VACCINOGEN

INVESTIGATOR'S BROCHURE RECEIPT ACKNOWLEDGMENT

OncoVAX® AUTOLOGOUS TUMOR CELLS/BCG VACCINE

Edition Number: 3.0
Release Date: 10 September 2008

I have thoroughly reviewed all aspects of this Investigator's Brochure. The Investigator's Brochure is ready for Institutional Review Board/Independent Ethics Committee (IRB/IEC) submission and will be sent to the IRB for addition to the project file.

Principal Investigator Name (printed): _____

Principal Investigator Signature: _____Date:_____

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OncoVAX® AUTOLOGOUS TUMOR CELLS/BCG VACCINE

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OncoVAX® AUTOLOGOUS TUMOR CELLS/BCG VACCINE

INVESTIGATOR'S BROCHURE

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CONFIDENTIALITY STATEMENT

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List of Abbreviations

ASI	active specific immunotherapy
BCG	Bacillus Calmette Guérin
CEA	carcinoembryonic antigen
CFU	colony forming units
cGMP	current Good Manufacturing Practices
C	Celsius
DCH	dermal cutaneous hypersensitivity
DNase	deoxyribonuclease
ECOG	Eastern Cooperative Oncology Group
F	Fahrenheit
FACS	fluorescence activated cell sorter
HBSS	Hanks' balanced salt solution
HBSS/G	Hanks' balanced salt solution with gentamicin
ID	intradermal
IEC	independent ethics committee
IRB	institutional review board
IV	intravenous
Mab	monoclonal antibody
mm	millimeter
mL	milliliter
OJT	on the job training
OP	OncoVAX personnel
OR	operating room
ORP	operating room personnel
PCR	polymerase chain reaction
PID	patient identification
PK	pharmacokinetics
PMN	polymorphonuclear
PPD	purified protein derivative
QA	quality assurance
QP	qualified person
RR	relative reduction
SDA	superficial distal axillary
SI	standing instructions
SOP	standard operating procedure
TNM	tumor, nodes, metastases

1. SUMMARY

OncoVAX is a form of active specific immunotherapy (ASI), a process by which the patient's immune response to tumor cells is stimulated and/or augmented. OncoVAX is an immunotherapeutic formulation composed of sterile, viable, irradiated, non-tumorigenic autologous tumor cells with or without fresh-frozen mycobacteria of the Bacillus Calmette Guérin (BCG) strain of *Mycobacterium bovis*. The tumor cells are derived from the patient's own solid tumor which, after surgical removal, has been enzymatically dissociated to a single cell suspension and cryopreserved using techniques to preserve cell viability. TICE® fresh-frozen BCG is an attenuated live culture preparation of the BCG strain of *M. bovis* packaged at a concentration of 2 to 8 x 10^8 CFU/vial. The TICE® strain is currently manufactured by Organon Teknika in Durham, North Carolina. The lyophilized form of TICE® BCG is currently approved by the FDA for the treatment of bladder cancer and for the prevention of tuberculosis. Fresh-frozen BCG has been shown to retain pre-freeze viability for up to 13 years when stored at -70°C. TICE® fresh-frozen BCG is packaged for Vaccinogen by Organon.

The OncoVAX manufacturing process reduces the bioburden inherent to colon-derived tumors yielding sterile cells without compromising the critical properties required for their immunogenicity. The four vaccine preparations are administered intradermally by an experienced and well-trained nurse or physician. The first two immunizing doses contain BCG as an immunological adjuvant. The third and fourth doses contain tumor cells only. The first three doses are given on a weekly basis starting approximately one month after surgery. A fourth, booster dose is administered 6 months after surgery to Stage II patients (or 1 month after completing chemotherapy for Stage III patients).

2. INTRODUCTION

2.1 Colon Cancer

Colon cancer is a common malignancy in developed countries. In the United States, excluding skin cancer, colon cancer is the third most commonly diagnosed cancer in both men and women and the second leading cause of cancer-related deaths.[2] More than 5% of Americans develop colon or rectal cancer.[1] The American Cancer Society estimates that 108,070 cases of colon cancer will be reported in 2008.[2] In Europe, the number of cases of colon cancer reported annually is even greater and exceeds 190,000.[3] The risk of colon cancer increases after the age of 40 and rises exponentially from the ages of 50 to 55; the risk then doubles with each succeeding decade.

Survival in patients with colon cancer is related to the stage of disease at the time of initial diagnosis. Surgery is the most common treatment for this disease, and for cancers that have not spread, surgical removal may be curative.[4] Surgery is curative of Stage I carcinoma of the colon; hence no adjuvant form of treatment has been found useful in this condition. In Stages II and III, surgical resection is also generally performed with the intent to cure. For Stage III (Dukes' C) disease (histologically detectable metastases in regional lymph nodes), the standard treatment of adjuvant chemotherapy with 5-

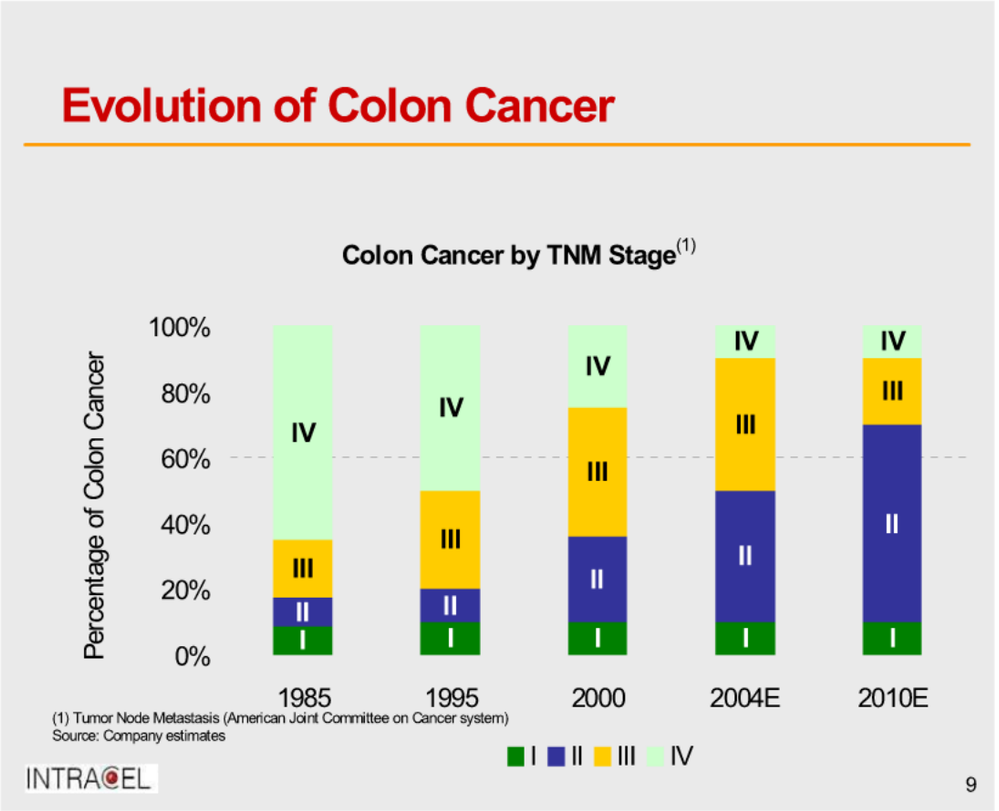
fluorouracil combined with levamisole or leucovorin has been linked to improved survival rates. [5,6,7,8] More recently the FOLFOX regimen (5-FU, leucovorin and oxaliplatin) has been approved for use in Stage III disease. For patients with Stage IV disease there are several essentially palliative treatments, many of which have been approved for use in recent years.

In the United States, mortality rates from colon cancer have declined over the past two decades, with a steeper decline in the most recent time period.[2] Earlier detection of primary tumors through the use of fecal occult blood tests, sigmoidoscopy and colonoscopy, and screening tests for serum carcinoembryonic antigen (CEA) levels have contributed to these reductions in mortality.[9] Finally, preventive measures, such as dietary modification, are still in an early developmental stage and will likely take decades to show benefits in clinical outcomes.

There are two important points to be emphasized looking forward. First, earlier detection of colon cancer has resulted in the redistribution of the TNM[10] (tumor, node, metastases) staging of colon cancers at first presentation. In less than a decade, there has been a major shift from Stage IV to Stage II colon cancer. For example, in 1995, Stage IV disease accounted for approximately 50 to 55% of all cases, Stage III accounted for 30%, and Stage II for less than 20%. For the year 2004, because of improved diagnostic methods, it is estimated that Stage IV cancers will account for approximately 10% of all cases, while Stage II disease will rise to 40% of all cases; Stage III cancer will remain at about 30% (Figure 1). This progression is expected to continue through the rest of the decade. Thus, the first point of emphasis is that colon cancer stages are transforming as a result of early detection, improved diagnosis, and more precise pathologic staging.

The second point of emphasis is that approximately 25% to 35% of the patients diagnosed with Stage II colon cancer, in spite of aggressive surgical resection, will recur with disseminated disease and adjuvant treatments with chemotherapeutic drugs have not shown significant prognostic benefits.[11,12]

Figure 1 Redistribution of the Incidence of Stages II Through IV Colon Cancer



Micrometastases or tumor cell seeding, while not detectable after surgery by conventional techniques, are generally responsible for disease recurrence and the eventual death of colon cancer patients. The histopathological detection of tumor in lymph nodes is, by definition, the hallmark diagnostic criterion for Stage III disease. Although not detectable by conventional pathological methods, micrometastases in regional lymph nodes in patients with Stage II cancer have now been detected by molecular techniques such as polymerase chain reaction (PCR). These occult micrometastases have been detected in one or more lymph nodes in 54% of Stage II patients. Analysis of the relationship between PCR-detectable metastases and survival has resulted in an adjusted 5-year survival (based on cancer-related deaths only) of 91% in patients without micrometastases and 50% in patients with micrometastases ($p=0.02$), with observed 5-year survival rates of 75% and 36%, respectively ($p=0.03$).[13] Hence, new methods of treatment to eliminate micrometastases in patients with Stage II colon cancer, and thereby delay or prevent recurrence, are particularly urgent given the increasing incidence of Stage II colon cancer.

2.2 Active Specific Immunotherapy (ASI)

The idea of controlling cancer by stimulating the immune system is not a new one. In the late 19th century, William Coley, a surgeon at the Memorial Sloan-Kettering Cancer Center, noted that rare events of tumor regression were preceded by infectious episodes.[14] Based on such observations, bacterial extracts were tested extensively in an attempt to stimulate tumor-specific immune responses. *Corynebacterium parvum* and Bacillus Calmette Guérin (BCG) have been tested extensively as immunostimulants against cancer.[15] It is now known that this approach to controlling cancer is active non-specific immunotherapy. BCG is registered as first line treatment for superficial bladder cancer.[16] In the past two decades, development and application of active specific immunotherapy (ASI) for the treatment of malignant diseases has been emphasized. It is anticipated that active immunotherapy may have advantages over passive immunotherapy with monoclonal antibodies or passive transfer of tumor reactive or engineered T-cells by inducing sustained immunity without significant toxicity.[17]

For Stage II and Stage III carcinoma of the colon, a new adjuvant therapy has been introduced as an investigational therapy: vaccination with autologous tumor cells, otherwise known as OncoVAX treatments. As opposed to prophylactic vaccinations to prevent disease such as polio, flu, or meningitis, autologous tumor vaccines are designed for the treatment of the disease with the intent to eliminate metastasis in a minimal residual disease condition, and thus, cure. The effects of treatment with tumor vaccines in cancer therapy differ from the manner in which chemotherapeutic drugs work. Drugs used for chemotherapy do not inherently distinguish between normal and cancerous cells, and have greater cytotoxicity to rapidly proliferating cells which results in immunosuppression and other debilitating sequelae. By contrast, the autologous tumor vaccine exerts its effects by eliciting an immune response directed exclusively against the tumor-associated antigens expressed by tumor cells. An additional advantage is that once the immunity against the tumor cells has been established, because of immunological memory, it may have a long duration. Not only are the remaining tumor cells under attack, but the same anti-tumor immunity process develops against tumor cells (and micrometastases) which may present themselves to the immune system long after the tumor vaccine treatment. In contrast, the therapeutic effects of chemotherapeutic drugs are of short duration and are, therefore, ineffectual against micrometastases that may present clinically after the cessation of treatment. One further advantage of an autologous vaccine is that it avoids the issue of introduction of adventitious agents.

The underlying basis for the OncoVAX autologous tumor cell vaccine is the assumption that there are distinct tumor antigens that are expressed by the patient's tumor cells that are either absent or in lower concentration than on normal cells. These tumor antigens may also be qualitatively and/or quantitatively different from those on tumors of other patients. OncoVAX therapy attempts to activate the host defenses against tumor-associated antigens by enhancing the immunogenicity of autologous tumor cells combined with the immunomodulating effects of BCG.

3. PHYSICAL, CHEMICAL, AND PHARMACEUTICAL PROPERTIES AND FORMULATION

3.1 Introduction

OncoVAX is being developed for the adjuvant treatment of Stage II and Stage III colon carcinoma after surgical resection. OncoVAX is a product prepared individually for each patient.

This product contains two distinct biological entities: viable, irradiated, autologous tumor cells and fresh-frozen, BCG bacteria. The first two vaccines contain 1.0 x 10^7 viable, metabolically active, non-tumorigenic, sterile tumor cells admixed with 1.0 x 10^7 colony forming units (CFU) of fresh-frozen BCG in a final volume of 0.2 to 0.4 ml sterile Hanks' Balanced Salt Solution (HBSS). The subsequent two vaccines are prepared similarly, but without the addition of BCG. The prepared patient dose is drawn into a 1.0 mL syringe labeled with appropriate patient information. The capped syringe is then packed in an insulated container and delivered to an appropriate site for administration of the vaccine to the patient. The vaccine must be administered within 4 hours of the thawing of the cells.

3.2 General Information on the Manufacturing Facility

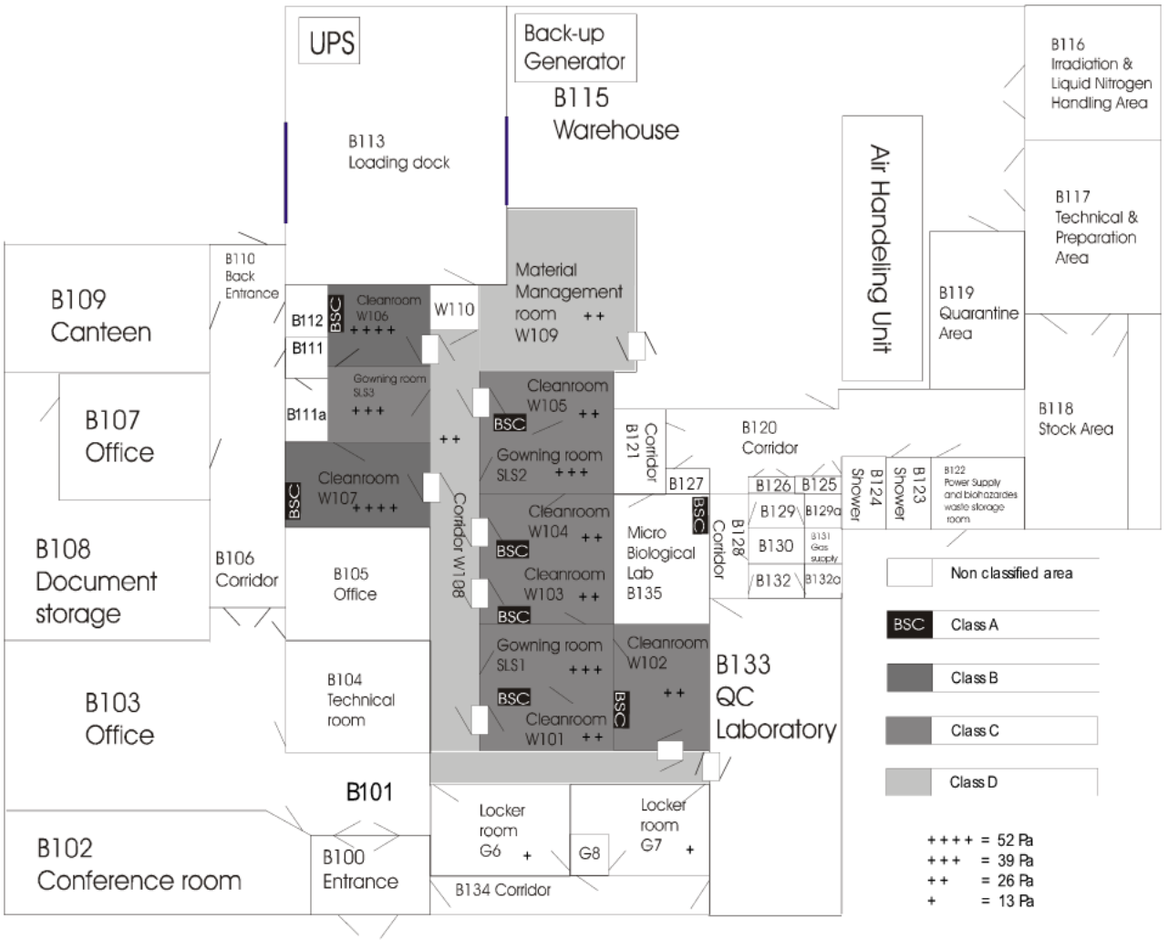
Vaccinogen B.V. in Emmen, The Netherlands, manufactures OncoVAX colon cancer vaccine. OncoVAX is an autologous whole cell vaccine comprised of sterile, viable, non-tumorigenic tumor cells. The source material for the manufacture of OncoVAX is the patient's tumor which is handled as a potential biohazard as is standard practice for human tissues.

