Journal of
Clinical & Cellular Immunology

Hanna, J Clin Cell Immunol 2014, 5:5
DOI: 10.4172/2155-9899.1000269

Commentary Open Access

Active (Patient Specific) Immunotherapy of Colon Cancer: A Transition from Preclinical Studies to Successful Clinical Trials

Michael G. Hanna Jr.*

Founder and Chairman Emeritus, Vaccinogen, Inc., USA

***Corresponding author:** Michael G. Hanna Jr., Chairman and Chief Executive Officer, Vaccinogen, Inc., USA, Tel: 301-793-7736; E-mail: mghannajr@vaccinogeninc.com

Received date: July 22, 2014, **Accepted date:** October 28, 2014, **Published date:** October 31, 2014

Introduction

There are a large number of experimental cancer immunotherapies being tested in clinical trials of advanced disease patients, an underserved patient population. Although there are heralded, isolated responses in some patients, most of these trials have shown minimal clinical benefit on a societal level. The current clinical and basic research is focused on reducing immune suppressive mechanisms that are present in tumors, and include treatment with monoclonal antibodies, ipilimumab and MDX-1106 which block Cytotoxic T Lymphocyte Activation-4 (CTLA-4) [1,2], Programmed Death-1 (PD-1) [3] inhibitory molecules on T cells, respectively. In addition to suppression via molecules such as CTLA-4 and PD-1, myeloid lineage cells constitute a network of immune suppressive cells that are present in most cancer patients and which profoundly inhibit the expression of anti-tumor immunity. This network includes myeloid-derived suppressor cells (MDSC), tumor-associated macrophages (TAMS), and dendritic cells (DC). Each of these cell populations has inherent immune suppressive activity, which is enhanced through their interactions with each other [4]. Most of the basic research on suppressor cell development and function originated in mouse models using transplantable tumors and the spleen has been implicated as the seat of this suppressor cell activity [5-8].

By blocking or circumventing these immune suppressive factors, these targeted therapies are designed to unleash the inherent immune response, either as monotherapies or in combination with traditional cytotoxic chemotherapy. The ultimate result of either strategy should/could improve the treatment of established, late stage disease patients. While these investigations have provided a novel direction for enhancing cancer immunotherapy, additional technologies still need to be developed to specifically identify tumor-associated antigens to mobilize the full power of an active anti-tumor immune response.

Active specific immunotherapy (ASI) has the potential to be that transformative technology by embracing the recently demonstrated genomic heterogeneity of tumor cells, through the use of live, metabolically active autologous tumor cells which represent the entire antigenic diversity of each patient's primary tumor.

ASI involves generating a robust, cytotoxic cell mediated, immune reaction against tumor cells. This concept is rooted in the reality that patient-derived vaccines can induce a potent and long-lasting immune response against TAAs capable of eliminating metastases and/or preventing recurrence of cancer. If immunomodulatory agents are capable of rearming the immune system against tumors, then ASI will serve as the guidance system.

The early claims of the role of the immune reactions for cancer treatment came from reports of infectious agents reducing or eliminating localized tumors both in animal models and man. More than a century ago, Dr. William Coley, a surgeon, was amazed that an aggressive sarcoma diagnosed in his patient, disappeared after the patient suffered a Streptococcus pyogenes infection following surgery. Dr. Coley speculated that the immune response to the bacterial infection played an integral role in fighting the disease [9]. Thus the innate immune response contributed too or provided antitumor therapy. Dr. Coley subsequently developed and tested the effect of injecting dead bacteria into the human tumors in an attempt to achieve the therapeutic effect while avoiding the risk of fatal infection.

Dr. Coley died in 1936. It is a fact that 54 years later, the first microbial vaccine approved by the US Food and Drug Administration and the European Medicines Agency, for the treatment of cancer was Bacillus Calmette-Guerin (BCG). In 1976, Morales et al. [10] were the first to report the use of BCG for treatment of non-muscle invasive superficial bladder tumors. Subsequently, numerous prospective randomized clinical trials demonstrated the efficacy of intravesicle BCG therapy for therapy of Carcinoma-in-situ (CIS) and later for preventing the recurrence with progression, of superficial papillary bladder cancer (Tables 1 and 2). The history of BCG and the application to treatment of bladder cancer is reviewed in reference [11]. It seems that the immune system is triggered by the admixing of the BCG attaching to the tumor at the wall of the bladder and this is often considered to be more inflammation by the innate immune response possibly with an adaptive immune response working together to provide immune mediated tumor elimination. As background to the findings of Dr. Coley, more than a century ago and the regulatory approval of BCG for treatment of bladder cancer in 1990, review of the history of BCG research in experimental animal models would be beneficial.

