Local administration via a recombinant vaccinia virus has several potential advantages over a systemic *in vivo* administration of IL-2:**

- It greatly reduces the risk of adverse side effects, because IL-2 secretion at the local source produces very low levels in the circulation [Konrad, 1990].
- It activates the cytotoxic function of LAK and NK cells and memory CTLs [Bubenik, 1993; Foa, 1992; Gansbacher, 1990].
- The virulence of the vaccinia virus (wild-type) can be attenuated by inserting the IL-2 gene. This has been observed in studies on nude mice [Flexner, 1987; Ramshaw, 1987] and on primates [Flexner, 1990; Ruby, 1990].

### 1.1.3. The vector: vaccinia virus (Copenhagen strain)

Several clinical trials in gene therapy using a replicative vaccinia virus have been performed. The choice of the vaccinia virus as a vector is based on the following criteria:

- **High immunogenicity:** this was demonstrated during the anti-smallpox vaccination campaigns. Thus, vaccinia virus should allow a good presentation of MUC-1 antigen through both the class I and II MHC molecules.
- **Low pathogenicity compared to that of the parental virus of most other vectors used in gene therapy. This low risk of pathogenicity with vaccinia has been demonstrated by extensive human experience with vaccinia virus.**
- Since vaccinia is a lytic virus, the possibility of DNA integration into the host genome is reduced. Moreover, the vector does not interact with the host cell genome because it remains in the cytoplasm until the cell has been destroyed.
- The vaccinia virus is large, allowing the insertion of several kilobases of DNA [Jolly, 1994]. Thus, the whole MUC-1 molecule can be expressed in this vector.
- **The vaccinia virus can be attenuated to a large degree by inactivation of the gene coding for thymidine kinase (TK) [Buller, 1985] and by insertion of the gene coding for IL-2 [Flexner, 1987]. Thus, TG1031 is the only recombinant vaccinia with two attenuation systems (by IL-2 insertion and TG gene disactivation) used in clinical trials.**
- **Good tolerance in man: around 150 patients were treated with recombinant vaccinia virus similar to TG1031. Good tolerance was observed with this type of vector and only minor side effects were reported (fever, chills, lesion at the site of injection site). [Graham, 1992 ; Graham 1994 ; Cooney 1991 ; Tsang, 1995 ; McAnemy, 1996 ; Borysiewicz, 1996 and Mastrangelo, 1995 ]**

## 1.2. Non-Small Cell Lung Cancer

This protocol is being conducted in patients with stage IIIB or IV non-small cell lung cancer, and is limited to those patients whose tumors express the MUC1 antigen, and who are either previously untreated, or have failed 1 prior regimen. The MUC1 cell surface glycoprotein has been found to be expressed in the majority of non-small cell lung cancers and may be the focus for a therapeutic target (references).