3.2.1 Short Description of the Site

Vaccinogen B.V. is located in Emmen, The Netherlands. A floor plan of the facility is shown as Figure 2. Emmen is situated in the north-eastern part of the Netherlands, about 200 km from Amsterdam. Vaccinogen B.V. is located in an industrial area. The area of the manufacturing facility is approximately 900 square meters. The building exterior is constructed of concrete brick walls, with glass windows, concrete floors and a flat roof. The clean room interior is constructed of gypsum board covered with a polyurethane coating and a Mipolam floor covering. The ceiling is constructed of coated panels and sealed with a silicone caulking.

Vaccinogen B.V. operates under cGMPs in all applicable aspects of its operations. The Vaccinogen Quality System consists of Corporate Quality Policies and Procedures, approved by Senior Management. These policies and procedures detail the standards of conduct and compliance expected in the company. The Quality Systems departments consist of Quality Assurance and Quality Control.

Figure 2 Floor Plan for Vaccinogen B.V.



The activities and responsibilities of each department are listed below:

- Operations is responsible for the timely and reproducible manufacture of tumor vaccines which are sterile and meet all other release specifications.
- Quality Control is responsible for performing all tests on raw materials, intermediates and final product in a timely and consistent manner.
- Quality Assurance is responsible to oversee all functions to ensure that all procedures are performed in compliance with cGMPs. Additionally, Quality Assurance is responsible for the release of raw materials and formulated products, review of documentation, control of training programs, communication with document control about document changes, handling deviations reports, and auditing. The Quality Assurance Manager is

responsible for assuring GMP compliance, release of final product, review of batch documentation, participation in training programs, auditing (internal, external, vendor), and review of calibration, validation and maintenance documents/reports. Final release of product is reviewed and approved by the Qualified Person, if all specifications are met. Regular meetings and conference calls of key personnel guarantee that the concept of quality assurance is shared throughout the company.

Self-inspection is considered to be an important contributor to maintain and/or improve the quality standards. Self-inspections are performed on a regular basis and are supplemented by audits conducted by Corporate QA as well as by independent, third-party consultants.

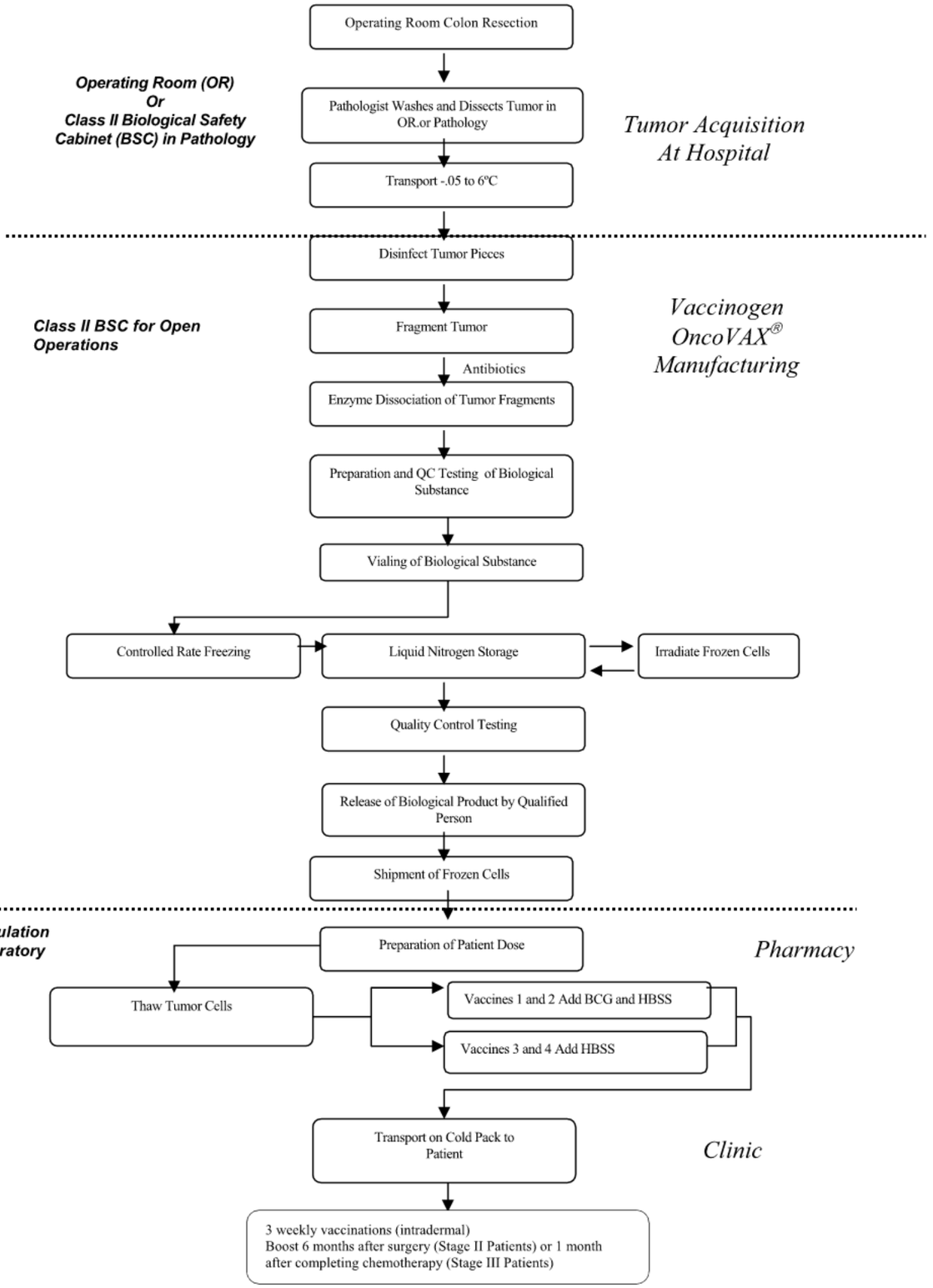
Batch release only takes place after the evaluation of the batch documentation and evaluation of the release of the raw materials and the intermediate products. Detailed batch release Standard Operating Procedures are available. The Qualified Person is responsible for the batch release. Prior to the actual release, the Manager of Operations or designee, the Quality Control Manager or designess, and the Quality Assurance Manager or designee review the batch records for correct documentation and compliance with current SOPs.

Vendor audits are conducted on priority basis. A vendor is audited starting from ISO-9001: 2000 and GMP standards, but a more strict system of vendor auditing is implemented. The recently updated ISO-9000 standard is the basis of the audit program. Product audits of vendors with a certified quality system are therefore considered adequate.

3.2.2 Description of the Production Operations

An overview of the OncoVAX manufacturing process is outlined in Figure 3. The tumor cells are derived from the patient's own solid tumor which has been surgically removed and processed to a single cell suspension which is then cryopreserved (frozen). The enzymatic dissociation of the tumor is performed in the presence of levofloxacin, amphotericin B, Primaxin (imipenem and cilastatin) and gentamicin sulfate to reduce endogenous bioburden inherent to colon-derived tumors. The frozen cells are rendered non-tumorigenic by irradiation. These manufacturing steps are described in greater detail below.

Figure 3 Overview of OncoVAX Manufacturing Process



3.2.2.1 Pre-Production

Pre-production involves the acquisition of the source material (tumors) for the manufacture of OncoVAX and, therefore, includes all of the handling of the tumor outside the production facility in Emmen. This consists of the surgical resection of the colon, the dissection and pathological processing of the tumor and the transport of the tumor to the manufacturing facility. Operating room and pathology personnel are trained in accordance with Vaccinogen protocols concerning collection and processing of the tumor. Training and ensuring compliance with the protocols are the responsibility of Vaccinogen B.V. After resection of the colon, the colon is placed in a sterile bag or basin. The resected colon may either be processed within the operating suite or within a controlled, aseptic environment in pathology. The resected colon is cut open, and washed in accordance with the standard operating procedure. The pathologist performs the dissection of the tumor after which the tumor is prepared for transport to the production facility in Emmen. For transport, the tumor is put in a tumor transport bottle containing Hanks' Balanced Salt Solution containing gentamicin (HBSS/G). The tumor transport bottle is packed in a transport box containing a temperature logger and cold packs to ensure maintenance of the specified transport temperature. The transport box containing the tumor and all applicable paperwork (tumor acquisition form) is labeled with a unique number which is used to track the material through all stages of manufacture, quality control testing and patient dose formulation.

3.2.2.2 Production

When the tumor arrives at the production facility, materials management receives the transport box, checks the tumor acquisition form and visually checks the transport box and tumor transport bottle. When conformance of the tumor to the required acceptance criteria has been verified, a patient ID (PID) number (first initial and first three letters of the patient's last name and birth date), in addition to the unique identifying number described above, is assigned to the tumor lot. This PID number is used as an additional identification safeguard prior to the administration of the vaccine. Current data support a 48-hour expiration time from surgical resection to onset of the manufacturing process. Depending on the time of arrival, processing will start immediately or at the first possible opportunity. In case of delayed processing, the tumor will be stored in a released tumor refrigerator maintained at 0 -6 °C.

During the production process, several acceptance criteria have to be met in order to continue processing. Minimum tumor weight will determine whether the tumor processing is initiated. Acceptance criteria for the biological substance include number and viability of the tumor cells, as well as identity and potency of the tumor cells.

A bag containing all necessary consumables, the tumor transport bottle, the manufacturing instruction and all controlled labels enter the clean room area via a pass through in W109. All tumor handling is performed under aseptic conditions in a biological safety cabinet (Class A/Class 100). Each biological safety cabinet and clean

room is dedicated to one single lot and is subjected to a line clearance and extensive cleaning before the next lot can be brought in for processing.

The tumor is transferred from the tumor transport bottle into a sterile container. The tumor is washed using HBSS/G and transferred to a sterile dish in which the extraneous tissue from the tumor is removed and discarded. The tumor is then transferred into a bottle containing a disinfectant. After this treatment, the disinfectant fluid is removed and the tumor is washed with HBSS/G.

The tumor is then trimmed into small pieces which are transferred into a stirred dissociation flask. Dissociation medium is then added. The dissociation medium consists of deoxyribonuclease (DNase) and collagenase. The flask is incubated at a temperature of 36 - 38°C for 35-45 minutes. After dissociation, the supernatant containing the cells is collected and then filtered into a centrifuge tube. The cells are centrifuged and the cell pellet is re-suspended in HBSS/G. Additional dissociation medium is added to the remaining tumor fragments and the dissociation procedure is repeated for a total of three dissociations. The cells from the three dissociations are pooled in one centrifuge tube and again centrifuged at 2 - 8°C. The cell pellet is re-suspended in a cryoprotective medium; the volume is based upon the number of viable tumor cells obtained and the desired cell density/vial. Up to a maximum of 17 vials are prepared for controlled rate freezing. During this freezing cycle, the cell suspension is frozen from +4°C to -90°C with a rate of decrease of 1°C/minute until a temperature of -40°C is reached. This procedure results in the successful cryopreservation of cell viability. The frozen tumor cells are then stored in quarantine under controlled conditions in the vapor phase of liquid nitrogen at a temperature ≤ -110°C.

3.2.2.3 Irradiation

Vials are taken from storage and are irradiated (200,000 rads) using a gamma radioactive source. The irradiation is performed by trained personnel. One vial will be used for quality control testing of the biological product. Eight of the vials comprise the four patient doses and are maintained in a separate quarantine area of the liquid nitrogen freezer until the successful completion of all release tests (appearance, tumor cell number and viability, purity, identity, potency, sterility and endotoxins). Upon release of the Biological Product by the Qualified Person, the patient doses, together with BCG for the first two vaccinations, and all applicable paperwork are shipped to the pharmacy or laboratory for formulation.

3.2.2.4 Post-Production

The first three vaccinations are administered, by the intradermal route, at weekly intervals, beginning 28 to 35 days after surgery. The first two injections are comprised of thawed irradiated tumor cells (1.0×10^7) admixed with BCG (1.0×10^7 CFU). The third injection of cells does not contain BCG. The fourth and final vaccination with irradiated tumor cells without BCG takes place 6 months after surgery (Stage II colon

cancer patients) or one month after the completion of chemotherapy (Stage III colon cancer patients).

For vaccine preparation, two vials of irradiated tumor cells and, if necessary, one vial of fresh frozen BCG are taken from storage and packed on dry ice. The vials are accompanied by a vaccine formulation procedure and sent to the pharmacy for compounding. The cells are thawed on a heat block set at 36 – 38 °C, washed twice with HBSS (without gentamicin) and the cell pellet is resuspended in a small volume. For the first two injections, BCG is added to the cell suspension. The dose is drawn into a 1cc syringe, packed on cold blocks and then transported to the physician or nurse for administration to the patient. The expiration of the formulated dose is four hours and begins with the thawing of cells.

Other than the training required for administering a proper intradermal injection described in section 6 of this Investigator's Brochure, there is no special equipment needed for vaccine administration. The treatment is administered on an "out-patient" basis.

3.2.4 Quality Control

The Quality Control (QC) department is responsible for the following quality control activities:

- Environmental monitoring of the manufacturing area at rest and during processing, incubating and counting of contaminants
- Analytical tests of intermediate and finished products
- Analytical tests of raw materials

Results of analytical and biological testing and release or rejection are documented in reports of analysis. Reports of analysis are authorized by the Quality Control Manager and are filed with the batch production record in case of products.

The laboratory documentation consists of Standard Operating Procedures (SOP) that describe general and specific laboratory processes such as Quality Instructions (QI/QCP/QCFM), Equipment Instructions (EI/EQP) and Standing Instructions (SI). There is also a SOP for Laboratory Investigations and Retesting of Out Of Specification results.

The OncoVAX QC Testing consists of the following assays:

- Tumor Cell Enumeration: microscopic test for the enumeration of viable tumor cells, non-viable tumor cells and viable non-tumor cells.

- Identity Assay: Fluorescence activated cell sorter (FACS) to determine the presence of adenocarcinoma cells using the tumor specific human monoclonal antibody 88BV59.
- Potency Assay: FACS to count tumor cells that are reactive to EpCAM and CEA, which are tumor associated antigens.
- Purity Assay: FACS to show ≥ 90% of live cells are tumor cells and/or lymphocytes in the biological product.
- Sterility: test to detect the presence of microbial and fungal contamination in the biological substance and biological product.
- Endotoxin Assay: test to determine the endotoxin level in the biological product.

3.2.5 Quality Assurance

Quality Assurance (QA) is responsible for the final review of the batch records and test report and for the release of the tested raw materials, intermediates and final products. The intermediate product (biological substance) undergoes QA review and internal release. The final product (biological product) release entails a review of all applicable batch records and test report for legibility, accuracy, completeness and any deviation from the approved process. Test results are compared to specifications to make sure they comply. The lot release of the final product is executed by the QA Manager (Qualified Person) by signing a Certificate of Analysis.

This certificate contains all relevant information on the biological product, assay results, lot number and irradiation date. As the information on the certificate is used for vaccine preparation at the pharmacy, information on dose is also given.

4. NONCLINICAL STUDIES

4.1 Introduction

The foundation of the preclinical (nonclinical) studies described in this document was the induction of tumor regression by intra-tumoral inoculation of BCG first published by the developers of the OncoVAX process in 1972. This experimental model, using the L-10 hepatocarcinoma of strain 2 inbred guinea pigs, evolved to the enzymatic dissociation of solid tumors for the preparation of tumor cell vaccines admixed with BCG. The therapeutic principles learned from these experiments were used for the development of procedures and regimens for the ASI of colon cancer as described in this document. The experimental results described below are taken from numerous peer reviewed articles and are referenced where appropriate.

The animal experiments, which served as the preclinical basis for the development of OncoVAX, were conducted and published over a 12-year period. It is, therefore, beyond the scope of this document to summarize the results of every published study. Instead, the salient features of the pivotal, most relevant, experiments will be described.

4.2 Non-Clinical Pharmacology

4.2.1 Antigenicity of Tumors as the Rationale for Immunotherapy

The specificity of the immune system, as opposed to generalized toxicity of standard chemotherapy or radiation therapy, makes ASI an attractive approach. The central tenet of this approach is that the tumor possesses immunologically recognizable molecules that are qualitatively or quantitatively different from those of normal cells and, following appropriate presentation, may be immunogenic in the host.

Tumor transplantation studies in animals have convincingly demonstrated the presence of tumor-associated transplantation antigens. Because some techniques cannot be used in humans for ethical reasons, various *in vitro* assays have been devised to study potential human antitumor immune responses. These studies involved mainly lymphocyte-tumor cell interactions and have been extensively reviewed.[18,19,20] The most important factor to influence cancer vaccine development was the recognition of the critical role that T-lymphocytes play in the anti-tumor response. *In vitro* studies have demonstrated that T-cells isolated from the tumor site can specifically lyse autologous tumor cells and can proliferate and secrete cytokines in response to tumor cells.

A second factor stimulating interest in cancer vaccine development was the recognition that tumor-associated antigens (TAA) can be shared by tumors from different patients and these antigens can be isolated, cloned, and manipulated. To enhance the immunogenicity of these antigens, they can be mixed with nonspecific immune adjuvants such as BCG. The ideal approach to the immunologic therapy of cancers, therefore, would be to surgically excise the primary tumor and use autologous tumor cells for inoculation. Residual micrometastases or tumors that later recurred would be recognized and then rejected by the immune system.