	Entered	Evaluable	CR*	CRNC**	Overall Response
No. of Patients	153	119	54	36	90
Percent Response		---	45.40%	30.20%	75.60%

*No evidence of disease on cystoscopy and a negative cytology (CR)

**No evidence of disease on cystoscopy and not validated by cytology (CRNC)

Table 1: Response of Patients to TICE BCG in CIS Bladder Cancer

Antitumor Activity of BCG in Experimental Animal Models

BCG has the potential of acting as a nonspecific immunopotentiating agent. This property of BCG probably is not initiated by a single mechanism but is a consequence of a cascade of

immunologic events involving a range of cells, including T-cells, B-Cells, macrophages and NK cells, all potentiated by a variety of induced lymphokines and cytokines [12-15]. Thus, BCG infection induces immune cell mobilization, homing and a wide range of protective effects against microorganisms and protozoans as well as various viruses [16-20]. A thoroughly studied BCG-mediated effect on macrophages includes increase in metabolic activity, release of extracellular enzymes, migration, chemotaxis and pinocytosis [21-22]. Macrophage activation seems at least partly a T-cell dependent phenomenon as reported for nonspecific resistance for bacteria [23] whereas in tumor-bearing mice, macrophage activation by BCG may occur independently of T-cells [24].

Treatment Schedule for Carcinoma *In Situ*					
Investigator*	**Indications**	**Induction**	**Maintenance**	**Dose (mg)**	**Route**
Guinan (1883)	CIS Prophylaxis	Weekly x 6	Monthly x 12	50	Intravesical
Lamm (1414)	CIS Prophylaxis Invasive Diagnosis	Weekly x 6	At 8, 10, 12 weeks; 6 months; every 6 months x 4 years	0.5 & 50	Intravesical
Khanna (2951)	Low Risk High Risk/CIS	Weekly x 6	Monthly x 12, Every 3 months x 8 Every 6 months x 4	50	Intravesical
Brosman (1111)	CIS Existing Tumor Prophylaxis	Weekly to CR	Every 2 weeks x 3 Months Monthly x 2 years	50	Intravesical
De Kernion (1571)	CIS Existing Tumor Prophylaxis	Weekly x 8	Monthly x 12	50	Intravesical
Williams (2427)	CIS Prophylaxis	Weekly x 6	Monthly x 12	50	Intravesical
*Investigational New Drug Application. Study numbers in parentheses.					

Table 2: TICE BCG Series: Summary of Study Features

Old et al. [25] demonstrated prophylactic activity of BCG resulting in prevention or delayed occurrence of tumors, sarcomas, and carcinomas tumor models in mice. Similar results were found for leukemia in the mouse [26] and epithelioma in the rat [27]. Subsequent studies in mice, rats, hamsters, and guinea pigs using transplants of spontaneously arising, viral-induced or chemically induced tumors confirm that systemic prophylaxis with BCG can exert an inhibitory or in some cases, stimulating effect on tumor transplants [28]. The complete suppression of tumor growth was observed in guinea pig [29-31] mouse [32-33] and rat [34-35] tumor systems when BCG was used locally or as an adjuvant in the administration of a mixture of BCG and tumor cells. In a relevant, inbred Strain 2 guinea pig model using the syngeneic Line-10 (L-10) hepatocarcinoma tumor, a tumor-specific immunity was observed when BCG was used intratumorally or as an admixture with transplantable tumor cells [36-37]. This is clear evidence of the BCG induced innate immune response working in tandem with the adaptive immune response and the resultant clinical benefit with respect to tumor elimination and/or the prevention of recurrent disease.

A major factor in interpreting the design of effective clinical immunotherapy trials with BCG was the dilemma of timing and dose.

The paramount influence of timing was shown in a study of transplantable L-10 hepatocarcinoma tumor cells in guinea pigs by Hanna et al. [38]. When animals were inoculated with live tumor cells on day 0 and were immunized with a tumor cell plus BCG vaccine on days 1,4,7 or 10, protection was inversely proportional to time of treatment and thus, tumor burden (Figures 1 and 2).

This important demonstration of optimum timing factors should provoke the reassessment of many past preclinical and clinical studies to optimally evaluate immunotherapy and its possible interaction with other conventional therapies.

