

Commentary

# Active Specific Immunotherapy with Autologous Tumor Cell Vaccines for Stage II Colon Cancer

## Logistics, Efficacy, Safety and Immunological Pharmacodynamics

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### KEY WORDS

OncoVAX, tumor cell vaccine, autologous tumor cells, BCG, stage II colon cancer, prevention of recurrence

### ABSTRACT

In the area of cancer treatment, immunotherapy with vaccines has suffered in the last five years, due to many clinical trial failures. One must keep in mind however, that many of the clinical trials conducted in the past decade were performed without the benefit of sound regulatory guidance or validated and compliant manufacturing processes. This has clearly been the case for patient specific, tumor cell vaccine therapy. The safety concerns that emanated within the regulatory agencies from the Somatic Cell Therapy concepts, translated to active specific immunotherapy with vaccines. Fortunately, in the past five years advances in understanding the immune system, improved design of clinical trials, improvement and compliance of manufacturing processes provided opportunities to significantly improve efficacy and safety. Clearly, the vaccine research establishment has learned the importance of not just selecting antigens but the requirement of tumor associated immunogens that can stimulate a functional immune response. Also, it has become clear that immunotherapy works best in situations of minimal residual disease. Finally, more realistic endpoints in clinical trials have been recognized and accepted by oversight review committees. This commentary describes the "trials and tribulations" of developing a patient specific, autologous tumor cell vaccine for therapy of Stage II colon cancer.

### INTRODUCTION

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. Approximately 8% of Americans develop the disease.<sup>1</sup> The American Cancer Society estimates that 106,680+ cases will be reported in 2006.<sup>2</sup> Among countries of the European Union (EU), the numbers are even greater. 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. To date, surgery is the primary treatment modality for this disease. 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.<sup>4-7</sup> 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 the last two years.

Between 1985 and 1997, death rates of colon cancer in the United States declined slightly. 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.<sup>8</sup>

There are two important points to be emphasized looking forward. First, earlier detection of colon cancer has resulted in the redistribution of the TNM<sup>9</sup> (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

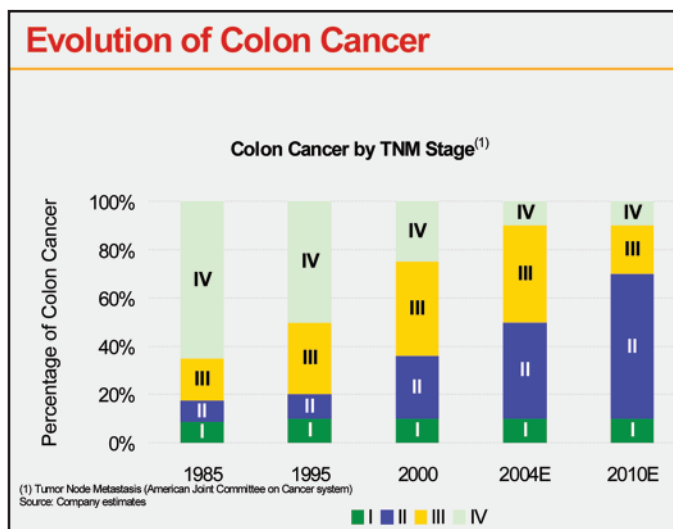


Figure 1. Redistribution of the Incidence of Stages II Through IV Colon Cancer

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 treatment with chemotherapeutic drugs have not shown significant prognostic benefits.<sup>10,11</sup>

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 five 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 five year survival rates of 75% and 36%, respectively ( $p = 0.03$ ).<sup>12</sup> 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.