In addition to tumor-reactive effector cells found in cancer patients, the development of tumor-reactive antibodies in patients provides additional support for the existence of TAA in human tumors. Although the presence of antibodies is by no means universal, their presence has been demonstrated in sufficiently diverse populations of cancer patients to suggest that this is not an infrequent occurrence.[21,22]

Studies by Haspel et al.[23] have shown that humoral immunity to autologous tumor is significantly increased in patients treated by specific inoculation with their own tumor cells. Haspel and colleagues reported the development of stable clones of human B lymphocytes that produced tumor-reactive monoclonal antibodies.[23,24] These clones were derived from peripheral blood lymphocytes of colorectal cancer patients who were

specifically immunized against their own tumors.[25,26] Although this approach of characterizing human monoclonal antibodies from immunized patients is relevant, absolute proof of specific and functional cellular recognition of tumor-cell antigens will require biochemical isolation of such antigens and successful active specific inoculation with these unique determinants.

4.2.2 Development of Active Specific Immunotherapy Pre-Clinical Model

A major contribution toward understanding the principles of ASI was the development and characterization of an experimental model that is relevant for the study of the immunotherapy of established tumors. The L-10 hepatocarcinoma was induced in inbred strain 2 guinea pigs following ingestion of the carcinogen dimethylnitrosamine.[27] The antigenic and biological properties of the transplantable ascites tumors derived from the primary hepatocarcinoma have been described.[28,29,30]

When strain 2 guinea pigs are injected intradermally with L-10 tumor cells, solid tumors grow progressively and metastasize first to the regional draining nodes then to the viscera, killing the host in 60 to 90 days. This natural tumor progression following intradermal inoculation, together with its weak immunogenicity and compatibility with its syngeneic host, make the L-10 model system particularly relevant for the study of autologous, weakly immunogenic human tumors. When injected intravenously, the tumor localizes primarily in the lungs, metastasizing to the viscera, the lymphatics, and the liver, killing the guinea pig in 60 to 90 days. When injected intraperitoneally into weanlings, the cells grow as an ascites suspension, killing the weanlings in 10 to 14 days.

4.2.3 Intralesional BCG Therapy

Earlier studies, including this one, into autologous immunotherapy used a guinea pig L-10 hepatocarcinoma model. The model demonstrated that BCG admixed with syngeneic tumor cells can induce systemic immunity capable of eradicating a limited disseminated tumor burden. This occurs, however, only if the processed therapeutic material is carefully controlled for variables such as the number of tumor cells, the ratio of viable BCG organisms to tumor cells, viability of the tumor cells, and treatment regimen.

For this study, seven days after intradermal inoculation of 10^6 L-10 cells, tumor cell infiltration of the first regional lymph node was detectable. The guinea pigs typically died from advanced metastatic cancer 60 to 90 days after inoculation. Despite excision of the intradermal tumor seven days after inoculation, the tumors progressed. By contrast, when the intradermal tumors were treated seven days post-inoculation with 2.4 $x10^7$ BCG, regression of the primary tumor and lymph node metastases resulted. Histopathological and ultrastructural studies suggested that cells of the macrophage-histiocyte compartment played a primary role in BCG-mediated L-10 tumor regression.[31,32,33,34,35]

Guinea pigs, previously treated with intralesional BCG, were challenged with L-10 tumor cells (10^6) injected contralateral to the original tumor. When injected one week after

intralesional treatment, little or no effect was observed on the growth of the contralateral tumor. By contrast, when the tumor challenge occurred two weeks after intralesional treatment, marked inhibition of tumor growth was observed. When the challenge was delayed for six weeks, contralateral tumors were not detected. These results indicate that the inoculation of BCG into an intradermal tumor results in a potent systemic effect.[36,37] This seminal observation served as the foundation for future studies of disseminated metastatic cancer.

Another important lesson, learned from these early studies, for the development of a clinically useful treatment was the significance of systemic tumor burden. The remission of an intradermal tumor induced by intra-tumoral BCG could be abrogated by the intravenous inoculation of an additional load of L-10 tumor cells. This phenomenon occurred when the additional tumor cells were injected six days after intradermal inoculation; this was one day prior to the intratumoral inoculation of BCG.[38] Similarly, the ability of previously treated guinea pigs to reject a contralateral challenge of tumor cells was diminished when this regimen of intravenous inoculation of tumor cells was followed.

The remaining studies outlined in this section deal with the biological response and the quantitative studies leading to the dose regimen using the tumor cells and BCG vaccine.

4.2.4 Intradermal Inoculation of L-10/BCG Vaccine (ASI)

The pathological changes that occur following primary and secondary intradermal inoculation (10^8 BCG combined with 10^7 L-10 tumor cells) were investigated in guinea pigs.[39,40] One day after primary inoculation, loose aggregates of tumor cells consisting of a few to several hundred, were distributed throughout the papillary and reticular region of the dermis. There was an extensive cellular infiltrate consisting mainly of polymorphonuclear cells (PMN), some monocytes, and occasional lymphocytes. At the edematous border of the reticular region of the dermis, there were many tumor cell ghosts or damaged tumor cells. PMN were still predominant by day three, but monocytes and lymphocytes became more numerous in the cellular infiltrate. At day four, a major architectural change occurred in the inoculation site. There was a transformation from a loosely organized tumor cell plus inflammatory cell infiltrate to a denser matrix in the dermis. At the cellular level, the majority of PMN cells contained BCG organisms within phagocytic vacuoles. At that time, L-10 cells interspersed with cellular infiltrate were highly vacuolated, and while in many cells intact membranes were common, the cytoplasm generally appeared to be degenerating and disorganized. By day seven, the inoculation site was devoid of tumor cells and consisted of a relatively solid fibrotic matrix interspersed with PMN, monocytes, and lymphocytes. The matrix was composed mainly of tissue-derived histiocytes which appeared to form syncytia. At this time, there was also an inflammatory response seen in the regional lymph nodes. Twice the number of lymphocytes and macrophages were seen in the lymph nodes in animals that received combined tumor/BCG vaccines compared with lymph nodes from control animals or animals injected intradermally with BCG or L-10 cells alone. During the second to fourth weeks after inoculation, the histologic changes at the vaccination site can best be

characterized as an epithelioid granulomatous response with a high level of nonspecific esterase activity detectable. Focal necrosis and giant inflammatory cells were seen.

Following the second inoculation, the composition of the cellular infiltrate in the second site was comparable to that of the primary vaccination site. However, the secondary vaccination site showed a more rapid evolution of the granulomatous response and an earlier development of focal necrosis.

This inflammatory response to the vaccine is the consequence of synergy between the tumor cells and BCG. When irradiated tumor cells alone are injected intradermally, little inflammation is observed. A similar inoculation of BCG results in leukocytic and monocytic infiltration. However, the inflammatory response to an inoculation of L-10 cells admixed with BCG was considerably greater than the response to BCG administered alone.[40] The increase in infiltration was synergistic rather than being additive. This synergistic effect was observed for the leukocyte (PMN), macrophage, and lymphocyte components of the inflammatory reaction. The degree of the cellular infiltration in the draining lymph nodes mirrored the enhanced response at the vaccination site. There are two lines of evidence that admixing the BCG with the tumor cells is preferable. First, when the cells and BCG were injected separately into adjacent sites that drained to the same lymph node, less infiltration in the draining lymph nodes was observed. Second, when injected together, the dermal vaccination site could be surgically excised 24 hours after inoculation without adversely affecting the degree of immunity. By contrast, if the cells and BCG were injected separately into adjacent sites, surgical excision prior to 96 hours after vaccination reduced the systemic effects (DCH and protection). Thus, the two components of the vaccine have to be admixed. As previously discussed, low viability tumor cells, when injected without BCG, do not persist at the inoculation site and as a consequence also failed to confer protection. Little or no synergy between L-10 cells and BCG in evoking an enhanced inflammatory response was observed when low viability cells were injected.

Thus, preclinical studies in a guinea pig model have shown the need for both BCG and viable (metabolically active but not tumorigenic) L-10 tumor cells for OncoVAX. This model established that a vaccine regimen of 10^7 viable, irradiated tumor cells admixed with 10^7 BCG organisms given at least twice, one week apart, was the minimum effective dose. Vaccination with irradiated tumor cells or BCG alone was ineffective. Tumor immunity was tumor-specific, as vaccination with an immunologically non-cross reactive syngeneic guinea pig hepatocarcinoma resulted in no protection against a challenge with L-10 tumor cells. Thus, the results of this sequential series of preclinical studies provided the basis for the dose and schedule of autologous tumor cells for the first studies of ASI in humans.

4.2.5 Development of a Model, Using Ascites-Derived L-10 Tumor Cells, for the Development of a Vaccine Treatment of Metastatic Disease: Dose Justification

Most tumors, with the exception of superficial melanoma and bladder cancer, are not accessible to the direct, intra-tumoral, administration of BCG used in the L-10 model of ASI. The analog of the direct administration of BCG into an intradermal tumor is the intradermal inoculation of tumor cells admixed with BCG. It was this experimental approach that served as the basis for the next series of process-development studies. The L-10 tumor cells were derived from ascites and were either freshly isolated or used after cryopreservation.

The critical elements investigated in the early studies[41,42] included the preparation of the tumor cells, dosage of tumor cells and BCG, and treatment regimen. The basic paradigm of the experiments was the establishment of distant metastases by intravenous inoculation of L-10 tumor cells. Intravenous inoculation of 10^4 L-10 tumor cells resulted in death in approximately 75% of the animals. Higher doses (10^5 and 10^6) of tumor cells consistently resulted in the death of all of the animals. Initially, the dose of 10^4 tumor cells was selected. In order to render the L-10 tumor cells non-tumorigenic, the cells were irradiated. Based on preliminary data, a dose of 20,000 rads was selected. This radiation dose was used for all preclinical studies. Several variants of immunotherapy treatment were evaluated, beginning either one or four days after the intravenous inoculation of (10^4) L-10 tumor cells. When two treatments were administered, the inoculations were given seven days apart.

Two inoculations of BCG (10^8) or two inoculations of irradiated L-10 cells (10^7) when administered alone were ineffectual in preventing death. A *single* inoculation of irradiated L-10 cells (10^7) admixed with BCG (10^6 or 10^8) was also ineffectual. However, an inoculation of irradiated L-10 cells (10^7) admixed with BCG (10^6 or 10^8), followed seven days later with a second inoculation of irradiated L-10 cells (either contralateral or into the inoculation site) or irradiated L-10 cells admixed with BCG, resulted in protection. Differences in the regimen became more evident when the intravenous tumor burden was increased from 10^4 to 10^5 or 10^6 L-10 cells. When guinea pigs were injected intravenously with 10^5 tumor cells, protection was obtained when the first vaccine inoculation had the higher concentration of BCG (10^8), and equivalent protection was obtained whether the second vaccine of irradiated L-10 cells contained BCG (80% survival) or not (90% survival). When the intravenous tumor burden was increased still further (10^6 cells), the greatest protection (60%) was observed when two inoculations of irradiated L-10 cells admixed with BCG (10^8) were administered. Only 30% of the animals survived when the second vaccine was composed of tumor cells alone. Thus, the minimal vaccination strategy for the maximum level of tumor burden in these studies would consist of two inoculations, given seven days apart, of irradiated L-10 cells (10^7) admixed with BCG (10^8). The effectiveness of this regimen was equivalent when the onset of the immunotherapy treatment was delayed from one to four days after intravenous inoculation of tumor cells.

An additional series of studies demonstrated that the active immunotherapy was cell/tumor line-specific. Another hepatocarcinoma line that is also syngeneic to the strain 2 guinea pigs, the L-1 tumor, is weakly cross-reactive with the antigens found in the L-10 tumor. When a vaccine comprising L-1 tumor cells admixed with BCG (10^8) was administered as a vaccine, the tumor cells were completely ineffective in preventing death due to intravenously administered L-10 tumor cells.

As the tumor cell vaccines are ineffective without the addition of BCG, parameters such as source and dosage of BCG are critical to achieving maximal, and consistent, therapeutic effects. The challenge of achieving effective immunotherapy is magnified by variations in the source, composition, and potency of various adjuvants (BCG).[43] The efficacy of different sources of BCG was, therefore, investigated.[44] No significant differences in efficacy were observed among three different sources of BCG.

Intradermal inoculation of BCG produces local side effects of inflammation and necrosis, in a dose-dependent manner, which can be severe at higher doses. A balance must, therefore, be achieved between maximal efficacy and minimal morbidity. Low doses of BCG (10^5 to 10^6) induce little or no protection. BCG induced significant levels of protection when administered at a higher dose (10^7 to 10^8). Equivalent protection was observed in animals treated with 10^7 as compared with 10^8 CFU of BCG. Less localized morbidity was observed following the administration of 10^7 CFU as compared to the higher dose. Therefore, 10^7 CFU was selected as the appropriate BCG dose for clinical trials. The tumor cell component of the vaccine was kept constant at 10^7, based on a determination that it would be difficult, if not impossible, to recover enough viable tumor cells from primary human tumors to administer multiple vaccinations with more than 10^7 tumor cells.

4.2.6 Treatment of BCG-Associated Toxicity

The intradermal administration of BCG and tumor cells in the animals results in ulceration and eschar formation at the inoculation site that can lead to secondary microbial invasion and regional lymphadenopathy. Isoniazid is the hydrazide of isonicotinic acid, which acts against actively growing tubercle bacilli and is commonly administered alone or in combination with other agents to patients for BCG-related complications. When added to the drinking water of the animals, isoniazid at a concentration of 25 μg/g of body weight markedly limited the severity of the necrosis of the derma without abrogating the efficacy of the L-10/BCG vaccine.[45,46]

4.3 Pharmacokinetics and Metabolism in Animals

The purpose of the pharmacokinetics testing for a biological product such as OncoVAX is to define, in a relevant animal species, the persistence and trafficking of the irradiated autologous cells and mycobacteria. OncoVAX is injected by the intradermal route, so therefore, the major PK studies in guinea pigs consisted of an evaluation of the distribution, persistence, metabolism, and excretion of the product, beginning in the

dermis. Studies of irradiated tumor cells alone, BCG alone, and irradiated tumor cells + BCG were performed first, followed by studies of nonirradiated tumor cells alone and in combination with BCG, for comparison by intradermal (ID) and intravenous (IV) routes. The pharmacokinetics of intralesional BCG into an established ID tumor and the effects of contralateral challenge on the immune response were also assessed.

Because OncoVAX is injected intradermally, histologic studies of the skin site, cellular infiltrate, and regional lymph nodes were necessary to understand the behavior of the product within the biological matrix of the dermis. Although it was already suspected that viable cells were necessary for effective immunizations, it was important to determine the fate of BCG, the lifespan of irradiated tumor cells in the skin, the types of effector cells that were recruited into the area, and their relative numbers and relationship to the initiation and completion of the cell-mediated response. Therefore, the pharmacokinetic findings are essentially the histologic evolution of the DCH response in the dermis and lymph nodes.

The experiments with irradiated autologous tumor cells + BCG injected twice at different sites as a vaccine after an L-10 cell challenge document the fate of the cells and mycobacteria in the skin until and after such time that they are no longer visible by histochemical methods. The irradiated tumor cells persisted at the skin site for approximately 1 week before undergoing apoptosis. The BCG organisms were phagocytized and were never observed after day 7. Irradiated tumor cells and mycobacteria were never observed in the draining lymph nodes at any time point.

In guinea pigs who received three ID immunizations of irradiated autologous tumor cells at a dose of 10^7 cells, with 10^7 BCG included in two of the vaccines, pulmonary tumor nodule size was influenced by the schedule of the vaccines. Intact tumor nodules were detected at 1 week after the first immunization in all necropsied animals that had received 10^6 L-10 cells on day 0. Percent survival of guinea pigs was directly related to the proximity of first vaccine and L-10 cell challenge. Guinea pigs immunized on days 1, 7, and 14 achieved the best survival protection (70%; <5 tumor cells on day 1), followed by days 4, 10, and 17 vaccination schedule (65%; 5-10 tumor cells on day 4)). In contrast, when the first vaccination was delivered on day 10, survival of guinea pigs was reduced to 20% (0.35 to 0.5 mm tumor nodule on days 21 to 24).

This study shows that the timing of vaccinations is critical in guinea pigs with a pre-existing tumor burden. Early immunization relative to the tumor challenge leads to fewer metastases and a better outcome.

In contrast, the pharmacokinetics of 10^6 intradermally inoculated non-irradiated tumor cells without subsequent vaccinations demonstrates that the tumor cells migrate from the ID site to the superficial distal axillary (SDA) nodes sometime after day 4 and by day 7. Tumors grow progressively, metastasize, and eventually kill the guinea pigs within 60-90 days.

4.4 Toxicology

4.4.1 Repeat Dose Toxicity Study

A repeat dose toxicity study in guinea pigs was performed using syngeneic L-10 hepatocarcinoma cells as the autologous cell component in combination with BCG as the adjuvant. The purpose of this study was to reproduce parameters of the human vaccine as closely as possible in the guinea pig with respect to the dose, schedule, components and excipients of the vaccine, and manufacturing process to demonstrate safety of the human clinical product, OncoVAX.

4.4.1.1 Materials and Methods

The age of guinea pigs in this study was 9 weeks or older. Equal numbers of male and female animals were entered into the study.