Another major factor in assessing the preclinical studies has been the problem of dose of BCG. A commentary by David Weiss [39] decried over dosages and overscheduling in immunotherapy trials because "overkill in immunology is reached with deceptive ease." There are experimental data that may be relevant to this point. These data are derived from two different tumor models. In the first model, BCG was used as an immunotherapeutic approach to immunize guinea pigs against L-10 tumor cells by admixture of BCG and tumor cells injected intradermal. This is not dissimilar to intravesical administration, where BCG is admixed with tumor cells in the bladder, thus stimulating an immunologic reaction that mediates bladder tumor regression. It has been established that variation in dosages of

BCG has a marked influence on percentage of survival of vaccinated guinea pigs injected with syngeneic tumor cells [40]. In general, the low adjuvant mixtures were ineffective and additional adjuvant above the optimum dose (10^7 BCG) was not proportionally beneficial (Figure 3). These findings were consistent with clinical studies of Lamm [41] in a murine transitional bladder cell carcinoma.

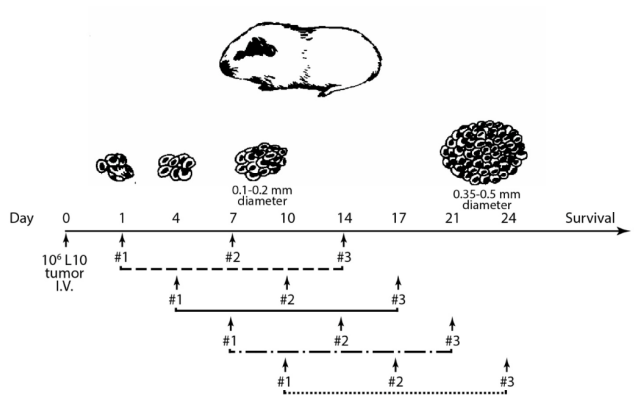


Figure 1: Experimental studies of active specific immunotherapy in the guinea pig tumor model system---Schema. Hanna Jr MG, Hoover Jr HC, Peters LC, Key ME, Haspel MV, et al. 1987. Fundamental and applied aspects of successful active specific immunotherapy of cancer. Principles of Cancer Biotherapy, edited by RK Oldham, Raven Press Ltd, New York.

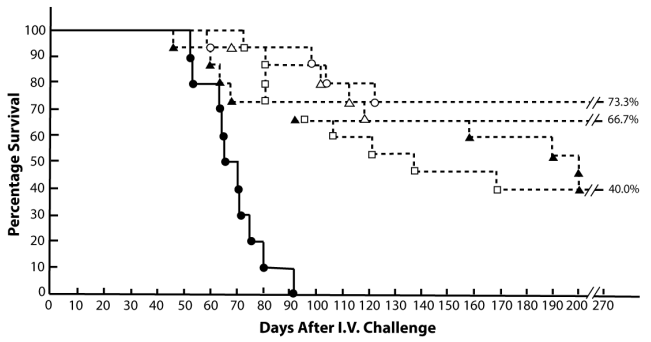


Figure 2: Experimental studies of active specific immunotherapy in the guinea pig tumor model system: Percentage survival as a function of time after challenge with 10^6 L10 cells i.v. Vaccinations 1+2=10^7 BCG + 10^7 L10; 3: L10 alone. (●) control; (○) 3 vaccinatgions, days 1, 7, 14; (Δ) 3 vaccinations, days 4, 10, 17; (□) 3 vaccinations, days 7, 14, 21 (▲) 3 vaccinations, days 10, 17, 24.

BCG was able to induce significant response rates in doses ranging from 10^5 to 10^7 colony-forming units (CFU) per animal, whereas doses in excess of 10^7 CFU were found to decrease the antitumor response. BCG and other immune modulators, are basic in action and, contrary to the major features of other systemic drugs, do not demonstrate a precise dose-response curve. Cytotoxic chemotherapy typically produces a dose-response curve that shows that the higher the dose, the higher the benefit. This is not the case with immunotherapy. Immune modulators do not demonstrate single-hit kinetics. Variations in timing, dosage and frequency of many biologic response modifiers (BCG in particular) can produce opposite effects that are critical to outcome. The rule is "more is not always better."