For stage II and stage III carcinoma of the colon a new adjuvant therapy was introduced: vaccination with autologous tumor cells, otherwise known as OncoVAX<sup>®</sup> treatments. As opposed to prophylactic vaccinations to prevent disease such as the polio, flu or meningitis, autologous tumor vaccines are designed for the treatment of the disease with the intent to cure. The effects of treatment with tumor vaccines in cancer therapy differ from the way cytostatic drugs work. Chemotherapeutic drugs inherently do not distinguish between normal and cancerous cells although the clinical toxicity, with respect to differing cell cycle period, cytoskeletal changes and signaling properties obviously differs for each type of cell. In contrast,

the autologous tumor vaccine is effective by eliciting an immune response, which instead of targeting a single pathway or selective difference between normal and tumor cells has the advantage of targeting multiple tumor specific differences (antigenic components of tumor cells). There is also the advantage that the immune system actively carries out surveillance through circulating cells, these can be amplified in the presence of tumor (metastases) and provide long-term memory. Cytotoxic drugs have a limited lifespan and do not express delayed effects.

## LOGISTICS

The underlying basis for the OncoVAX<sup>®</sup> 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 on normal cells. These tumor antigens may also be qualitatively and/or quantitatively different from those on tumors of other patients. Active specific immunotherapy (ASI) is a strategy to induce a functional anti-tumor immune response against tumor cells and tumor antigens. A variety of strategies or technology platforms exists to achieve this goal. Peptide-based vaccines, recombinant viral vaccines, nucleic acid vaccines, and dendritic cell vaccines are some of the new innovative approaches being tested. A possible disadvantage of these antigen-specific vaccines is that they utilize only known tumor antigens. Many of these known tumor antigens have not been validated to be functional immunogens and/or may lack important immunogens not yet identified. Using tumor cells derived from each patient to be vaccinated would obviate this problem. OncoVAX<sup>®</sup> 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 TICE Bacillus Calmette-Guérin (BCG). This TICE strain of BCG is used as an adjuvant in the autologous tumor cell vaccine to activate the innate immunity to promote antigen processing, presentation and T-cell activation.

OncoVax<sup>®</sup> contains two distinct biological entities: viable, irradiated, autologous tumor cells and fresh-frozen, Mycobacterium bovis (BCG). The first two vaccines contain viable, metabolically active, non-tumorigenic, sterile tumor cells admixed with 107 colony forming units (CFU) of fresh-frozen BCG. The subsequent two vaccines are prepared similarly, but without the addition of BCG. The 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 four hours of the thawing of the cells.

Over the 15 years of clinical trials with this vaccine there have been some critical changes in the manufacturing and logistic methodology. The most significant is that the colon tumor vaccine as a final Drug Product is sterile and all procedures are cGMP and/or aseptic. In addition we have developed matrix associated potency and product characterization assays that are automated using Flow Cytometry. We have also developed an automated Flow Cytometric Identity assay that uses a human monoclonal antibody that reacts with human colon tumor associated antigens.

The manufacturing of OncoVAX involves the acquisition of the source material (tumors) and therefore, includes all of the handling of the tumor outside the production facility. The initial steps in the manufacturing process consists of the surgical resection of the colon, the dissection and pathological processing of the tumor in the

surgical suite and the transport of the tumor to the manufacturing facility. Operating room and pathology personnel are trained in accordance with validated procedures.

Intracel's OncoVAX<sup>®</sup> manufacturing facility operates under cGMPs in all aspects of the operation. The Intracel 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 department consists of Quality Assurance and Quality Control.

The tumor cell component of the vaccine is derived from the patient's own solid tumor, which has been surgically removed and processed to a single cell suspension, which is then cryopreserved. The enzymatic dissociation of the tumor is performed in the presence of several antibiotics to reduce endogenous bioburden inherent to colon-derived tumors. The frozen cells are rendered non-tumorigenic by irradiation. These steps are performed in Intracel's cGMP facility in Emmen, The Netherlands. From the time the tumor arrives at the facility until irradiation of the cells is approximately five hours.

In this context, OncoVAX<sup>®</sup> is a form of ASI in which a formulation is administered comprised of (1) sterile, live, irradiated, non-tumorigenic autologous tumor cells; and (2) with or without fresh frozen BCG bacteria. Thus, the product contains two distinct biological entities: viable, irradiated, autologous tumor cells and fresh-frozen, BCG bacteria. 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<sup>8</sup> CFU/vial. The TICE strain is currently is manufactured by Organon 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 manufactured by Organon, Inc., West Orange, NJ.