This toxicology study utilized solid tumors (hepatocarcinoma) from guinea pigs that required dissociation, like human colon tumors. These solid tumors gave similar survival protection results compared to ascites-derived tumor cells. Tumor cells derived from both the original non-sterile manufacturing process and newly revised sterile manufacturing processes were evaluated to show that no changes with respect to safety could be detected. The results of the toxicology studies were indistinguishable between the two manufacturing processes.

The treatment groups received either three or four vaccines, all by the intradermal route. The first two vaccines contained BCG (10^7 CFU) + autologous cells (10^7, 3 x 10^7, or 10^8); the third and fourth vaccines contained only autologous cells, comparable to the clinical treatment regimen. The first three vaccines were administered weekly, exactly mimicking the human schedule, and the fourth was given 4 weeks after the third vaccination, sooner than the fourth vaccine in humans (approximately 5 months after the first vaccination). There was also an interim necropsy group that did not receive the fourth injection.

The interim groups were necropsied one week after the third vaccination (day 21). The terminal necropsy groups were sacrificed 4 weeks after the fourth dose (day 70). Although the fourth vaccination was given 3 ½ months earlier than in the clinical regimen, the desired cell-mediated immune response to the test antigen in guinea pigs does not require four vaccinations, but does appear to require at least two vaccines of 10^7 autologous L-10 cells + 10^7 BCG for optimal activity.

4.4.1.2 Results

Although there were several significant differences in body weight among the groups, most were due to age-related differences or did not appear to be treatment-related. Almost all animals exhibited laboratory values within normal reference ranges. Those

values that fell outside the normal range or when elevated for both control and treatment groups were not considered to be test related.

The primary pathology was local skin toxicity. The reason for the differences in local toxicity at the interim and terminal sacrifices was associated with progression and healing of the DCH reaction, mainly at the sites that received BCG. By 21 days (sacrifice of the interim groups), the reaction had evolved from a histiocytosis to an epithelioid granuloma, which is characterized by local ulceration. By 70 days, the lesion had almost or completely healed, leaving scar tissue at the site. The most severe lesions occurred in interim necropsy animals receiving BCG and autologous tumor cells, regardless of the multiplicity of the dose (1X, 3X or 10X) of tumor cells. In this study, there was evidence of an additive effect of the inflammatory responses of the admixed product as compared with BCG or tumor cells injected alone. This phenomenon was observed during developmental preclinical research and appears to be idiosyncratic to L-10 tumor cells; other tumor cells did not exhibit an additive effect to the inflammatory response to the BCG component. It was postulated that there may be cross-reactive or shared antigens between L-10 cells and BCG.[40] These effects were also found irrespective of the manufacturing process used to prepare the inoculum. Pathology noted in other tissues was considered incidental or secondary to the inoculation site pathology. No malignancy was found in any necropsied animal.

The BCG component is clearly responsible for the majority of the local dermal toxicities. Induration is an expected and desirable sequel to intradermal or percutaneous inoculation of BCG.

4.4.2 Other Toxicology Testing

Genotoxicity, carcinogenicity, reproductive, and developmental toxicity studies were not performed. OncoVAX is composed of irradiated autologous cells and BCG. The irradiated autologous cells become necrotic and die within a week of administration into the dermis. The BCG organisms are phagocytized and inactivated within one week of injection. While it is not believed that the components of the vaccine would produce reproductive or developmental toxicities, prerequisites for treatment with OncoVAX mandate that all premenopausal women have a negative pregnancy test result before initiation of treatment and must utilize an effective method of contraception during the treatment period.

A tumorigenicity study was performed in nude mice that showed that 20,000 rads of irradiation left tumor cells metabolically active but unable to replicate. The current manufacturing process involves irradiation at a 10-fold higher dose (200,000 rads) providing an even greater level of assurance that the product is non-tumorigenic.

The main concern with a product irradiated at the higher dose was whether it would retain its immunogenicity and efficacy in guinea pigs and humans. An efficacy/protection study was performed in the seminal L-10 -strain 2 guinea pig model. No differences in efficacy were observed between vaccines manufactured by the two procedures.

5. EFFECTS IN HUMANS

5.1 Introduction

Over the last 20 years, investigators have translated the principles and procedures of ASI as observed in the L10 model into phase I, II, and III adjuvant therapy clinical trials in patients with colon and rectal cancer. These clinical trials were designed based on the evidence in the animal models that the immune system can be manipulated to control a limited systemic tumor burden remaining after surgical excision of solid tumors. Since the biologic essence of the therapeutic process is the presence in the vaccine of a minimum number of viable, metabolically active, autologous tumor cells, the processing procedures of the product is the limiting factor.

Clinical testing of OncoVAX as an adjuvant to surgical resection began in 1980 with a single center pilot study of 5 subjects with colon cancer. To date 757 subjects with colorectal cancer, of which 720 had colon cancer, have been enrolled in trials of OncoVAX (see Table 1 for summary). In addition, a bioequivalency study enrolled 15 patients with colon cancer. This study, discussed in section 5.2.4, was conducted to compare the immunogenicity, as determined by the magnitude of dermal cutaneous hypersensitivity (DCH) responses to tumor cells alone, of vaccines manufactured by the current,, sterile process with historical data from the phase III clinical study (8701). The results from this study unequivocally support the premise that the immunogenicity of vaccines produced by either process was comparable.

In these trials, excluding the bioequivalency study, 385 subjects were randomized to receive OncoVAX, of which 353 received at least one vaccination; 372 subjects were treated with surgery alone. In addition, subjects with colorectal cancer were enrolled in three separate trials that assessed the effects of ASI with OncoVAX in combination with chemotherapy as adjuvant therapy to surgical resection. Lastly, two trials have been performed using autologous tumor cells/BCG in melanoma (n=86) and renal cell carcinoma (n=14).

Table 1 OncoVAX Clinical Trials for Colon Cancer

Protocol Number	Trial Phase	Number of Patients	Number of Vaccine Doses	Disease and Stage	Manufacturing*
8101	I	5 Treated	3	Colon Cancer Stage III/IV	Centralized
8102	II/III	47 Control 50 Treated	3	Colorectal Cancer Stage I-IV	Centralized
5283	III	207 Control 205 Treated	3	Colon Cancer Stage II/III	Decentralized
8701	III	118 Control 125 Treated	4	Colon Cancer Stage I-III	Centralized
ASI-2002-01	I/II	15 Treated	4	Colon Cancer Stage II/III	Centralized

* Centralized = Manufacture at a single, centralized location; Decentralized = manufacture at each investigational site.

This summary will focus on the three randomized multi-center trials of surgery vs. surgery and OncoVAX: Study 8102[47], Study 5283[48], and Study 8701[49]. Study 8102 used a three vaccine regimen as did 5283. In Study 8102, manufacture was conducted at the institution of the principal investigator, Dr. Herbert Hoover, although this institution changed twice during the study, while in 5283, manufacture was decentralized. Neither of these studies demonstrated statistically significant outcomes. In Study 8102, a subset of subjects (discussed in section 5.2.1.1) underwent DCH testing that revealed a deterioration of the immune response at 6 months. It was believed that a fourth booster immunization at 6 months after surgical resection would enhance the waning immune response to autologous tumor cells. Hence a four vaccine regimen was tested and manufacturing was centralized at the Free University of Amsterdam (Study 8701). In this study, subjects with Stage II disease had both clinically meaningful and statistically significant outcomes in both recurrence-free interval (p=0.008) and disease-free survival (p=0.015). The 5-year event-free rates also demonstrated a clinically and statistically meaningful outcome in overall survival.

5.1.1 Tumor-Associated Antigens (TAA)

One important objective of the OncoVAX studies was to determine if OncoVAX-treated patients were able to mount an immune response to TAA present in their autologous vaccines. The studies also attempted to determine whether there were common TAA present in the tumors of different patients and, if so, whether these were antigens capable of stimulating a T cell response. The lymphocyte-tumor cell interactions have been extensively reviewed.[18,19,20] The most important factor to influence cancer vaccine development was the recognition of the critical role that T lymphocytes play in the anti-tumor response. T cells that accumulate at the tumor site can specifically lyse autologous tumor cells *in vitro* and proliferate and secrete cytokines in response to stimulation by tumor cells. A second factor to influence cancer vaccine development was the recognition that TAA can be shared by tumors from different patients and these antigens can be isolated, cloned, and manipulated. If present, these TAA, once isolated and characterized, could be used in the development of a generic vaccine. The generic vaccine could be used: to supplement treatment of patients for whom there were insufficient viable tumor cells to produce an optimal autologous (live) tumor cell vaccine; to boost patients that have already received the autologous OncoVAX vaccine in order to extend the period of remission; or to eventually replace the autologous vaccine for all treatments. The antigens can be mixed with nonspecific immune adjuvants, such as BCG, to enhance their immunogenicity. TAA have been evaluated in clinical trials and will be reviewed below.

Currently, however, the ideal approach to the immunologic therapy of cancers is surgical resection of the primary lesion and administration of autologous tumor cells for inoculation. The autologous tumor cells include all of the patient's relevant antigens.

5.1.2 Characterization of TAA Recognized by Human Monoclonal Antibodies

Using peripheral blood lymphocytes from patients immunized with autologous tumor cells during clinical studies of OncoVAX, a panel of human monoclonal antibodies (MAb) reactive with colon carcinoma tissues and colon carcinoma cell lines was generated. Some of these antibodies have been radiolabeled and used in the clinic for the radioimmunodiagnosis and radioimmunotherapy of cancer.[50,51] Since the antibodies were derived from patients immunized with their own tumors, they are valuable tools for identifying TAA that should be more relevant than antigens identified by antibodies obtained from the inoculation of animals such as mice.

Eleven TAA were identified by MAb generated from peripheral blood lymphocytes from patients undergoing OncoVAX treatment for colorectal cancer.

Using peripheral blood lymphocytes from tumor-immunized individuals, it has been possible to evaluate the relevance of these antigens to cellular immunity. Lymphoproliferative assays were performed using lymphocytes obtained from patients prior to and after inoculation with autologous tumor cells. Three of the antigens (CTAA 28A32-32K, CTA #6 and CTA #8) induced T cell proliferation in samples obtained after completion of inoculation. These antigens induced proliferation in 21% (CTA #6) to 47% (CTAA 28A32-32K) of the patient samples. CTAA 28A32-32K did not induce responses in lymphocytes obtained prior to inoculation. There is evidence that CTA #6 and CTA #8 are complementary to CTAA 28A32-32K. Furthermore, CTAA 28A32-32K was able to induce DCH responses in immunized patients. These antigens are candidates for further evaluation for generic vaccine development.

5.2 Safety and Efficacy

5.2.1 Study 8102

A Randomized, Open-Label, Controlled Clinical Trial Comparing Surgical Resection Alone with Surgical Resection followed by Active Specific Immunotherapy with OncoVAX in Subjects with Colon or Rectal Adenocarcinoma.

In this phase II/III multicenter trial, subjects with Stages II, III and IV disease were randomized, after surgical resection, to either observation (n=47) or to OncoVAX (n=50). Subjects assigned to the OncoVAX group received three intradermal (ID) injections with the vaccine. The first inoculation was administered 28 – 35 days after surgery. First and second doses contained 10^7 irradiated tumor cells and 10^7 BCG, while the third contained only autologous tumor cells.

Subjects with colon cancer and rectal cancer were in separate but parallel studies that were identical except that postoperative radiation therapy was performed starting several days after the third inoculation in subjects with rectal cancer. The compositions of the randomized groups are shown in Table 2. However, one subject with colon cancer and

one with rectal cancer were assigned to the control group but were given three vaccinations by mistake. This was dealt with by including them in the OncoVAX group in the "as-treated" analysis.

Table 2 Study 8102 - Composition of Randomized Groups

Treatment Group	All Patients	Colon	Rectal
Control	47	28	19
OncoVAX	50	32	18

The randomization of subjects was stratified according to disease location and disease stage. Baseline characteristics of the randomized groups were similar with respect to gender, age, performance status and stage of disease.

Subjects enrolled in the study were monitored at regular intervals for signs of tumor recurrence and were followed until death or termination of the study (6.5-year median follow-up). The primary efficacy variables were disease-free survival and overall survival.

Table 3 Study 8102 - Colon Cancer Subjects, Intent-to-treat Analysis: Overall Survival

TNM Stage	Crude Death Rates		Log Rank Analysis	
	Control n/N (%)	OncoVAX n/N (%)	P-Value	HR (95% CI)
All*	14/28 (50.0%)	8/32 (25.0%)	0.17	0.53 (0.21, 1.33)
I	0/1 (0%)	0/1 (0%)	-	-
II	4/14 (28.6%)	2/15 (13.3%)	0.49	0.55 (0.10, 3.06)
III	7/10 (70.0%)	4/14 (28.6%)	0.07	0.33 (0.09, 1.15)
IV	3/3 (100%)	2/2 (100%)	-	-

* Stratified by TNM stage

Table 4 Study 8102 - Colon Cancer Subjects, Intent-to-treat Analysis: Disease-Free Survival

TNM Stage	Crude Death and Event Rates		Log Rank Analysis	
	Control n/N (%)	OncoVAX n/N (%)	P-Value	HR (95% CI)
All**	14/26* (53.9%)	12/32 (37.5%)	0.14	0.54 (0.23, 1.25)
I	0/1 (0%)	1/1 (100%)	-	-
II	6/14 (42.9%)	4/15 (26.7%)	0.39	0.58 (0.16, 2.05)
III	7/10 (70.0%)	5/14 (35.7%)	0.23	0.47 (0.14, 1.64)
IV	1/1* (100%)	2/2 (100%)	-	-

*Two patients (83 and 93) were deleted because the date of disease recurrence was recorded as being on or before the date of tumor resection
**Stratified by TNM stage

In an analysis of the intent-to-treat population (colon and rectal combined), the risk of recurrence or death was not reduced by vaccination with OncoVAX. As shown in Table 3 and Table 4, while the results did not reach statistical significance, the overall percentage of subjects with colon cancer who died or had a recurrence was lower in the OncoVAX treated group than in the control group. One theory as to why this difference was not seen in subjects with rectal cancer is that the pelvic irradiation may have killed effector cells in the inguinal draining lymph nodes.

Forty-four subjects died during the study: 25 (53.2%) in the control group and 19 (38.0%) in the OncoVAX group. No death was considered related to OncoVAX treatment. Disease progression was the primary cause of death in each group. Nineteen subjects (40.4%) in the control group died of metastases or regional cancer vs. 14 (30%) in the OncoVAX treated group.

Among the colon cancer patients, 22 patients died during the study, 14 (50%) in the control group and 8 (25%) in the OncoVAX group. This improvement in overall survival was not statistically significant. As determined by DCH reactions to irradiated autologous tumor cells, it was demonstrated that immunized patients showed a significant increase in DCH response to irradiated autologous tumor cells as compared to autologous mucosa as a control. The response was of short duration. The response waned by six months after immunization.[52] These results support expanded Phase III trials in colon cancer patients and a booster immunization at 6 months after OncoVAX treatment.

Two subjects experienced a non-fatal serious adverse event. While both were treated with OncoVAX, these were not deemed by the investigator to be related to treatment. A 55 year old woman developed aphasia and hemiparesis as a result of a cerebral infarct. The outcome is unknown. A 66 year old man developed abdominal pain and vomiting, classified as serious, which resolved.

OncoVAX was generally well tolerated (see Table 5). Injection-related reactions were the most common treatment-related events. All subjects experienced local induration to vaccinations and most developed ulceration to the first two inoculations, containing BCG. Approximately 60% of subjects developed palpable bilateral inguinal lymphadenopathy. Transient low grade fever and chills were also commonly experienced within the first 2 days following vaccines containing BCG.

Table 5 Study 8102 - Number and Percentage of Patients with an Adverse Event by Preferred Term

Adverse Event /Preferred Term	OncoVAX		Control	
Total number of patients	**N = 50**		**N = 47**	
Patients with at least one event	**N = 19**		**N = 14**	
Pyrexia	5	10.0 %	2	4.3%
Diarrhea	4	8.0%	2	4.3%
Fatigue	4	8.0%	2	4.3%
Dizziness	2	4.0%	0	0.0%
Malaise	2	4.0%	0	0.0%
Nausea	2	4.0%	0	0.0%
Rigors	2	4.0%	0	0.0%

This study showed the importance of giving a "booster" dose of the autologous tumor cells at 6 months after the third inoculation. The presence of a significant DCH response to tumor cells after vaccination with an autologous/BCG vaccine is a measure of T-cell mediated immunogenicity of the vaccine and has been correlated with survival. This phenomenon may reflect the adequacy of the vaccination (cell number and viability; intradermal placement of the inoculum), or some factor relating to the capacity of the patients to be immunized by their autologous vaccines, or some combination of the two. In this study, a time-course of DCH skin testing was performed on all treated and control patients with 10^6 irradiated autologous tumor cells, and 10^6 irradiated autologous normal colon mucosal cells. First of all, significant differences were seen between DCH reaction to tumor and mucosal cells at 6 weeks and 6 months after immunization. The treated patients also had significant elevations in reaction to tumor cells post vaccination when compared to unimmunized controls. Secondly, the noted decline in DCH reactivity at 6 months lead to the incorporation of booster immunizations at 6 months in all future clinical studies of ASI.