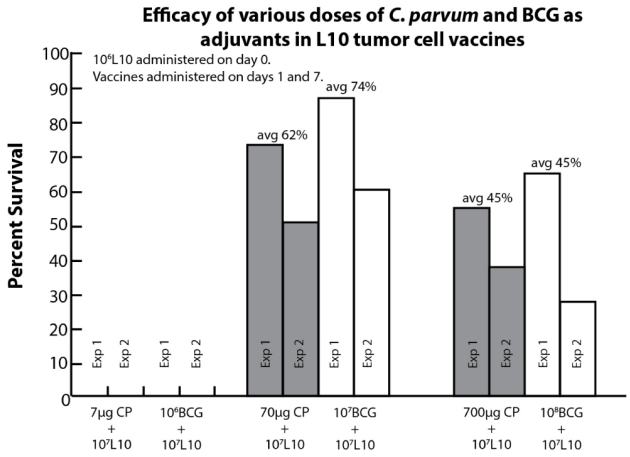


Figure 3: Efficacy of various doses of *C. parvum* and BCG as adjuvants in Line-10 (L-10) tumor cell vaccines.

	No. of Survivors/ Total No. of Animals/ Group at Following i.v. Tumor Cell Dose	
Treatment[a]	**1 × 10^5**	**1 × 10^6**
None	0/10	0/10
(10^8 BCG) (10^8 BCG)	0/10	0/10
(10^7 L10) (10^7 L10)	0/10	0/10
(10^7 BCG + 10^7 L10)[b]	1/10	0/10
(10^8 BCG + 10^7 L10)[b]	2/10	0/10
(10^6 BCG + 10^7 L10) (10^6 BCG + 10^7 L10)	1/10	1/10
(10^7 BCG + 10^7 L10) (10^7 BCG + 10^7 L10)	10/10	3/10
(10^8 BCG + 10^7 L10) (10^8 BCG + 10^7 L10)	10/10	5/10
(10^8 BCG + 10^7 LV[c] L10) (10^8 BCG + 10^7 LV L10)	6/10	1/10

These experiments were terminated at 240 days after tumor injection. All non-treated controls in the 10^5 group died by 95 days and all non-treated controls in the 10^6 group died by 77 days. Significance of differences in survival was calculated by the Fisher 2-tailed exact test.

[a]Vaccinations were administered i.d.,6 days apart on opposite sides.

[b]Vaccination was administered i.d., as a single injection.

[c]Low viability tumor cells.

Table 3: Survival of Guinea Pigs Given i.v. Injections of 1 x 10^5 or 10^6 Syngeneic L10 Hepatocarcinoma

Dose and timing of treatment was tested in guinea pigs with regards to BCG injections alone, tumor cell injections alone or admixture of

BCG and tumor cells. Two modes of immunization and 3 ratios of viable BCG to tumor cells were tested in guinea pigs injected intravenously (iv) with L-10 tumor cells. The BCG tumor-cell ratios were 1:10, 1:1, and 10:1, respectively. The doses were injected as single intradermal (id) injections or two id immunizations separated by 6 days. The survival results are shown in Table 3.

Compared to the untreated tumor-bearing guinea pigs, no significant difference in survival was detected in animals treated with 2 id injections of BCG or tumor cell alone. Single BCG + tumor cell immunizations at a ratio of 1:10 or 10:1 did not lead to significant protection from systemic disease. Compared to those animals that received single injections of BCG + L-10, BCG or tumor cells alone, and the untreated controls, significant differences in survival were achieved in tumor bearing guinea pigs when the second vaccination was an identical BCG L-10 mixture. Survival in these treatment groups was clearly a function of the BCG L-10 cell ratio.

These studies in the inbred strain 2 guinea pig model using the transplanted, progressor, syngeneic L 10 hepatocarcinoma demonstrated that BCG, admixed with tumor cells, could induce a degree of systemic tumor immunity that would eliminate a small disseminated tumor burden. To be effective though, careful control of such variables as the number of viable, but nontumorigenic tumor cells (10^7 optimal), at the ratio of viable BCG organisms to tumor cells (1:1) and the vaccination regimen three vaccinations, one week apart was required. BCG was not essential in the third vaccination. This allowed for the measurement of delayed-type hypersensitivity (DTH) measurements as a measure of vaccine potency and immune status. The magnitude of the DTH directly correlated with clinical benefit.

The percentage of viable tumor cells in the vaccine was strongly correlated with the efficacy of the vaccine. Vaccines with low tumor cell viability (less than 30%) were less effective than vaccines with high tumor cell viability (more than 80%). Another critical factor in vaccine efficacy was the dose of the adjuvant BCG. Variation in dosage with constant tumor cell dosage (10^7) had a marked influence on percentage survival of tumor bearing guinea pigs (Table 3, Figure 3).