All vaccinations are administered, by the intradermal route, at weekly intervals, beginning 28 to 35 days after surgery. The intradermal route of injection was demonstrated in preclinical studies to be superior for immunization. It is believed that this is due to the presence of discriminating antigen presenting Dendritic cells (Langerhans cells). The first two injections are comprised of sterile, thawed irradiated tumor cells (1.0 x 10<sup>7</sup>) admixed with BCG (1.0 x 10<sup>7</sup> CFU). The third injection of cells does not contain BCG. The fourth and final vaccination with irradiated tumor cells without BCG takes place six months after surgery. Forty-eight hours after intradermal injection of the tumor cell alone, third and fourth vaccination, significant cutaneous Delayed Type Hypersensitivity (DTH) indurations can be measured in over 95% of the treated patients. A DTH response is indicative of a tumor-specific T lymphocyte infiltration in response to the tumor antigens present in the vaccine. Where as the first two injections in the presence of BCG break tolerance and prime the immune response, the second two injections of tumor cells alone both demonstrate a tumor-specific T cell response, as well as providing antigen to further boost the tumor-specific immune response.

## STRATEGY FOR CLINICAL TRIALS

The first successful completion of the Phase II/III OncoVAX<sup>®</sup> clinical trial by Hoover et al.,<sup>13,14</sup> albeit in a small number of patients warranted further study of OncoVAX<sup>®</sup> in larger well-conducted, randomized, multicenter Phase III trials. Hoover et al., demonstrated

that three vaccinations are necessary but may not be sufficient for a prolonged and sustained tumor specific immune response. This was determined by measurements of the *in vivo* surrogate endpoint for immunological T-cell response, DTH, measured 48 hours after the third, tumor cell alone, immunization. It was observed that the magnitude of DTH response waned by six months after treatment.

For larger follow-on clinical studies, the next consideration was product manufacturing. Consistent manufacturing of the autologous vaccine in a centralized laboratory presented several rate limiting issues including transportation of patient materials between the clinical and manufacturing sites, a reproducible and cost-effective manufacturing process for individualized therapy on a large scale and automated quality control process to reproducibly release product. The first two issues were evaluated in the next two clinical trials while the last and final issue was put to rest in preparing for the pivotal study.

From a marketing and distribution perspective an immunotherapeutic process where the vaccine can be manufactured, formulated and administered in each hospital appeared to be desirable. 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<sup>®</sup> using this de-centralized approach. However, it was recognized that the quality control and quality assurance that could be achieved through this de-centralized approach was grossly overestimated and therefore the results of the Hoover study could not be achieved.<sup>15-17</sup> The results of the ECOG Study were negative in terms of intent-to-treat analysis; however, a subset analysis comparing patients who received vaccines that met specification compared to deficient vaccines showed that the former had significantly improved prognosis.

When this study of the de-centralized 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 as part of the study of the centralized manufacturing conducted by the Free University in The Netherlands. This study also differed from the ECOG study in that it utilized a centralized approach to the manufacture, quality control and quality assurance of vaccines in a facility located at the University hospital in Amsterdam. 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 adequate sample for clinical diagnosis and staging of the patient's tumor and a four vaccine study was conducted by Drs. H. Pinedo and Jan Vermorken at the Free University in the Netherlands.<sup>18</sup> The protocols were essentially the same for both studies except for the additional booster vaccination in the Amsterdam trial.

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 six-month booster inoculation after the initial three weekly treatments because a sub study of study 8102 suggested that immune response begins to wane at month six.

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 three inoculations was identical to that in studies 8102 and 5283 (described above) except that a fourth dose, considered a booster treatment, was administered six months after surgical resection. The median follow-up in this study was 5.8 years.

The 12 sites enrolled between five and 34 subjects each. 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 (Table 1).

In the OncoVAX® group, 102/128 subjects received all four vaccinations. To determine the extent of DTH reactivity, vaccination sites were measured for indurations 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.