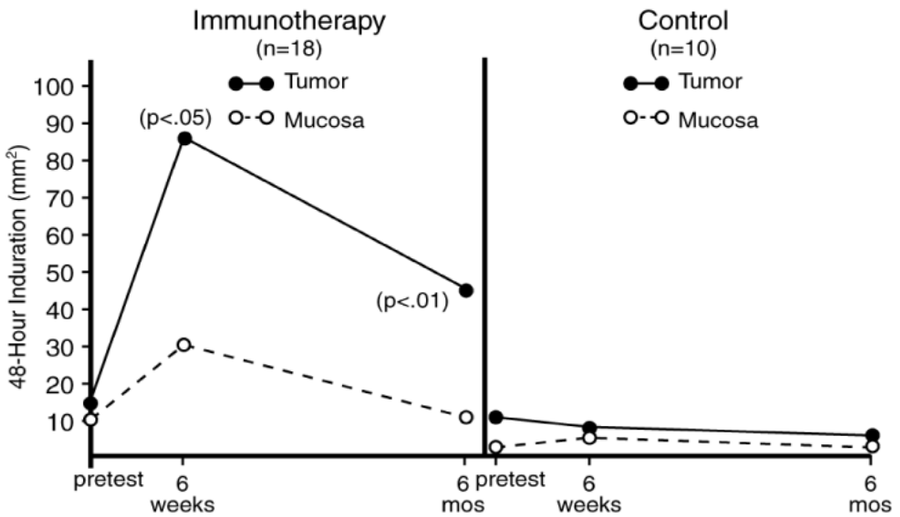
5.2.1.1 Study 8102 Subset

Since the size of human colon tumors available for dissociation and the cellularity of the tumors are variable, it may not be possible to obtain sufficient cells to immunize with 10^7 cells. To examine this requirement, a clinical research project was conducted by Jessup *et al.* at M.D. Anderson Hospital[53]. The patients were immunized with autologous colon tumor cells made with the OncoVAX process but a modification of the immunization regimen was used. Patients were coded and received two injections of either 10^7 (high dose) or 3 x 10^6 (low dose) tumor cells admixed with BCG. Patients in both treatment groups received a third immunization one week later with 3 x 10^6 tumor cells. The patients were also injected, at that time, with 3 x 10^6 autologous normal mucosal cells. Induration at the third vaccination site and the site that was injected with normal mucosal cells were examined 24 and 48 hours later. The patients were also skin tested after 3 and 6 months. A positive delayed cutaneous hypersensitivity (DCH) response was considered to be an induration of at least 7 mm that was observed at 24 hours and that persisted for 48 hours after injection. Although six of the seven patients that received the low dose

developed a positive DCH to the third immunization, none of the patients exhibited positive DCH responses when tested at 3 months after immunization. By contrast, all four patients that received the high dose exhibited positive DCH to the third immunization; three of the patients had positive DCH responses at three months and two of the patients had positive DCH responses at six months. Thus, short term, transient immunity was the result of immunization with fewer that 1 x 10^7 cells. The number of viable tumor cells, therefore, remains an important requirement for vaccine efficacy.

Another important observation derived from this study was the result of the DCH tests conducted on 28 patients. Skin test reactivity to tumor cells peaked at six weeks and began to wane by six months, as seen in Figure 4. This observation led to the inclusion of the fourth "booster" vaccination in the planning for the pivotal Phase III study conducted in Amsterdam (Study 8701).

Figure 4 Study 8102 - DCH Response to Tumor Mucosa



There were no serious side effects demonstrated in the immunized patients. A few subjects reported minimal elevation of temperature in the first two days after receiving the first two vaccines containing BCG. All patients developed superficial ulceration at the sites of the first and second vaccinations containing BCG. The ulcers usually occurred around the third week, were 1.5 to 2 cm in diameter, and usually healed within three months. Sixty percent of patients developed palpable ipsilateral inguinal lymph node adenopathy. These are all normally observed sequelae to percutaneous administration of BCG. In each case, the adenopathy resolved within three months. Satellite ulcers within 5 cm of the vaccine site occurred in two patients. These resolved without any treatment.

One patient had an exaggerated response to the first vaccine with an induration of 15 cm and 8 cm of central ulceration. Healing occurred over a period of three months. She received no additional vaccines. Liver and renal function tests were not altered by the immunotherapy. Total lymphocyte counts and absolute lymphocyte counts did not change significantly.[54]

These encouraging results established the rationale behind conducting larger phase III trials. Two phase III clinical trials were conducted; first an ECOG sponsored study at multiple clinical sites in the United States and second, at the Free University in Amsterdam.

5.2.2 Randomized Phase III Clinical Studies

The completion of the Phase II/III OncoVAX clinical trial (8102) by Hoover, *et al*., albeit in a small number of patients, warranted further study of OncoVAX in well conducted, randomized, multicenter Phase III trials. First, it was imperative to achieve the level of results observed in the Hoover study. However, what was learned from this trial is that three vaccinations are necessary but may not be sufficient for a prolonged and sustained tumor specific immune response. The next consideration was product manufacturing. The immunotherapeutic process is unique and consistent manufacturing of the autologous vaccine in a centralized laboratory presented rate-limiting and manufacturing issues with regards to transportation that made the process problematic at best. Thus, two primary fundamental issues had to be considered in the next clinical trials.

From a marketing and distribution perspective, an immunotherapeutic process where the vaccine can be manufactured, formulated and administered in each hospital appeared to be manageable. This de-centralized approach was of interest to the Eastern Cooperative Oncology Group (ECOG). They took the challenge to conduct a Phase III clinical trial of OncoVAX using this decentralized approach. However, it was recognized that the requirements for quality control and quality assurance that could be achieved through this decentralized approach were grossly underestimated and, therefore, the results of the Hoover study could not be consistently achieved. This ECOG study was performed under Protocol 5283.[55,56,57] When this study of the decentralized approach was initiated by ECOG, the importance of the inclusion of the fourth, booster immunization had not yet been realized; the booster immunization was later implemented in the pivotal study of centralized manufacturing conducted by the Free University in The Netherlands.

The pivotal study (Study 8701) utilized a centralized approach to the manufacture, quality control testing and quality assurance that resulted in a consistent level of product quality. This study also provided for a four vaccine regimen that had a six month booster inoculation after the initial three weekly treatments. This required a centralized manufacturing laboratory in a limited geographical area and some modifications of the practice of medicine by the pathologists to provide the maximum amount of tumor to the manufacturing laboratory, but still allow an adequate sample for clinical diagnosis and staging of the patient's tumor. These methods were developed by Dr. Chris Meijer, Head of Pathology at the Free University Hospital, Amsterdam, and a four vaccine study was

conducted by Dr. H. Pinedo and Dr. Jan Vermorken at the Free University in Amsterdam, The Netherlands.[49] The centralized manufacturing laboratory was also established in the Free University. The protocols were essentially the same for both studies except for the additional inoculation in the Amsterdam trial. Both of these clinical trials are described in the following two sections.

5.2.2.1 Study 5283

Phase III Study for the Evaluation of Combined Modalities in the Treatment of Colonic Carcinoma with Positive Nodes, Dukes' C, or with Penetration through the Muscularis Propria or Serosa (Nodes Negative), Dukes' B_2, B_3; Surgical Resection Alone Versus Postoperative Immunotherapy (Autologous Irradiated Tumor Cells Plus BCG).

The second randomized, multicenter study (5283) was conducted by ECOG. Subjects with Stage II (n = 297) and Stage III (n = 115) colon cancer were randomized to receive OncoVAX after surgery (n = 205) or observation post surgery (n = 207). The 22 participating centers enrolled between 1 and 109 subjects. The treatment regimen of three inoculations was identical to that in Study 8102.

As shown in Table 6, baseline characteristics were well matched across groups.

Table 6 Study 5283 - Baseline Characteristics

Characteristic	OncoVAX N = 205		Control N = 207	
Gender				
Male	105	51.2%	109	52.7%
Female	94	45.9%	97	46.9%
Unknown	6	2.9%	1	0.5%
Age (years)				
Mean	63.9		65.3	
Range	20-87		26-90	
ECOG Performance Status				
0	135	65.9%	124	59.9%
1	63	30.7%	81	39.1%
2	1	0.5%	0	0%
Unknown	6	2.9%	2	1.0%
Stage at Randomization				
TNM Stage II	148	72.2%	149	72.0%
TNM Stage III	57	27.8%	58	28.0%

Subjects enrolled in the study were monitored at regular intervals for signs of tumor recurrence and were followed until death or termination of the study. The primary efficacy variables were disease-free survival and overall survival. In the intent-to-treat analysis, rates of disease-free survival and overall survival were comparable in the treated and control arms. No statistically significant differences were noted when the analysis

was performed by TNM stage. One possible explanation for the lack of effect was that manufacture of vaccine done in a decentralized fashion had poor quality control; thus, many vaccines did not meet the required specifications.

No death was considered related to treatment with OncoVAX. The percentage of subjects who died was comparable in each treatment group: 85 (41.5%) in the OncoVAX group and 82 (39.6%) in the control group. Thirty-five subjects (17.1%) inoculated with OncoVAX and 37 subjects (17.9%) in the control group died from disease progression, the primary cause of death.

Fifty-three subjects experienced at least one non-fatal serious adverse event during the study. The control group had a slightly higher percentage of patients with an event (15%) other than the OncoVAX group (10.8%).

Treatment with OncoVAX was generally well tolerated. The majority of subjects experienced mild to moderate induration with each inoculation with a substantial number of subjects showing mild induration after the third inoculation. Severe induration that extended beyond the injection site occurred in a small percentage of subjects. Ulceration was evident after the first and second inoculations which included BCG organisms. Only one subject sustained ulceration after the third inoculation that was devoid of BCG. Lymphadenopathy was observed in 10-15% of subjects after the first two inoculations but most subjects had no regional reactions. Systemic reactions were infrequent with fever and chills occurring in less than 15% of subjects after the first two inoculations. In the above studies (8102 and 5283), the treatment regimen consisted of three vaccinations, two with BCG and the third consisting of irradiated autologous cells only.

5.2.2.2 Study 8701

Evaluation of Combined Modalities in the Treatment of Colonic Carcinoma with Positive Nodes, TNM Stage III (Dukes' C_1, C_2, C_3) or with Penetration through the Muscularis Propria or Serosa (Nodes Negative), TNM Stage II (Dukes' B_2, B_3): Surgical Resection Alone Versus Surgical Resection and Active Specific Immunotherapy

This multi-center, randomized trial was performed at 12 cooperating hospitals in The Netherlands, and used a central vaccine preparation facility located at the University Hospital, Vrije Universiteit (Free University), Amsterdam. Colon resections were performed at each of the 12 sites, and all tumor samples were sent to the University Hospital's vaccine production laboratory for dissociation, cryopreservation, irradiation and administration.

This study, unlike all of the previous studies, consisted of a four vaccine regimen that included a 6 month booster inoculation after the initial three weekly treatments because a substudy of Study 8102 suggested that immune response begins to wane at month 6. Subjects with Stage II and III colon cancer randomized to the control group (n=126)

received no further treatment after surgical resection and were followed according to scheduled assessments. For subjects randomized to OncoVAX (n=128), the timing of the first 3 inoculations was identical to that in studies 8102 and 5283 (described above) except that a 4th dose, considered a booster treatment, was administered 6 months after surgical resection. The median follow-up in this study was 5.8 years.

The 12 sites each enrolled between 5 and 34 subjects. Randomization into the study was stratified based on TNM stage, tumor location and institution. As can be seen, a small number of subjects were enrolled who had Stage I or Stage IV disease. Patients were well matched with regard to their baseline characteristics (see Table 7).

Table 7 Study 8701 - Baseline Characteristics (Stage II/III Patients only)

	Stage II		Stage III	
	OncoVAX (N=81)	**Control (N=77)**	**OncoVAX (N=44)**	**Control (N=40)**
Gender				
Male	45 (55.6%)	43 (55.8%)	21 (47.7%)	22 (55.0%)
Female	36 (44.4%)	34 (44.2%)	23 (52.3%)	18 (45.0%)
Age (years)				
Mean	64.6	64.2	62.1	60.2
Range	35 - 83	32 - 86	37 - 88	34 - 78
WHO Performance Status				
0	50 (61.7%)	59 (76.6%)	33 (75.0%)	30 (75.0%)
1	21 (25.9%)	11 (14.3%)	8 (18.2%)	7 (17.5%)
2	2 (2.5%)	0 (0%)	0 (0%)	0 (0%)
Missing	8 (9.9%)	7 (9.1%)	3 (6.8%)	3 (7.5%)
Tumor Location				
Right colon	34 (42.0%)	30 (39.0%)	20 (45.5%)	15 (37.5%)
Transverse colon	4 (4.9%)	5 (6.5%)	2 (4.6%)	3 (7.5%)
Left Colon	43 (53.1%)	42 (54.6%)	22 (50.0%)	22 (55.0%)

In the OncoVAX group, 102/127 subjects received all 4 vaccinations. To determine the extent of DCH reactivity, vaccination sites were measured for induration 48 hours after the third and fourth immunizations. Subjects with an average of the two diameters ≥ 5 mm were considered to have effective cellular immunity; 97% of patients after the fourth inoculation achieved effective cellular immunity.

The original protocol stated that recurrence free interval and overall survival would be the primary efficacy endpoints to be analyzed in the study using the log-rank statistic; the secondary efficacy endpoint would be disease-free survival. The amendment to the protocol dated December 12, 1995, discussed overall survival as the primary endpoint.

Revised sample size calculations were based on disease-free survival. Both the protocol and the amendment indicated that analyses would be done both overall and within TNM Stages II and III separately. In a presentation to the Oncologic Drugs Advisory Committee in May 2004, Daniel Sargent of the Mayo Clinic analyzed 15 adjuvant colon cancer trials with a median follow-up of 8 years and 5-year data on 93% of patients. He showed an excellent correlation between 3-year disease-free survival and 5-year overall survival, and concluded that on an arm by arm basis, 3-year disease-free survival is an excellent predictor for overall survival.[58] We have, therefore, analyzed overall survival and disease-free survival as the primary endpoints, both overall and separately within TNM stage. Recurrence free interval, which was analyzed as a secondary efficacy endpoint, is also presented overall and within TNM stage. Subjects who did not have disease progression and died due to a cause unrelated to their primary disease or to an unknown cause were considered censored on their date of death.

With the approval of new agents to treat Stage III and Stage IV colon cancer, it will be difficult, if not impossible to conduct controlled studies in Stage II disease with overall survival as the primary endpoint, because drug treatments likely to be used following tumor recurrence would confound the analysis. Disease-free survival will be a more appropriate endpoint for future Stage II colon cancer trials.

The intent-to-treat analyses included all subjects who received 0-4 vaccinations. The following tables and figures provide summaries of the key protocol-specified efficacy endpoints (disease-free survival Table 8 and Figure 5; overall survival Table 9 and Figure 6; recurrence-free interval Table 10 and Figure 7).

Table 8 Study 8701 - Disease-Free Survival

TNM Stage	Crude Rates		Log Rank Analysis		5-Year Event-Free Rates[2]		
	Control n/N (%)	OncoVAX n/N (%)	p-Value	RR (95% CI)	Control	OncoVAX	p-Value
I-IV[1]	49/126 (38.9%)	39/127 (30.7%)	0.08	0.69 (0.45 – 1.05)	56.9%	68.8%	0.08
I	2/8 (25.0%)	1/3 (33.3%)	0.91	1.16 (0.10 – 12.80)	75.0%	100%	-
II[1]	29/77 (37.7%)	18/80 (22.5%)	0.015	0.48 (0.27 – 0.88)	57.1%	79.2%	0.008
III/IV	18/41 (43.9%)	20/44 (45.5%)	0.99	1.01 (0.53 – 1.90)	52.6%	48.5%	0.73

[1] Patient 25 is excluded (OncoVAX treatment group) because he did not have a post-surgery tumor evaluation.
[2] An event is the first occurrence of tumor recurrence or death due to any cause.