Clinical Application of Cancer Vaccines with BCG: Active Specific Immunotherapy

The result of the immunotherapy studies in animal models and treatment of human bladder cancer with the BCG vaccine supported the enthusiasm for the specificity of ASI as a rational modality for cancer treatment and developing cancer vaccines as a means of achieving tumor-specific immune responses for disseminated disease. However, the majority of cancer vaccines have failed in practice [42]. Over the last decade, the failure rate of these treatments in phase II/III clinical trials is over 70%. If we intend to make meaningful progress with vaccine-based cancer treatments, we need to resolve this glaring discrepancy between theory and practice.

First, almost all of the failed vaccine trials were conducted in patients with advanced, late stage disease as a primary or salvage treatment to improve overall survival. This is not the patient population that successful vaccines have achieved clinical benefit. These patients are often heavily pretreated and have extensive disseminated disease. In this setting, these immune-base treatments are expected to be effective within a well-established, tumor microenvironment that is often immunosuppressive. As mentioned earlier, we now have considerable evidence that tumor-infiltrating lymphocytes (TILs) demonstrated an "exhausted" phenotype initiated by molecular interactions within the tumor cells. Specifically molecules such as members of the PD-1/PDL-1 axis negatively regulate the efficacy of these immune responses [3]. These critical interactions prevent cytotoxic T-cell responses against cancer cells, essentially cloaking them from the immune system. Thus, even with a systemic, robust immune response, the functional immunocompetant cells are suppressed within the primary tumor.

It is well established that a general consideration in vaccine development is antigen discovery, the selection of the most informative targets. With respect to cancer vaccines the ideal targets should be tumor specific. The history of cancer vaccines is replete with the failure of cancer vaccines that were constituted by too limited or nonrepresentative antigen targets. Also, it is important to use the intended study population to assess the proportion of tumors that express the targets of choice and the proportion of cells within each tumor that express it. Thus, it should be a goal in the antigen discovery phase of vaccine development to actively search for a maximum number of shared antigens that most effectively define a patient population of interest.

However, this stipulation would require a disease with significant inter- and intra-patient homogeneity. This flawed approach is complicated by the fact that in all cancers, there is a staggering degree of heterogeneity within established tumors and between patients of a given cancer type.

Tumor Heterogeneity and the Impact on Antigen Discovery in Cancer Vaccine Development

Based on genomic analysis there is validation of both intra-tumor and inter-tumor heterogeneity. An excellent example of the inter-tumoral heterogeneity inherent to cancer was provided by Wood et al. [43]. To answer the question, these investigators asked "how many genes are mutated in a human tumor?" Applying the latest DNA sequencing technology to a cohort of breast and colorectal tumors, they reported roughly 80 mutations that alter critical amino acids were evident in a typical tumor.

About 95% of these mutations are single-base substitutions, whereas the remainders are deletions or insertions. By definition, the resulting altered proteins are unique from the perspective of the immune system and all are candidates for potent immunological markers or TAAs. However, when the sequencing results of individual tumors are visualized as mutational landscapes, a troubling view emerges (Figure 4). Despite sharing a similar number of mutations, breast and colorectal cancers demonstrated very different results with respect to the type of mutations and specific genes mutated. Of the ~80 mutations in an individual tumor, only about 3 of these mutations were shared between two different tumors. Additionally, many of the most common mutations are observed within intracellular signaling molecules (p53, P13K, etc.) that may not be effectively presented to the immune system. Consequently, a polyvalent cancer vaccine is technically limited from providing the diversity required to stimulate an appropriately robust and therapeutic immune response across a given patient population. Based on these results, antigen discovery for the development of "off the shelf" cancer vaccines takes on a new level of complexity and is fraught with logistical hurdles.

We have simultaneously gained a greater appreciation for the troubling degree of intra-tumoral heterogeneity inherent in this disease. Recently, two definitive studies have proven that individual tumors are comprised of many distinct clonal populations. Yachida et

al. [44] were able to demonstrate this in pancreatic tumors while Swanton and colleagues [45] showed this in renal-cell cancer. Based on multiple biopsy samples from each patients primary and metastatic tumor sites, about two thirds of the mutations that were found in single biopsies were not uniformly detectable throughout all the sampled regions of the same patients tumor.