While favorable trends were observed, there were no statistically significant differences in recurrence-free survival, overall survival or recurrence-free interval between all subjects, Stages I-IV in the control group and those who received OncoVAX®. The randomization was stratified so that a prospective analysis by stage of disease was performed. Subjects with Stage II disease had both clinically meaningful and statistically significant outcomes in both recurrence-free interval and recurrence free survival. When five year event-free rates were measured, clinically and statistically significant outcomes in overall survival were observed.

Forty-six TNM Stage II (B2, B3) patients (29 control, 17 OncoVAX® treatment) were reported as having disease progression or having died during the study. Kaplan-Meier estimates of colon cancer rates show a statistically significant improvement of recurrence-free survival in the Stage II treated patients (Figure 2). The percentages after five years of follow-up were 21.3% and 37.7% for the treatment and control groups, respectively. The favorable 16.4% difference

**Table 1 Baseline Characteristics (Study 8701)**

|                               | Stage II<br>OncoVAX®<br>(N = 81) | Control<br>(N = 77) | Stage III<br>OncoVAX®<br>(N = 44) | Control<br>(N = 41) |
|-------------------------------|----------------------------------|---------------------|-----------------------------------|---------------------|
| <b>Gender</b>                 |                                  |                     |                                   |                     |
| 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%)          |
| <b>Age (years)</b>            |                                  |                     |                                   |                     |
| Mean                          | 64.6                             | 64.2                | 62.1                              | 60.2                |
| Range                         | 35 - 83                          | 32 - 86             | 37 - 88                           | 34 - 78             |
| <b>WHO Performance Status</b> |                                  |                     |                                   |                     |
| 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%)            |
| <b>Tumor Location</b>         |                                  |                     |                                   |                     |
| 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%)          |

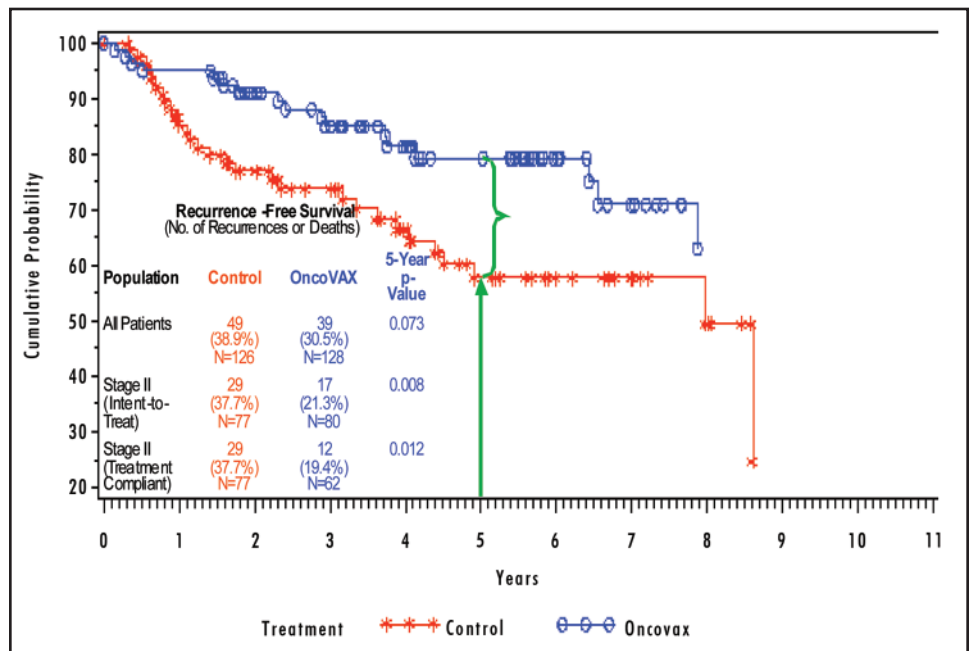


Figure 2. 8701 Study—Recurrence Free Survival in Stage II patients.

represents a 41.4% relative risk reduction of disease progression (5-year survival p=0.008; log-rank analysis p=0.018).