Figure 5 Study 8701 - Kaplan-Meier Estimates of Disease-Free Survival: Stage II Patients

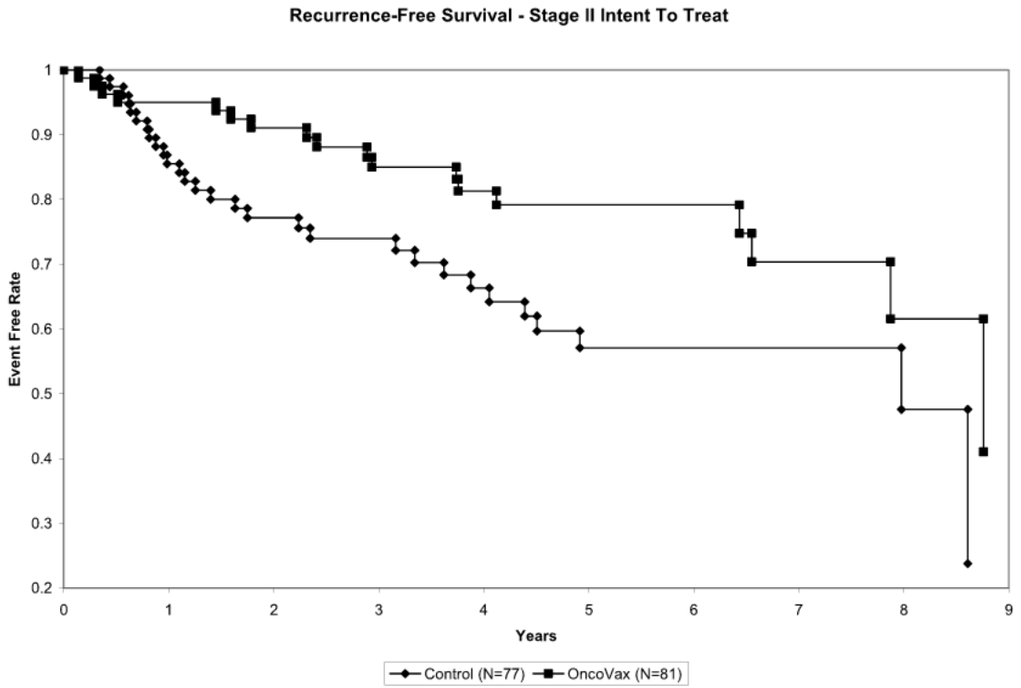


Table 9 Study 8701 - Overall Survival

TNM Stage	Crude Rates		Log Rank Analysis		5-Year Event-Free Rates[1]		
	Control n/N (%)	OncoVAX n/N (%)	p-Value	RR (95% CI)	Control	OncoVAX	p-Value
I-IV	36/126 (28.6%)	32/128 (25.0%)	0.38	0.81 (0.50 – 1.30)	69.0%	77.8%	0.15
I	2/8 (25.0%)	1/3 (33.3%)	0.94	1.10 (0.10 – 12.16)	87.5%	100%	-
II	21/77 (27.3%)	15/81 (18.5%)	0.12	0.60 (0.31 – 1.16)	69.4%	87.0%	0.014
III/IV	13/41 (31.7%)	16/44 (36.4%)	0.72	1.14 (0.55 – 2.38)	63.6%	59.1%	0.70

[1]An event is death due to any cause

Figure 6 Study 8701 - Kaplan-Meier Estimates of Overall Survival: Stage II Patients

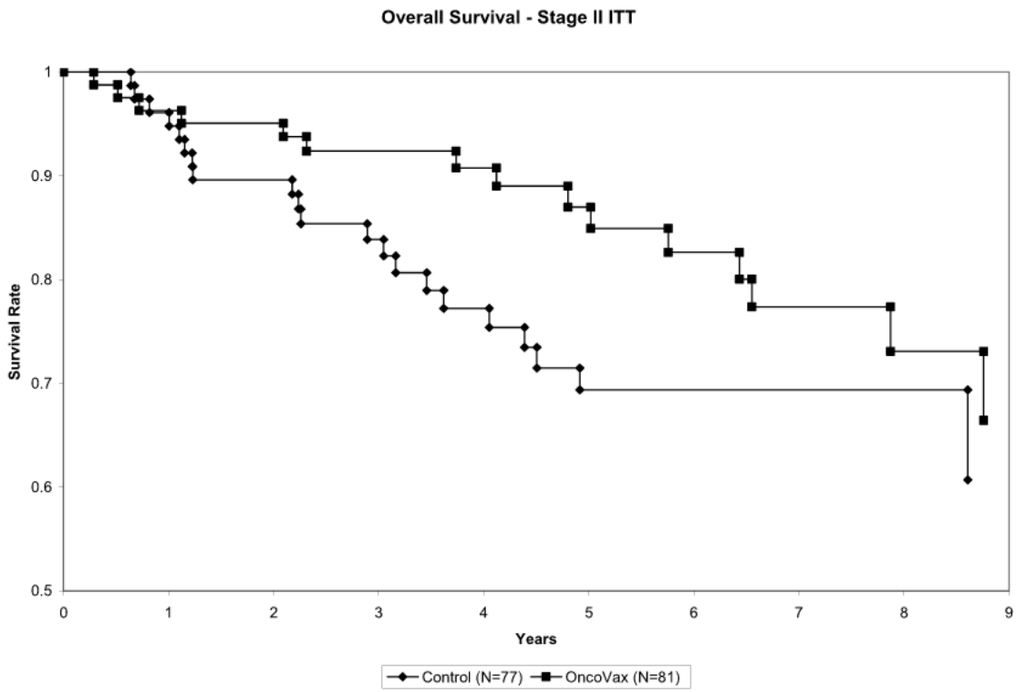


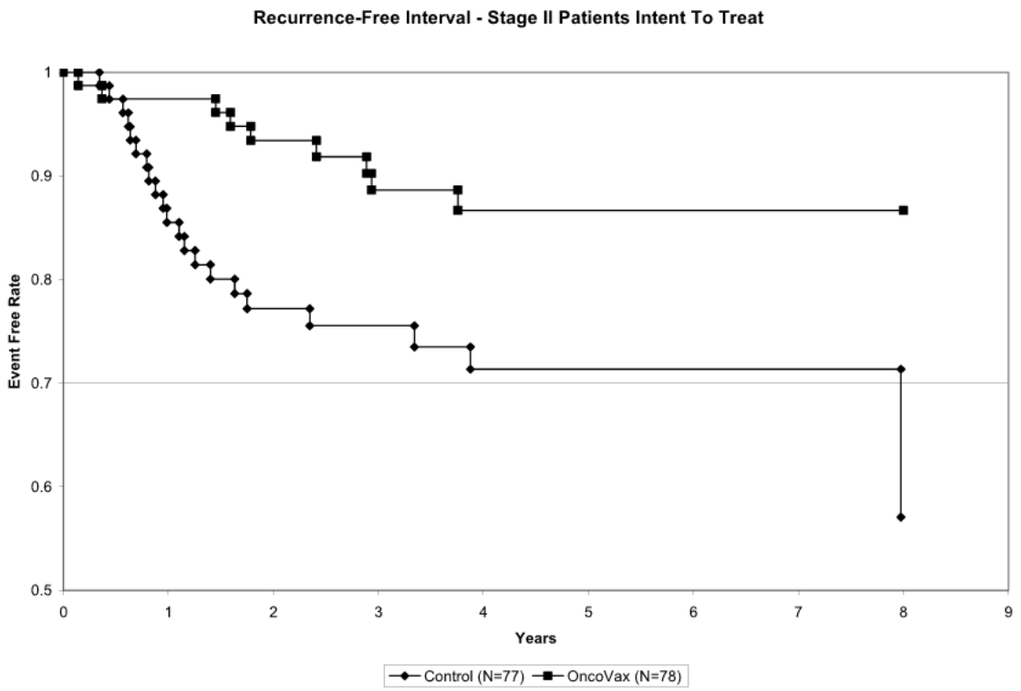
Table 10 Study 8701 - Recurrence-Free Interval

TNM Stage	Crude Rates		Log Rank Analysis		5-Year Event-Free Rates[2]		
	Control n/N (%)	OncoVAX n/N (%)	p-Value	RR (95% CI)	Control	OncoVAX	p-Value
I-IV[1]	41/126 (32.5%)	25/125 (20.0%)	0.018	0.55 (0.34 – 0.91)	65.2%	76.8%	0.06
I	2/8 (25.0%)	0/3 (0%)	0.37	0.0 (-)	75.0%	100%	-
II[1]	21/77 (27.3%)	9/78 (11.5%)	0.008	0.36 (0.17 – 0.79)	71.3%	86.7%	0.028
III/IV	18/41 (43.9%)	16/44 (36.4%)	0.54	0.81 (0.41 – 1.59)	52.6%	57.6%	0.67

[1] Patients 17, 24, and 25 are excluded (all in OncoVAX treatment group) because they did not have a post-surgery tumor evaluation.

[2] An event is the first occurrence of tumor recurrence or death due to the disease. Patients who died due to unrelated or unknown causes, without having had a prior tumor recurrence, are considered censored at that time.

Figure 7 Study 8701 - Kaplan-Meier Estimates of Recurrence-Free Interval: Stage II Patients



While favorable trends were observed in the overall population, there were no statistically significant differences in disease-free survival, overall survival or recurrence-free interval between subjects in the control group and those who received OncoVAX. Subjects with Stage II disease had both clinically meaningful and statistically significant outcomes in both recurrence-free interval and disease-free survival. When 5-year event-free rates were measured, clinically and statistically significant outcomes were observed.

In a *post-hoc* analysis, outcomes of subjects who received all 4 inoculations were evaluated. In such a case, the combined OncoVAX treated cohort achieved a clinically meaningful and statistically significant outcome in terms of recurrence-free interval and disease-free survival. Using this analysis, subjects with Stage II disease also achieved a statistically significant difference in overall survival (p=0.046); 85.5% in the OncoVAX treated group survived vs. 72.7%.

While there were trends toward improved efficacy outcomes in subjects <65 years of age, these trends did not reach statistical significance. There were no differences in efficacy outcomes based on gender.

The percentage of subjects who died was comparable in the control and OncoVAX treated groups: 32 patients (25.0%) in the OncoVAX group and 36 patients (28.6%) in the control group. Disease progression was the primary cause of death in each group. Sixteen subjects (12.6%) treated with OncoVAX died from disease progression compared with 24 subjects (19.0%) in the control group. Cardiovascular events accounted for 5 deaths (4.0%) in the control group and 9 deaths (7.0%) in the OncoVAX group.

The control group had a higher percentage of patients with a non-fatal serious adverse event than the OncoVAX group. Thirty-three patients in the OncoVAX group (25.8%) and 46 patients in the control group (36.5%) experienced at least one non-fatal serious adverse event. One serious adverse event was considered related to treatment with OncoVAX. A 72-year old woman developed a flu-like syndrome approximately one week after her last vaccination. She was hospitalized for treatment and the event resolved nine days later. In addition, treatment with OncoVAX was discontinued in a 71-year old woman who developed a 21 x 32 mm ulceration after the second inoculation (from which BCG had been omitted because of adverse events after the first inoculation). The area of ulceration became necrotic and required surgical excision.

As in previous studies, the most common non-serious adverse events were inoculation-related (erythema, induration, ulceration, lymphadenopathy, fever and chills). A comprehensive review of these events can be found in section 5.3 Overall Safety Summary.

5.2.3 Clinical Trials of ASI in Combination with Chemotherapy

An initial pilot study leading to a Phase II trial was conducted by The Free University Hospital, Amsterdam, to determine if chemotherapy abrogated any or all of the vaccine-

mediated immune response to the tumor, when both therapies were combined in Stage III colon cancer patients. Fifty six (56) patients were treated with the autologous tumor cells + BCG regimen (two vaccines with BCG and two without BCG) followed by 5-FU/leucovorin therapy. The delayed cutaneous hypersensitivity (DCH) response (mean diameter of induration) was measured after the third vaccine (prior to chemotherapy) and at eight months, after completion of chemotherapy and administration of the fourth vaccine. Little difference in the mean diameter of induration was observed between the third (median DCH = 20.3 mm) and fourth vaccine (median DCH = 18.4 mm), indicating that patients were equally able to mount an immune response to their tumor antigens prior to and after this particular chemotherapy regimen.[59]

Northwestern University also conducted a pilot study (8513) combining OncoVAX and 5-FU in metastatic colorectal carcinoma. Of the 31 colorectal patients enrolled, 27 were considered evaluable for efficacy. Patients received one intradermal vaccination per week for two weeks consisting of irradiated autologous tumor cells with BCG and one vaccination of autologous tumor cells alone in the third week. During the fourth week, patients received 5-FU therapy (12 mg/kg/day for 5 days, repeated monthly). Responses were observed in 11 of 27 patients. Four of 27 patients had greater than 50% tumor reduction. Two patients had a near complete response and one patient had therapy discontinued at the time of disease progression and subsequently demonstrated a spontaneous regression of his lesions. Seven of 27 patients showed minor responses or stable disease. The mean response duration for the 12 patients was 15.6 months (range 6-42 months).[60]

A total of 23 of the 27 evaluable patients developed ulceration at the autologous tumor cell/BCG site and 19 of 27 patients demonstrated a local reaction at the site injected with vaccine alone. Grade 3 and 4 toxicities included mucositis, diarrhea, and leucopenia, all of which were associated with the chemotherapy.[30]

Anecdotally, patients that are PPD positive due to previous BCG exposure exhibit ulceration and local reactions similar in nature to those described, with the only exception being that these reactions tend to have a more rapid onset than seen in BCG naive patients.

A third OncoVAX/chemotherapy study (EST 1290), sponsored by ECOG, was initiated in 1990, comparing OncoVAX + 5-FU + levamisole versus 5-FU + levamisole. The study closed in January 1996 due to insufficient accrual.

5.2.4 Bioequivalence Study (Sterile vs. Non-Sterile Product)

A Phase I/II study (ASI-2002-01) was conducted to evaluate the safety and immunogenicity of the current, sterile autologous tumor cell vaccines admixed with BCG in patients with Stage II or Stage III primary adenocarcinoma of the colon, and to demonstrate that the immune response to the sterile vaccine formulation is equivalent to that of the non-sterile formulation used in previous clinical trials.

To meet the primary endpoint (DCH response measured at 48 hours after the third vaccine, which excludes BCG), a patient is considered to have had a response to the vaccine if he/she achieves an induration of at least 5 mm. Local, regional and systemic adverse events were monitored after each vaccine and full safety evaluation including physical examination, performance status, complete blood count with differential, blood chemistries, CEA and urinalysis were conducted 3 months after surgery, after the 4th vaccination (6 months after surgery) and 90 days after the 4th vaccination.

All 15 patients treated and evaluated in the ASI-2002-01 study had an immune response with DCH reactions of >5 mm. Moreover, 13 of 15 patients (87%) treated with the sterile vaccine had DCH reactions of at least 10 mm. In a previous trial in which non-sterile vaccine were administered to 128 patients, the percentage with DCH response of at least 10mm was also 87%. However, the degree of erythema is dramatically reduced compared to that in Study 8701, which used the earlier, non sterile formulation. The erythema is a humoral response, and its reduction presumably reflects the removal of bioburden from the current sterile formulation.

The immune response to the sterile vaccine, therefore, appears comparable to that of the previous non-sterile product. The results further show that the immunogenic response achieved to this immunization is to the tumor-associated antigens and not to a bioburden or contaminant of the product.

In addition, no deaths, no serious adverse events attributed to OncoVAX and no clinically-significant changes in laboratory results were reported. The adverse events reported are those expected from intradermal administration of an immune stimulant, and generally similar to those seen in previous studies and to those reported for BCG. Adverse events reported to vaccines 3 and 4, which contained irradiated colon tumor cells without BCG, are largely limited to erythema and induration.

5.3 Overall Safety Summary

The safety of OncoVAX was assessed in three randomized, multicenter studies that involved 383 patients randomized to treatment with the vaccine (see Table 11). Colon cancer was the diagnosis in 365 patients and rectal cancer was the diagnosis in 18 patients. Demographic characteristics of the treated and control patients were similar in each of the studies.

Two hundred and ninety-four patients (76.8%) randomized to OncoVAX and 260 patients (68.4%) in the control group experienced at least one adverse event. Injection site reactions, consisting of pain, erythema, and induration, and lymphadenopathy were the most frequent adverse events and are considered probably related to treatment with OncoVAX.

Two serious adverse events were attributed to OncoVAX. A 72 year old woman was hospitalized with flu syndrome and a 71 year old woman developed ulceration at the

injection site that became necrotic and required surgical debridement. Both patients recovered.

No patient died as a result of treatment with OncoVAX. Disease progression was the cause of death in 16.9% of patients treated with OncoVAX and 21.1% of patients in the control group. Cardiovascular events were the primary cause of death in patients who died for reasons unrelated to disease progression. Fatal cardiovascular events occurred with essentially equal frequency in both treatment groups.

The collective safety data from the three studies indicate that OncoVAX has an acceptable safety profile and is safe for the treatment of patients with Stage II colon cancer.

Table 11 Controlled Studies

Study No. Investigator	Location of Study	Objective	Design	Subjects	Age Range (yrs)	M/F	Number of Vaccinations
8701 Herbert M. Pinedo, M.D., Ph.D.	Netherlands	To determine the efficacy and safety of immunotherapy with autologous tumor cells and BCG (OncoVAX) in improving survival and delaying or preventing tumor recurrence in patients with surgically resected Stage II or Stage III colon cancer.	Randomized, parallel group, open-label, multi-center trial	Control: 126 OncoVAX: 128	33-88	136/128	4
5283 Herbert C. Hoover, M.D.	United States	To determine the efficacy and safety of immunotherapy with autologous tumor cells and BCG (OncoVAX) in improving survival and delaying or preventing tumor recurrence in patients with surgically resected Stage II or Stage III colon cancer.	Randomized, parallel group, open-label multi-center trial	Control: 207 OncoVAX: 205	20-90	214/191[1]	3
8102 Herbert C. Hoover, M.D.	United States	To determine the efficacy and safety of immunotherapy with autologous tumor cells and BCG (OncoVAX) in improving survival and delaying or preventing tumor recurrence in patients with surgically resected Stage II or Stage III colon and rectal cancer.	Randomized, parallel group, open-label multi-center trial	Colon Control: 28 OncoVAX: 32 Rectal Control: 19 OncoVAX: 18	31-87	60/37	3

[1] Gender was not recorded for seven patients

5.3.1 Objectives and Design

The primary objective of the studies was to determine whether inoculation with autologous irradiated tumor cells evoked a cellular immune response sufficient to prevent or delay the recurrence of tumor in patients with colon or rectal cancer following surgical resection.

Each trial was a prospective, randomized, two-arm, open-label study in which patients, after curative resection of the bowel, were assigned to receive active specific immunotherapy (OncoVAX) or be followed by observation only. Immunotherapy started 28 to 35 days after surgery and consisted of two intradermal doses of irradiated autologous tumor cells together with 10^7 colony-forming units of BCG administered one week apart. A third inoculation consisting only of autologous tumor cells was administered a week later. In Study 8701, six months after colon resection, patients received a fourth (booster) inoculation consisting only of autologous tumor cells.