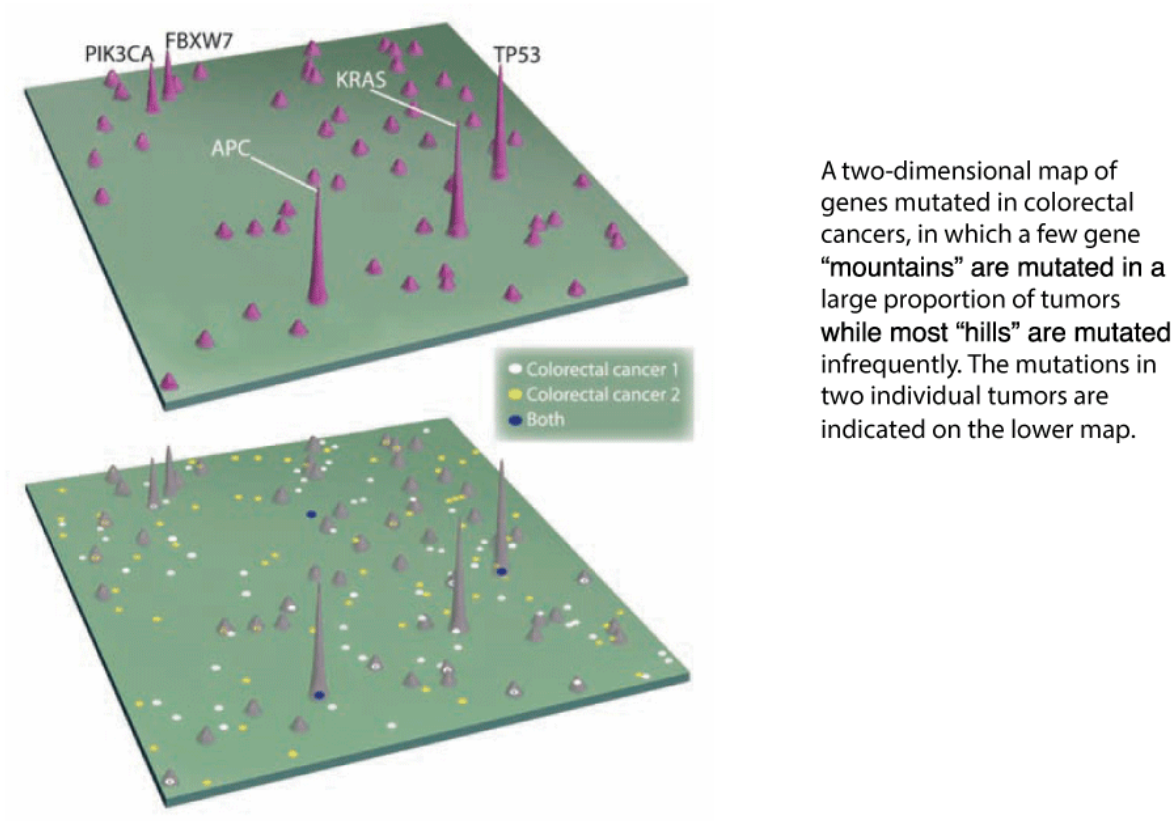


Figure 4: Genomic Landscape of Colorectal Cancer, Wood et al. [43]. A two-dimensional map of genes mutated in colorectal cancers, in which a few genes "mountains" are mutated in a large proportion of tumors while most are mutated infrequently. The mutations in two individual tumors are indicated in the lower map. Note that only 3 mutations (blue dots on bottom landscape) were common to both tumors indicating a potential for weak common immunogenicity.

Undoubtedly, future studies will demonstrate that this level of intra-tumoral heterogeneity is a general feature of cancer. While inter-tumoral heterogeneity calls into question the logic of the cancer vaccine trials of the past, intra-tumoral heterogeneity challenges the promise of "personalized medicine." A major focus of cancer research today is profiling patient-specific mutations such that appropriate targeted agents can be used in a rational manner to treat primary disease. Given the degree of intra-tumoral heterogeneity how can a random biopsy be expected to adequately represent the complexity of the entire tumor? How many biopsies are required? What clones with known resistance lay undetected in the remaining tumor? This leads to the provocative yet critical question, is tumor heterogeneity of any practical value and how does one embrace heterogeneity in cancer treatment? With respect to cancer vaccines, the answer is employing a means of antigen discovery that is inclusive, highly adaptable and exquisitely sensitive utilizing the entire array of parenchymal tumor cells as source material.

An autologous cancer vaccine, or the process of using a patient's own tumor as source material for an individualized treatment, is not a new endeavour. However, given what we now know about tumor heterogeneity, we are primed to deploy these tools in the appropriate way. Using powerful, genomic sequencing technology and an updated understanding of tumor-immune system interactions, we now have the ability to design tools capable of addressing the biological realities of cancer. We are at the cusp of a renaissance for ASI, assuming we follow a basic set of guidelines.