Thirty-five TNM Stage II (B2, B3) patients (21 control, 14 OncoVAX® treatment) died during the study. Overall survival showed a statistically significant improvement in the Stage II OncoVAX® treated patients (17.5%) over those patients in the control group (27.3%) (Figure 3). The favorable 9.8% difference represents a 33.3% relative risk reduction (5-year survival p=0.014; log-rank analysis p=0.074).

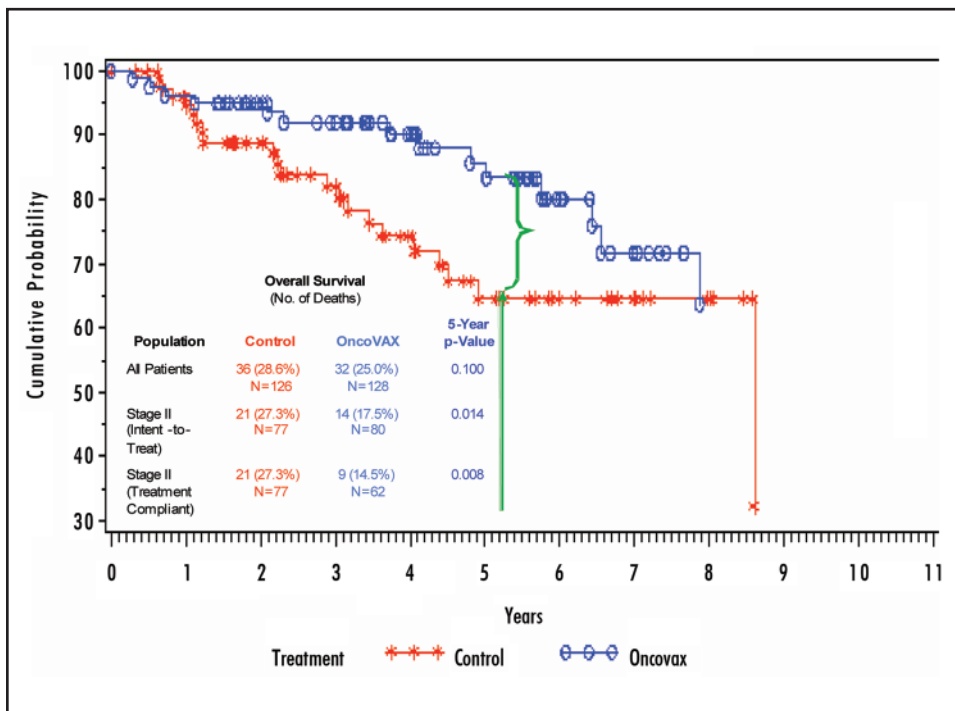


Figure 3. 8701 Study—Overall Survival in Stage II Patients.

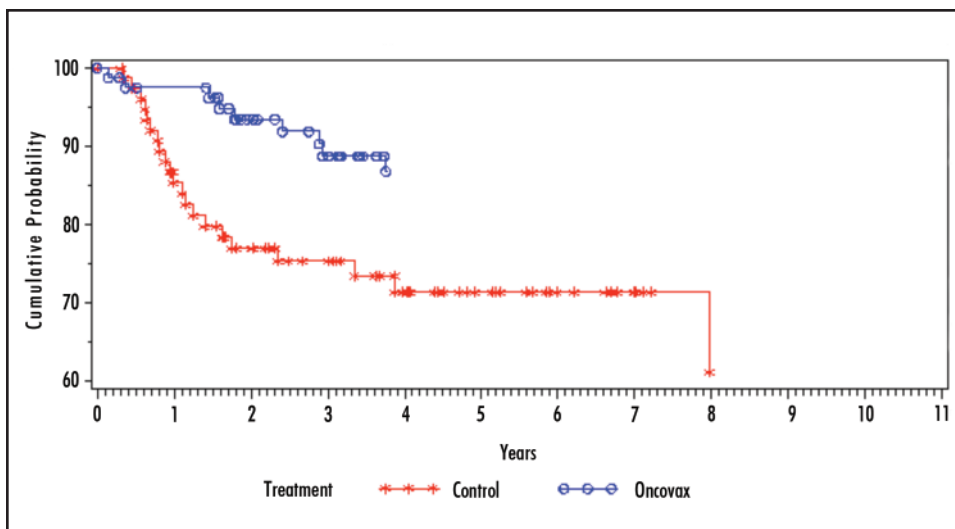


Figure 4. 8701 Study—Recurrence Free Interval in Stage II Patients.