5.3.2 Patient Population

A total of 763 patients participated in the three studies. The principal criteria for inclusion were:

- Stage II or III adenocarcinoma of the colon with histologic evidence of clear margins after excision of the tumor. Patients with rectal cancer were also included in Study 8102.
- Performance status of 0 or 1
- Adequate bone marrow, hepatic, and renal function
- Normal CEA within 21 days after surgical resection

Patients were excluded for any of the following reasons:

- Evidence of residual tumor or distant metastases at the completion of surgery
- Distant metastases including liver metastases
- Prior chemotherapy or radiotherapy
- Postoperative complications that prevented initiation of therapy
- Therapy with steroids or cytotoxic immunosuppressant drugs
- Other diseases of the bowel such as Crohn's disease, Gardner's syndrome, or Turcot's syndrome

The demographic characteristics of patients in each of the studies were similar (Table 12). The mean age across studies was between 60 and 65 years. There were a slightly higher percentage of men in each study except 8102 in which the percentage of men was substantially higher. The majority of patients had a performance status of 0 or 1 and a TNM stage of II or III. Fourteen patients with Stage I disease and seven patients with Stage IV disease were enrolled in violation of the protocol and included in the analyses.

Table 12 Demographic Characteristics of Patients

Demographic Characteristics	8701				5283				8102			
	OncoVAX N=128		Control N=126		OncoVAX N=205		Control N=207		OncoVAX N=50		Control N=47	
Gender												
Male	67	52.3%	69	54.8%	105	51.2%	109	52.7%	32	64.0%	28	59.6 %
Female	61	47.7%	57	45.2%	94	45.9%	97	46.9%	18	36.0%	19	40.4 %
Unknown	0	0%	0	0%	6	2.9%	1	0.5%	0	0%	0	0%
Age (years)												
Mean	63.7		63.1		63.9		65.3		62.6		61.4	
Range	35-88		32-86		20-87		26-90		31-87		36-81	
Performance Status												
0	85	66.4%	95	75.4%	135	65.9%	124	59.9%	31	62.0%	24	51.1 %
1	30	23.4%	21	16.7%	63	30.7%	81	39.1%	1	2.0%	1	2.1%
2	2	1.6%	0	0.0%	1	0.5%	0	0%	0	0%	0	0%
Unknown	11	8.6%	10	7.9%	6	2.9%	2	1.0%	18	36.0%	22	46.8 %
Stage at Randomization												
TNM Stage I	3	2.3%	8	6.4%	0	0%	0	0%	1	2.0%	1	2.1%
TNM Stage II	81	63.3%	77	61.1%	148	72.2%	149	72.0%	19	38.0%	21	44.7 %
TNM Stage III	44	34.4%	40	31.7%	57	27.8%	58	28.0%	27	54.0%	22	46.8 %
TNM Stage IV	0	0.0%	1	0.8%	0	0%	0	0%	3	6.0%	3	6.4%
No. of Positive Nodes (Stage III and IV)												
≤3	34	77.3%	28	70.0%	36	63.2%	45	77.6%	19	63.3%	16	64.0 %
>3	10	22.7%	12	30.0%	20	35.1%	13	22.4%	7	23.3%	8	32.0 %
Unknown	0	0%	0	0%	1	1.8%	0	0%	4	13.3%	1	4.0%

5.3.3 Extent of Exposure

OncoVAX was administered by intradermal injection. The first inoculation was administered 28-35 days after surgical resection of the tumor. The second and third inoculations were administered at weekly intervals after the first. In Study 8701 a fourth inoculation was administered six months after surgery. The first two vaccinations in each study contained irradiated autologous tumor cells together with 10^7 colony-forming units of BCG. The third and fourth inoculations contained only irradiated tumor cells. Extent of exposure is summarized in Table 13.

Table 13 Extent of Exposure

Number of Vaccinations	Number of Patients			
	8701	5283	8102	Total
4[1]	102	-	-	102
3	5	193	47 [2]	245
2	1	0	0	1
1	2	2	1	5
0	18	10	4	32

[1] Only patients in 8701 received 4 vaccinations

[2] Includes two patients randomized to the control group who received OncoVAX by mistake

5.4 Adverse Events

5.4.1 Display of Adverse Events

The number and percentage of patients who experienced an adverse event are listed by body system in Table 14. A summary of adverse events classified by preferred term, in decreasing order of frequency in the OncoVAX group, is contained in Table 15. Events that occurred in less than 5% of patients treated with OncoVAX are omitted from the table.

The percentage of patients reporting at least one adverse event was similar in each treatment group; 76.8% of patients treated with OncoVAX and 68.4% of patients in the control group reported at least one adverse event. General disorders and gastrointestinal disorders were the most frequently reported adverse events. The incidence of gastrointestinal events was similar in each treatment group which is to be expected in patients who had bowel surgery. Events classified as general disorders and events involving the blood and lymphatic system occurred with greater frequency in patients treated with OncoVAX. General disorders include injection site reactions and the higher incidence of such events in the OncoVAX group (48% vs. 22.1%) is expected since patients in the control group received no injections. Events involving the blood and lymphatic system occurred in 17.8% of patients inoculated with OncoVAX and are an expected reaction to vaccines.

Table 14 Number and Percentage of Patients with at Least One Adverse Event Classified by Body System

Body System	OncoVAX N=383		Control N=380	
Number and percent of patients with at least one event	294	76.8%	260	68.4%
Blood and lymphatic	68	17.8%	12	3.2%
Cardiac	23	6.0%	35	9.2%
Congenital/familial/genetic	0	0.0%	4	1.1%
Ear/labyrinth	4	1.0%	4	1.1%
Endocrine	1	0.3%	1	0.3%
Eye	7	1.8%	13	3.4%
Gastrointestinal	171	44.6%	168	44.2%
General disorders/administration site	184	48.0%	84	22.1%
Hepatobiliary	10	2.6%	16	4.2%
Immune system	3	0.8%	2	0.5%
Infection/infestations	69	18.0%	52	13.7%
Injury/poisoning/procedural complications	28	7.3%	19	5.0%
Investigations[1]	36	9.4%	47	12.4%
Metabolism/nutrition	21	5.5%	21	5.5%
Musculoskeletal	89	23.2%	73	19.2%
Neoplasms	32	8.4%	33	8.7%
Nervous system	51	13.3%	38	10.0%
Psychiatric	27	7.0%	16	4.2%
Renal/urinary	26	6.8%	30	7.9%
Reproductive/breast	33	8.6%	27	7.1%
Respiratory/thoracic	40	10.4%	45	11.8%
Skin/subcutaneous	53	13.8%	29	7.6%
Social circumstances	3	0.8%	0	0.0%
Surgical/medical procedures	12	3.1%	13	3.4%
Vascular	26	6.8%	22	5.8%

Injection site reactions were the most frequent event and occurred in 27.2% of patients who received an injection of vaccine. Lymphadenopathy, fatigue, fever, headache and arthralgia are characteristic reactions to vaccines and are considered related to treatment.

Table 15 Number and Percentage of Patients with Specific Adverse Events Classified by Preferred Term

Adverse Event/ Preferred Term	OncoVAX N = 383		Control N = 380	
Injection site reaction	104	27.2%	0	0.0%
Lymphadenopathy	60	15.7%	4	1.1%
Fatigue	50	13.1%	32	8.4%
Abdominal pain	41	10.7%	34	8.9%
Pyrexia	36	9.4%	11	2.9%
Diarrhea	30	7.8%	28	7.4%
Arthralgia	29	7.6%	14	3.7%
Back pain	24	6.3%	18	4.7%
Constipation	23	6.0%	25	6.6%
Headache	20	5.2%	7	1.8%

5.4.2 Serious Adverse Events

A summary of the number and percent of patients who sustained non-fatal serious adverse events is reported in Table 16. The incidence of non-fatal serious adverse events was slightly higher in the control group

Table 16 Serious Adverse Events

OncoVAX	Control
N = 383	N = 380
58 15.1%	77 20.3%

One serious adverse event was considered related to treatment with OncoVAX. The patient (Study 8701) was a 72 year old woman with a Stage II colon tumor resected 31-Jan-89. Her last vaccination was administered 14-Aug-89. On 20-Mar-90, the patient developed flu syndrome and was hospitalized for treatment. The event resolved by 29-Mar-89. The investigator considered the event definitely related to treatment.

A second patient in Study 8701 sustained two serious adverse events that were not assessed for causality by the investigator. Neither event is considered by the sponsor to be related to treatment with OncoVAX. The patient was a 73 year old man with a Stage II tumor resected on 19-Jul-90. He received all four inoculations with the last vaccine administered 17-Dec-90. The patient was hospitalized because of a hernia on 11-Jan-91 and the event resolved with treatment by 24-Jan-91. The investigator did not assess the causality of the event. However, the sponsor considers the relationship of the hernia to treatment with OncoVAX to be biologically improbable. On 10-Apr-91, four months after the last inoculation, the patient developed facial paralysis and was hospitalized. The event did not resolve. The investigator did not assess the causality of the event.

Although the relationship to OncoVAX cannot be ruled out, the sponsor considers the relationship to be improbable because the event occurred four months after the last inoculation.

Treatment with OncoVAX was discontinued in a 71 year old woman with Stage III colon cancer because of skin ulceration. She received her first inoculation on 15-Jun-92 and the second on 22-Jun-92. One week after the first vaccination, the patient developed lymphadenopathy, severe induration, and erythema at the injection site. Consequently, BCG was omitted from the second inoculation. One week after the second inoculation the patient developed fever, chills, induration, erythema, and ulceration measuring 21 x 32 mm. The area of ulceration became necrotic and required surgical excision. No further vaccinations were administered. The event was considered related to the vaccine.

5.4.3 Deaths

No death was considered related to treatment with OncoVAX. Table 17 is a summary of the causes of death in each study. Disease progression was the primary cause of death. Cardiovascular events accounted for the majority of deaths classified as unrelated to cancer. However, the nature of the event varied and there was no consistent pattern across studies that would suggest that OncoVAX was responsible for cardiovascular events. Table 18 contains a list of fatal cardiovascular events in each study. The nature of the event was determined by reviewing written notes in the patient records. The terms are verbatim terms recorded by the investigator.

Table 17 Causes of Death

Cause of Death	OncoVAX N = 383	Control N = 380
Disease progression	65 16.9%	80 21.1%
Unrelated to cancer	42 10.9%	31 8.2%
Complications of surgery	1 0.2%	1 0.2%
Unknown/missing data	28 7.3%	31 8.2%
Total	136 35.5%	143 37.6%

Table 18 Cardiovascular Events Resulting in Death

Cardiovascular Event Resulting in Death	OncoVAX N = 383	Control N = 380
Number (%) of Cardiovascular Deaths	**26 (6.8%)**	**21 (5.5%)**
Myocardial infarction	7	3
Congestive heart failure	5	6
Cardiac decompensation	2	1
Cerebrovascular event	2	0
Pulmonary embolus	1	0
Myocardial insufficiency	1	0
Arrhythmia	1	0
Cardiac failure	1	0
Probable cardiac event	1	1
Cardiac arrest	1	2
Aortic embolus	1	0
Atherosclerotic heart disease	1	0
Myocardial rupture	1	0
Stroke	1	1
Embolism	0	1
Venous thrombosis	0	1
Multiple infarcts	0	2
Aortic stenosis	0	1
Aneurysm	0	1
Cerebral hemorrhage	0	1

5.5 Conclusions

A total of 757 subjects with colon and rectal cancers have been enrolled in trials of OncoVAX. In Study 8701, in which a booster vaccination at month 6 was added and manufacture of vaccine was carefully controlled at a central facility, subjects with Stage II disease achieved both clinically meaningful and statistically significant benefits in terms of disease-free survival and recurrence free interval. Inoculation with OncoVAX has been demonstrated to be well tolerated and may provide a beneficial alternative to more toxic chemotherapies which have not been established to be efficacious as adjuvant therapy in patients with Stage II disease.

As discussed previously, the focus of Study 8701, which was initiated in 1987, was on overall survival. However, new agents for the treatment of Stage III and Stage IV colon cancer make overall survival as a primary endpoint impossible in controlled studies. These newer agents would be used as soon as tumor progression occurs in a Stage II patient, confounding the analysis of survival. Disease-free survival is now the most appropriate endpoint.

OncoVAX has an acceptable safety profile in patients with colon cancer and is considered safe for treatment of patients with Stage II colon cancer. No patient died as a result of inoculation with OncoVAX and only two patients sustained a serious adverse event that was considered related to treatment. Injection-site reactions consisting of pain, induration, erythema, and ulceration were anticipated OncoVAX-related events. Other anticipated events were lymphadenopathy, fever, and flu-like symptoms. Most events were mild to moderate in severity and resolved with or without treatment. After the second inoculation, one patient developed an ulcerative lesion at the injection site that became necrotic and required debridement. It was considered related to treatment and the patient did not receive further vaccinations.

6. GUIDANCE FOR THE INVESTIGATOR

6.1 Tumor Acquisition Training

At the time of study start up, prior to the first tumor collection, Vaccinogen will ensure that training regarding the appropriate steps and methods for tumor collection is conducted for all of the applicable nurses, physicians, coordinators and pathology assistants. This training will be followed by a practical demonstration at the time of the first colon resection. Qualified OncoVAX training personnel will visit the facility on the day of the first colon resection and work directly with the pathology team to ensure a successful tissue collection. The fundamental purpose of such "real time" training is to ensure that participating individuals understand the requirements and techniques for effectively collecting tumor tissue. Training will address the documentation requirements, the preparation of the colon and tumor prior to packaging, dissection of the tumor, and the packaging and shipping procedures. Although initial procedural training will be completed at the time of the first tissue collection, additional training may be conducted when new personnel have been identified to participate in OncoVAX activities or when existing personnel require refresher training. Such training may be conducted in a group training session, as on the job training (OJT) with supervision or as a self-study.

6.2 Tumor Specimen Handling, Shipping and Processing

Tumor acquisition, handling, processing, shipping and tracking procedures have been put in place in order to ensure that the OncoVAX product prepared from the colon cancer specimen of a given patient is returned to that specific patient. Specific procedures will

be given to each site in the form of an External Operating Procedures (EOP) Manual. This manual will be updated as needed.

6.2.1 Tumor Specimen Handling, Shipping and Processing Overview

The Pathology department staff at participating institutions will be required to handle and process resected colon specimens and resultant tumor tissue using aseptic techniques in a controlled environment. The operating room (OR) personnel will be required to maintain the integrity of the colon after resection and to provide the pathologist, if so needed, with a sterile area within the OR and all material required for dissection. Trained pathology staff will be required to open the colon specimen, to wash the colon and tumor according to Vaccinogen procedures, to provide sections of the tumor for vaccine preparation and to retain sections for routine histopathologic staging. Figure 6-1 depicts the process flow for tumor acquisition and pathological handling and processing. Given the multiple organizations involved in acquiring and processing tumor specimens, detailed procedural steps are specified for each responsible group, to ensure applicable actions occur when required. These procedural steps are collectively housed in the form of External Operating Procedures Manuals. These manuals will be supplied to each site and distributed to the clinical coordinator, formulation lab / pharmacy and the pathology department. Periodically, Vaccinogen will update procedures and the updated materials will be sent to each site for review and training. All training should be documented.

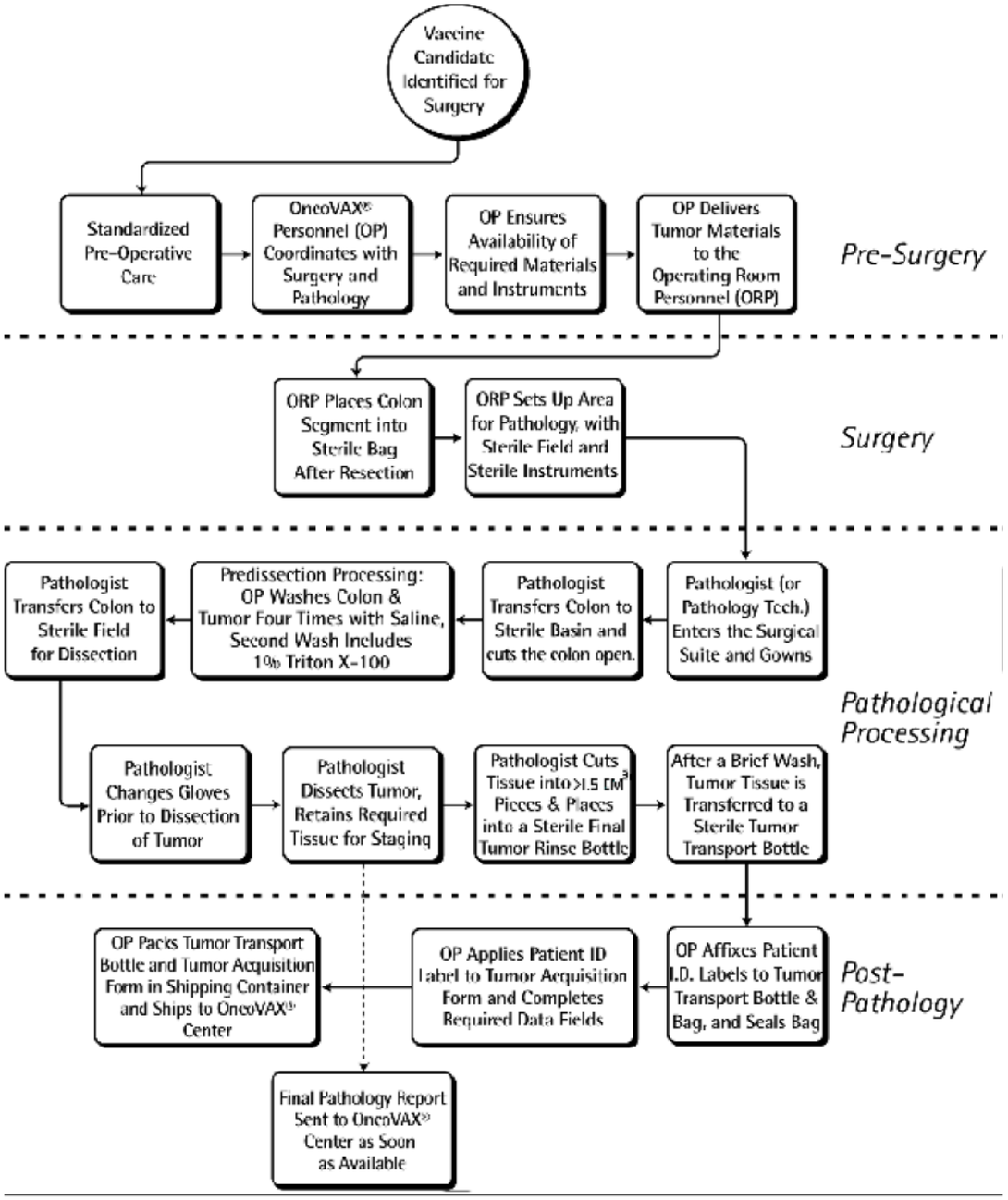
6.2.2 Actions By Vaccinogen Specialist or Study Coordinator (henceforth referred to as OncoVAX Personnel or OP)

When a patient has been identified as a surgical candidate for colon tumor resection, all available information is recorded by the Coordinator, Specialist, or designee in the Subject Screening and Enrollment Log.