- While antigen discovery platforms of the past emphasized the use of common antigens, based on tumor homogeneity, there is now indisputable evidence cancer is comprised of extreme genetic diversity from an inter- and intra-tumoral standpoint. It is now illogical to treat a heterogeneous disease with homogeneous tools.
- As immunologists, we are aware on one highly adaptable, exquisitely sensitive tool provided by evolution to address the magnitude of cancer diversity – the immune system.

- No longer can we use cancer vaccines to inappropriately treat established or advanced disease. We must be focused on preventing recurrence in the adjuvant setting by curing minimal residual disease (MRD). In this way, latent disease, which has not yet established a tumor microenvironment, but is certainly capable of doing so later, would be the therapeutic target. This has opportunity of significantly impacting cancer mortality as the majority of cancer patients (~80%) die due to recurrence.
- In the clinical setting described above, extending recurrence-free survival (RFS) should be the primary endpoint of autologous cancer vaccines. Overall survival will serve as a secondary clinical endpoint. A schematic that emphasizes this last point is provided in Figure 5.

An Autologous Tumor Vaccine: OncoVAX

OncoVAX immunotherapy is a patient-specific (personalized) vaccine composed of irradiated, but metabolically-active, autologous tumor cells compounded with TICE® BCG, a live, attenuated mycobacteria which serves as a potent adjuvant. Using a proprietary method for dissociating and purifying cancer cells from a resected tumor, this autologous vaccine induces a robust and functional immune response. By using the entire tumor and relying on the immune system to determine which epitopes are unique, the vaccine provides a treatment in which no preconception of "known" or shared tumor antigens is needed. However, a series of steps were required to bring this treatment from proof of concept to therapeutic reality.

The first randomized, multicenter, Phase III clinical trial [46] for OncoVAX was attempted in stage I/II/III colon cancer patients under the auspices of the Eastern Cooperative Oncology Group (ECOG). While the final results showed no significant clinical benefit, this study was instructive for a number of reasons.

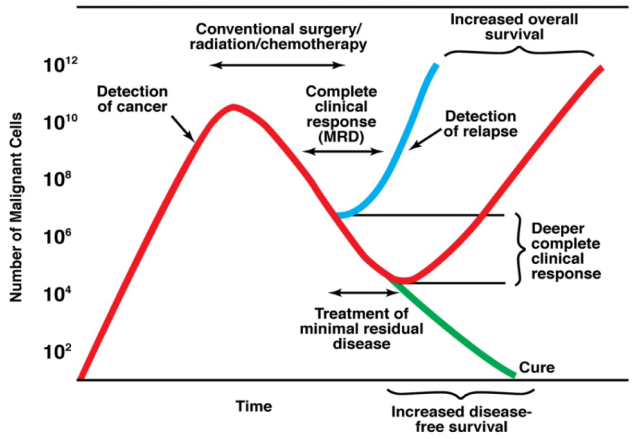


Figure 5: Best Hope for Significant Progress with Solid Tumors is via Treatment of Minimal Residual Disease.