In the intent-to-treat (ITT) population of all patients randomized as Stage II, there were 43 recurrences, (Fig. 4). The five-year recurrence-free interval p-value (0.010) and the log-rank analysis p-value (0.004) was highly significant, it was discovered in referee pathology diagnosis that this included a proportion of B1 patients (9 control and 4 treated patients). These were excluded in the separate Stage II (B2, B3) analysis.

Thirty Stage II (B2, B3) patients had recurrences (21 control, 9 OncoVAX<sup>®</sup> treated; Fig. 4). Recurrence-free interval showed a statistically significant improvement in the Stage II OncoVAX<sup>®</sup> treated patients. Kaplan-Meier estimates of colon cancer rates of recurrence after five years of follow-up were 27.3% and 11.3% for

the control and OncoVAX<sup>®</sup> treatment groups, respectively. When compared to the control group, the favorable 16% difference represents a 57.1% relative risk reduction in the recurrence of colon cancer in the OncoVAX<sup>®</sup> group (five-year survival  $p = 0.026$ ; log-rank analysis  $p = 0.008$ ).

The control group had a higher percentage of patients with a non-fatal serious adverse event than the OncoVAX<sup>®</sup> group. Thirty-three patients in the OncoVAX<sup>®</sup> 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<sup>®</sup>. A patient was hospitalized for treatment of a flu-like syndrome and the event resolved nine days later. In addition, treatment with OncoVAX<sup>®</sup> 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.

In a post-hoc analysis, outcomes of subjects who received all four inoculations were evaluated. In such a case, the combined OncoVAX<sup>®</sup> treated cohort achieved a clinically meaningful and statistically significant outcome in terms of recurrence-free interval and recurrence-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<sup>®</sup> treated group survived vs. 72.7%.

## SPECULATION ON IMMUNOLOGICAL MECHANISM OF PROTECTION

The presence of a significant DTH response to tumor cells after immunization with an autologous tumor cell/BCG vaccine has been shown to be a measure of the immunogenicity of the vaccine and has been correlated with survival.<sup>13,14,17</sup> Historically, numerous investigators have correlated immune reactivity with prognosis.<sup>19,20,21,22</sup> We will, however, focus on ASI with OncoVAX<sup>®</sup> in colon carcinoma. The magnitude of the immune response, and its correlation to prognosis, is dependent upon the adequacy of the vaccine's potency (cell number and viability), proper administration (intradermal) of the vaccine and patient related factors such as the ability to mount an immune response to autologous tumor cells. Hoover, et al.<sup>13</sup> skin tested 24 treated and 11 control patients with 106 irradiated autologous tumor cells, and 106 irradiated autologous normal colon mucosal cells. Significant differences were seen between

DTH reactions to tumor and normal mucosal cells at six weeks and six months after immunization. The treated patients had significant increases in DTH reaction after vaccination. By contrast, non-immunized control patients did not exhibit significant immune responses to either their tumor or normal cells. It is generally recognized that DTH is a consequence of infiltration and proliferation of primed T-memory cells.

During the course of immunization memory T-cells are the protective surveillance immune component possibly for years after treatment. During the course of time that the patient is protected, we can presume that these cells contain rearranged T-cell receptors responding to tumor associated antigens. This will trigger the proliferative expansion of cytotoxic T-cells. These cells should be abundant in the peripheral circulation after the final induction vaccine (3rd) and after the booster vaccination (4th).

Libraries of these memory T-cells should express the molecular basis for both the target as well as the immunological products and stimulation to kill the tumor cells. The *in vivo* DTH, which we consider the surrogate for a successful immunization series also is an induced expression of the relevant T-memory cells directed at tumor antigens in the third, and fourth, tumor cell vaccine. This suggests that the majority of these tumor reactive T-cells are created in the induction phase of the vaccination regimen and continue to play a role after the third vaccination.