The OP coordinates with the OR and Pathology Laboratory staff to ensure proper scheduling of resources and to assist in the resolution of any problems that may occur.

Figure 8 depicts the process flow for tumor acquisition. Detailed instructions regarding preparing the resected colon for tumor collection, collection procedures, specimen labeling, packaging and shipping are provided to each site in the form of External Operating Procedure EOP-0001.

Figure 8 Process Flow for Tumor Acquisition

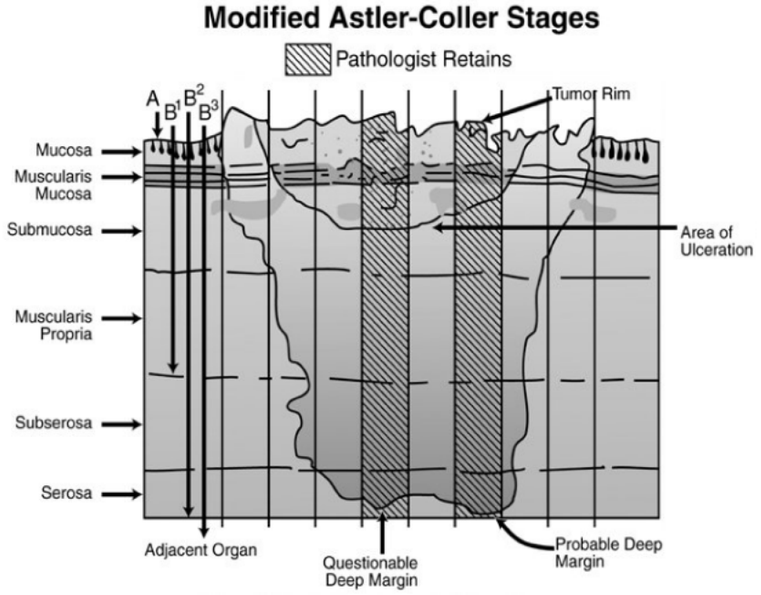


6.2.3 Actions by the Pathologist and Pathology Personnel

In order to ensure the overall safety and efficacy of the vaccine product, the tumor tissue acquired for vaccine preparation must be handled and processed in accordance with current Good Manufacturing Practices, as outlined in the External Operating Procedures (EOP) Manual provided to each site.

It is recognized that tumor acquisition for vaccine preparation must occur simultaneously while obtaining sufficient tissue for routine histopathologic staging. An adequate vaccination regimen requires targeting an approximate minimum of 3.5 cm^3 of tumor however, since tumors differ in cellularity and viability, as much tumor tissue as is available is collected for processing. Following standard techniques for the purpose of determining the deepest penetration margin, the pathology staff member makes a minimum number of parallel slices through the tumor, using a new sterile scalpel, when appropriate, to avoid potential contamination through the tumor tissue. Once the probable margin of deepest penetration has been established, the pathology staff member retains two sections of 2 - 4 mm each for diagnosis and the remaining tumor tissue will be used for vaccine manufacturing. (Figure 9 diagrams the sections that would be retained for diagnostic purposes.)

Figure 9 Pathological Staging for Diagnosis



Using a new sterile scalpel, the pathology staff member cuts any large pieces of tumor tissue into no smaller than 1.5 cm^3 pieces to facilitate rapid cooling and nutrient availability during transport. **Since the tumor tissue will be subjected to a disinfection step during the manufacturing process, cutting the tumor into smaller than 1 cm^3 pieces should be avoided.**

The Pathologist places the tumor tissue into the sterile tumor transport container. Only tumors ≥ 3.5 g should be shipped. If the weight of the collected tissue is <3.5 g, then the pathologist should, if possible, increase the quantity of tumor provided.

The tissue is placed in the tumor transport bottle and prepared for shipping in the Tumor Transport Kit. (A complete description along with the diagram depicting the proper packaging method is outlined in the EOP Manual.) **It is critical that the tumor transport container be packed precisely as outlined in the External Operating Procedure (EOP-0001).**

6.3 Vaccination Training

At the time of study start up, prior to the first patient inoculation with an OncoVAX vaccine, Vaccinogen will ensure that applicable nurses or physicians have been trained to administer OncoVAX. The fundamental purpose of such training is to demonstrate that participating individuals understand the requirements and techniques for administering an effective inoculation of the vaccine. Qualified OncoVAX personnel/Vaccinogen staff members will typically conduct training as a documented in-service training program within each hospital/facility with applicable participating personnel. Training will address the documentation and identification requirements, the time limitations, and the inoculation procedures and specific anatomical sites for the OncoVAX vaccine series. Initial procedural training must be completed prior to the target implementation date of OncoVAX activities at participating facilities. Additional training may be conducted when new personnel have been identified to participate in OncoVAX activities or when existing personnel require refresher training. Such training may be conducted in a group training session, as on the job training (OJT) with supervision or as a self-study, and should be documented accordingly.

Each site will be provided an External Operating Procedure (EOP-0002) which gives detailed information as to how to appropriately deliver an intradermal inoculation of OncoVAX.

Table 19 Vaccination Schedule

	YEAR 1											
	Baseline				**M1**	**M2**	**M2**	**M2**	**M 3**	**M 6**	**M 9**	**M 12**
Treatment	Pre-	W	W	W	W	W	W	W	W	W	W	W
Schedule	Study	0	2	3	4	5	6	7	13	26	39	52
OncoVAX						x1	x2	x2		x5		
BCG						x	x					
Wheal measurement						x3	x3	x3		x3		
Erythema, induration, ulceration						x	x	x, x4		x, x4		

x1 = 28-35 days after surgery
x2 = 7 days after the previous immunization
x3 = within 2 minutes after administration of vaccine
x4 = 48 hours after vaccine administration
x5 = six months after surgery (+/- 1 week)

Vaccine #1 (with BCG): The first vaccination will be administered 28-35 days after colon resection. Wheal formation and local toxicity at the vaccination site will be observed immediately (within 2 minutes of vaccine administration) and again one week after injection.

Vaccine #2 (with BCG): One week (7 days) after vaccine #1, the patient will be evaluated for local (erythema, induration, ulceration), regional and systemic reactions to vaccine #1. The second vaccine will be given and the wheal formation and local toxicity will be observed immediately, and again one week later.

Vaccine #3: One week (7 days) after Vaccine #2, the third vaccine will be administered. Wheal formation and local toxicity will be observed immediately and again two days later. *DCH assessment:* 48 hours after Vaccine #3, the patient's reactions (local, regional, systemic) to ALL THREE vaccines will be assessed. The induration and erythema and local toxicity at the third vaccine site will be measured.

Vaccine #4: Approximately 6 months after the date of resection, the fourth vaccine will be administered. Wheal formation and local toxicity will be observed immediately and again two days later. *DCH assessment:* 48 hours after Vaccine #4, the patient's reactions (local, regional, systemic) to ALL FOUR vaccines will be assessed. The induration and erythema and local toxicity at the fourth vaccine site will be measured.

6.5 Inoculation Process

In order to prevent delays that may result in the expiration of the vaccine before administration, the physician or nurse who will administer the vaccine must closely coordinate the schedule with the individual who will prepare the vaccine. Usually, only four doses of vaccine are available for each patient. Once the cells for each dose are thawed they cannot be refrozen. The cells must be used or discarded by the expiration date and time indicated on the label. Please notify Vaccinogen immediately by telephone if the product has expired and return product to the Pharmacy for proper disposition.

The following are Critical Points:

- **The vaccine MUST be administered by the intradermal route, otherwise the treatment will not be efficacious.** An intradermal injection of 0.2 mL will make a raised wheal of approximately 5 mm. The skin surface will be uniformly raised across all axes. A larger volume inoculation will produce a larger wheal.
- In order to give a successful intradermal injection, please keep in mind the following directions:
  - Hold skin taut between thumb & index finger.
  - Hold needle bevel up at an angle 10-15 degrees (almost parallel to skin).
  - Insert needle into dermis for 2-3 mm of length.
  - Considerable resistance is felt from a correctly given intradermal injection. If this is not felt, and it is suspected that the needle is too deep, it should be removed and reinserted before more vaccine is given.
- The entire vaccine must be inoculated from the syringe. This can best be achieved by having the patients lie flat on their back. Ensure that the needle is very secure on the syringe and visually inspect the cell suspension for clumps. If the cells have aggregated in the syringe they can be dispersed somewhat by allowing a bubble to flow back and forth through the fluid a couple of times. Allow an air bubble large enough (approximately 0.1 mL) to rest between the plunger and the vaccine fluid to expel the entire inoculum. Injections are performed by standing on the right side of the patient for the right thigh inoculum and on the left side of the patient for the left side inoculum. Place the syringe in a downward angle with the inner thigh, keeping the needle level with the skin surface, which facilitates the intradermal injection.
- Back-flow after the needle is removed can be prevented by injecting the vaccine very slowly allowing pressure to dissipate and allowing the needle to remain in position for several seconds after the injection is completed.
- Do not recap needles - dispose used empty syringe with needle attached in an approved "sharps" container.

6.6 DCH Response Measurements

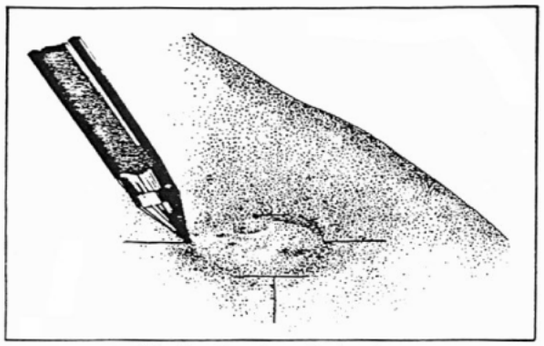
The third and fourth vaccines, which consist of tumor cells alone (no BCG), provide an opportunity to measure DCH. The DCH response is an indicator of the degree of cell-mediated immunity conferred by the initial vaccinations. Two days after vaccine injection, the induration at the site of injection is measured in two perpendicular diameters by the pen method, a standard method for measuring indurations. Please refer to Figure 10 on the next page for a visual depiction of the measurement method.

A line is drawn with a medium ballpoint pen beginning from a point 1 to 2 cm away from the margin of the skin test reaction. The pen is held at a 45 angle and moderate pressure is applied. The pen is advanced until resistance is met, signaling the edge of the area of induration. The procedure is repeated from the opposite angle. The termination points of each set of two lines are then measured by a centimeter ruler or caliper and recorded on the case report form.

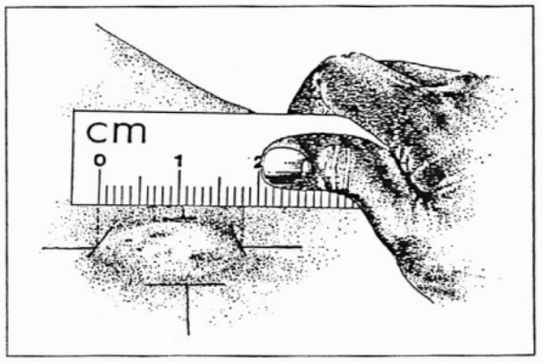
Local reactions are evaluated after each vaccination. In brief, all vaccinations are examined immediately after injection and within the following week. In addition, any local adverse event or ulceration of Grade 2 or greater (moderate or severe) will be examined and evaluated by a physician or trained staff member within 24 hours.

Figure 10 Measurement of DCH Response

Fig



Marking the edge of induration.



Measuring the distance between the lines at the exact edge of induration.

6.7 Instructions for the Patient on Care of the Vaccination Site

√ Take a fever reducer such as acetaminophen (paracetemol) for fever greater than 99.8°F (37.7°C). Contact your doctor if your temperature rises over 101°F (38.5°C).

√ Bathe daily as usual using a mild soap. You may take a shower or a bath until the vaccine area starts to ulcerate. Then it is preferable to shower. Clean out the bathtub with disinfectant after use during the time the vaccine area is draining.

√ To alleviate discomfort that may result when clothing rubs against the draining ulcer (and to protect clothing), use an antibacterial ointment and cover the area loosely with a gauze pad. However, it is best to leave the area exposed to the air as much as possible so that it can dry and form a scab. So remove constrictive clothing in the evening or when convenient and leave the area exposed to air.

√ While the ulcer is draining, cover the area during intimate contact. This will eliminate the rare chance that BCG organisms could be transferred to the other person.

√ Follow your doctor's orders regarding any dietary restrictions; otherwise eat a regular well-balanced diet.

- √ Engage in normal activity, with exercise as tolerated and recommended by your doctor.
- √ Contact your doctor for any additional information you may require regarding fever, the healing of your vaccination areas and any other concerns.
- √ A copy of EOP-Form-0002.1 "Care of Skin Reactions during Vaccine Therapy" will be provided.

6.8 Anticipated Side Effects

The local reactions to the OncoVAX treatments, including induration, erythema and ulceration are expected consequences of the BCG bacteria that are admixed with the tumor cells for the first two treatments. Induration and erythema are the expected local reactions to the third and fourth OncoVAX treatments administered without the addition of BCG bacteria. These local reactions are desirable as they indicate the development of antitumor immune responses. Adenopathy, the only regional reaction reported in the clinical trials, is also a normal consequence of inoculation with BCG bacteria. Mild systemic reactions, including self-limiting fever and chills, are also expected outcomes related to immunostimulation. Only one serious adverse event considered related to OncoVAX, severe induration with ulceration, was reported in a patient in Study 8701. No other serious adverse events were attributable to treatment.

To preclude any risk of localized infection with normal enteric microorganisms, only sterile vaccines are released for patient treatment. To minimize risks that may be associated with BCG, the dose is carefully calculated to be 10^7 CFU/vaccine dose. Systemic BCG infection has not been reported, and such an occurrence is considered a remote possibility at this low dose of BCG. BCG is very sensitive to isoniazid, which could be utilized should any patient develop symptoms of systemic BCG infection.

Although risk of tumor enhancement is a theoretical possibility, none was observed in any of the previous clinical trials. Enhancement has not been suggested in any of the patients treated. To minimize risks that may be associated with autologous tumor cell therapy, the tumor cells are irradiated with 200,000 rads (2000 Gy), which is a dose in far excess of the minimum required to render the cells non-tumorigenic.

The majority of the reported adverse events (other than those related to immune response) were related to the abdomen or digestive tract including: abdominal pain, diarrhea, constipation, and abnormal stool. These adverse events occurred among OncoVAX-treated patients at a rate similar to the rate of occurrence among control patients. These events are expected in this patient population whose underlying disease and previous surgical treatment results in a predisposition to gastrointestinal dysfunction. All adverse events should be recorded in the patient's medical record and again in the case report forms.

6.9 Precautions and Warnings

Although to date, there have been no reports of hypersensitivity reactions in patients treated with OncoVAX, this autologous vaccine product is contraindicated in patients with a history of severe hypersensitivity and/or allergic reaction to gentamicin sulfate, amphotericin B, imipenem, cilastatin and/or levofloxacin.

OncoVAX is contraindicated in patients who have a history of congenital or acquired immune deficiency diseases such as HIV. Patients diagnosed with hepatitis B, C, and/or a history of tuberculosis are not eligible for treatment. OncoVAX is contraindicated in patients requiring steroids, cytotoxic drugs, or other immunosuppressive agents from the time of pre-surgery screening through the conclusion of the third (3^{rd}) vaccine inoculation (Vaccine #3).

Prophylactic antibiotics can be given prior to surgery and topical antibiotic treatments during the vaccination period are acceptable; however systemic antibiotic therapy during the period of immunization is unacceptable.

The OncoVAX vaccine should not be administered to women who are pregnant or breastfeeding. Individuals of childbearing potential should be using effective contraceptive measures.

The OncoVAX vaccine should not be administered to patients with an acute infection characterized by a fever (defined as oral or tympanic temperature of > 38°C (>100.4 °F) or greater) within 5 days of first vaccine treatment.

7. REFERENCES

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