Considerable attention has been devoted to the tumor infiltrating T-cells, both the CD4 cells with their toxic cytokine production and CD8 memory T-cells. However, the cells while they may retard or suppress the growth of primary tumors are not capable of, based on their short half-life, eliminating any or all primary tumors and/or metastatic seeds, which establish at distant sites. This function is clearly the role of T-memory cells.

The question can be raised as to when these T-memory cells develop? Based on the studies of Pagès, et. al.,<sup>23</sup>, they develop during oncogenesis and it is a function of the number of CD8 cells. The retrospective data shows that tumors with the largest number of T-memory cells correlate to the best prognosis after surgery. Thus, OncoVAX® may level the playing field by stimulating a higher more constant and clinically relevant number of T-memory cells in circulation to prevent metastatic seeding. Keep in mind that these cells are efferent in both nature and function and that CD4 cytotoxic T-cells act primarily at the primary immunogenic site. The number and efferent nature of the T-memory cells becomes the keystone of preventing recurrence thus prolonging time to progression and possibly cures. The immunologic anti-tumor properties of these cells are expressed over several years unlike the short duration effect of chemotherapeutics. This biotherapeutic approach can only be accomplished through active patient specific immunotherapy using all of the tried and tested methods of vaccinology.

## THE BEGINNING

The dialogue between Intracel and the U.S. Food and Drug Administration (FDA) regarding the approval of OncoVAX®, took place between January 2000 and May 31, 2006 and centered on the pharmacological aspects of the drug product. The issues resolved were sterility and complete product characterization studies using flow cytometry. The latter resulted in developing an automated matrix associated potency and identity assay.

Finally, the FDA requested a second, confirmatory, randomized controlled phase III trial of OncoVAX® in stage II colon cancer.

Based on a protocol approved by the FDA, this study will be carried out under a Special Protocol Assessment (SPA). An SPA granted by the FDA provides a mechanism for the sponsors and the FDA to reach agreement on, size, execution and analysis of a clinical trial that is intended to form the primary basis for regulatory approval. The primary endpoint of this pivotal phase III trial is recurrence-free survival (RFS) with an interim and final primary analysis one and three years after following the full enrollment respectively. The study is powered at 90% to detect a 50% reduction in RFS versus resection only control for final analysis with adjustment for interim analysis. If a robust p value is achieved at the interim analysis, the Biologic License application can be filed. Past clinical trials using the optimum four immunization regimen will be accepted as supportive studies during the FDA review of the BLA.

It must be noted that the period between 2002 and 2005, while we were performing the work described in this text; seven biotechnology companies attempted and failed in pivotal phase III clinical trials of various platforms of ASI. Some of these studies were performed under FDA granted SPAs. This does not include several other attempts that failed in phase II clinical trials or were put on clinical hold for failing to accomplish pharmacological or manufacturing requirements. The failure of these studies not only negatively affected the economic status of the companies involved, but also have in general negatively affected the status of the field of cancer immunotherapy.

Despite these failed efforts, we remain cautiously optimistic for several reasons. First, OncoVAX® is the only immunotherapy platform using autologous tumor cells to treat minimal residual disease. Second, in all of our dose and regimen finding clinical trials, we had randomized surgery only controls and thus more comprehensive clinical data for OncoVAX® compared to other immunotherapy products. Finally, we are confident that in all of the attempts to satisfy the compliance aspects of the manufacturing and quality control requirements, we were conscientious to maintain the immunologic essence of the effective vaccine. Major process changes were required to produce sterile autologous tumor cell vaccines. However, we carefully performed a clinical bioequivalence using DTH to the third and fourth (booster) vaccine as a surrogate for immunogenicity, which demonstrated there were comparable DTH responses to those generated by previous non-sterile vaccines. We hope that this critical and careful approach to the clinical development of cancer vaccines should allow us to generate positive clinical results for stage II colon cancer that is a true “unmet medical need.”

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