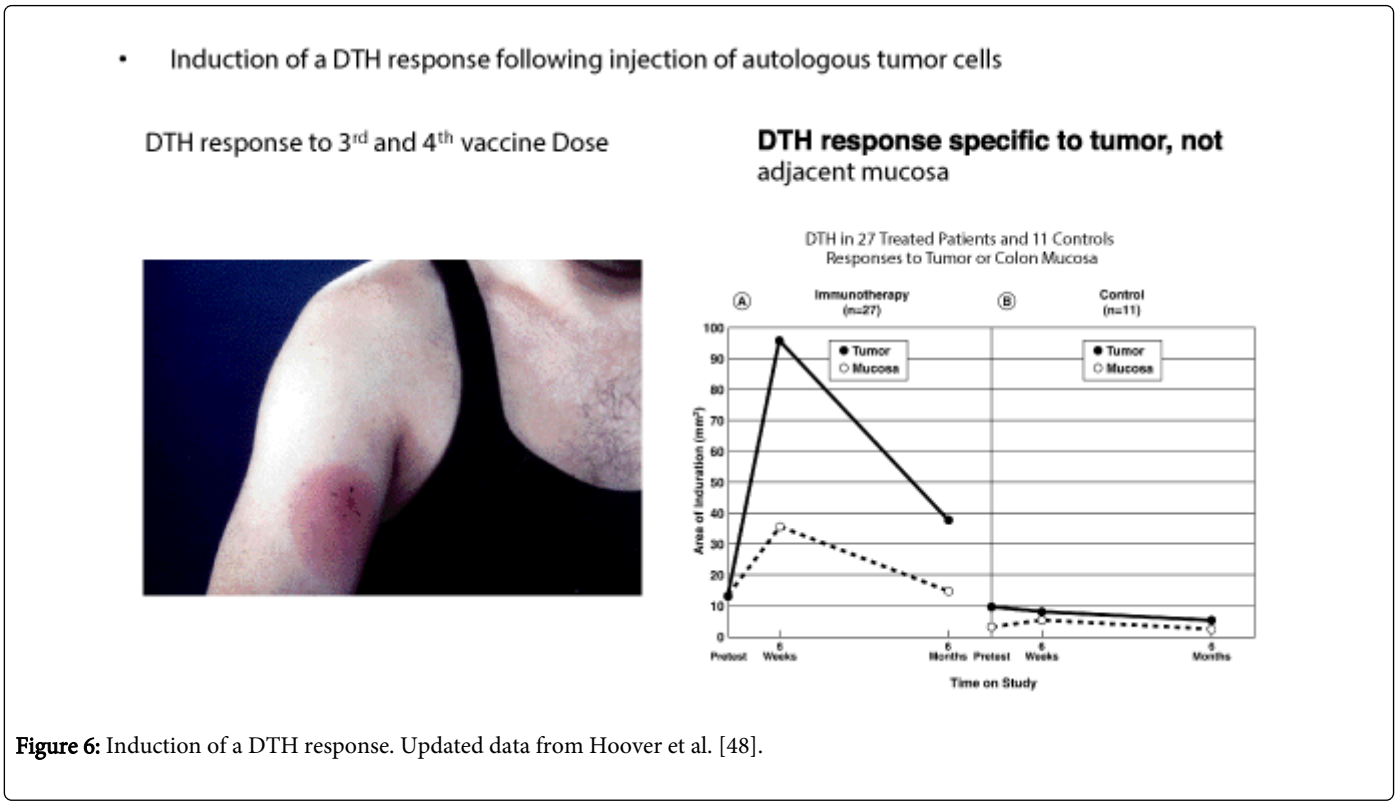


Figure 6: Induction of a DTH response. Updated data from Hoover et al. [48].

First, vaccine preparation was accomplished in a decentralized fashion, with each clinical site manufacturing the autologous vaccine in their respective pathology departments. Due to the logistical realities of OncoVAX preparation, this study clearly demonstrated the requirement for a central manufacturing facility to assure adequate quality control (QC) and quality assurance (QA), providing a more standardized approach to vaccine production. Additionally, this oversight needed to extend from the primary facility to the clinical sites where the final vaccine was compounded with TICE® BCG. Secondly, based on the results in the guinea pig model, the treatment

protocol for this study only involved three intradermal vaccine injections, delivered each week beginning 28 to 35 days after tumor resection. The first two injections were compounded with TICE® BCG while the third vaccination was comprised autologous tumor cells alone. The final injection without adjuvant is critical for monitoring whether the immune system has been trained to react to cells previously defined as "self." Active and potent immune responses toward these cells manifest as a DTH reaction visible at the site of injection (Figure 6). This visible response is still the best *in vivo* indication of T-cell specificity and activity. Indurations greater than 5 mm are considered a significant indication of a specific T-cell response. Additionally, this reaction serves as proof of concept that with prior adjuvant stimulation the immune system has been trained to recognize these cells, and hopefully any MRD remaining after surgery. Not surprisingly, induration size correlates well with patient outcome (Figure 6).

Lessons learned from the previous study were incorporated into the next Phase III clinical trial (8701). This study [47] utilized a centralized manufacturing facility to address the QC and QA issues encountered in the previous trial. This required processing to occur within a reasonable geographical area, consequently, production was centralized at the Free University in The Netherlands, a reasonable distance from the 12 Dutch hospitals participating in the trial. Additionally, pathologists participating in the study needed to modify their standard sampling procedures to provide maximum tumor material for vaccine production while allowing for adequate diagnosing and staging. Following resection and staging, tumor samples were sent to the production facility for dissociation, cryopreservation, irradiation, and administration. The treatment protocol was also augmented to include a four vaccine regimen: three initial weekly treatments (two with TICE® BCG, one without) and a six-month follow-up booster inoculation.

The follow-up booster was added based on the results of a side Phase II trial [48] that suggested initial immune responses begin to wane 6 months after the induction vaccinations (Figure 7). However, due to the addition of a fourth inoculation, larger tumors were required for sufficient vaccine production. With a minimum requirement of 3-3.5 grams of tumor, this trial was logistically limited to stage II/III patients. An additional study change involved stratifying patient randomization by tumor stage to power for a prospective analysis.

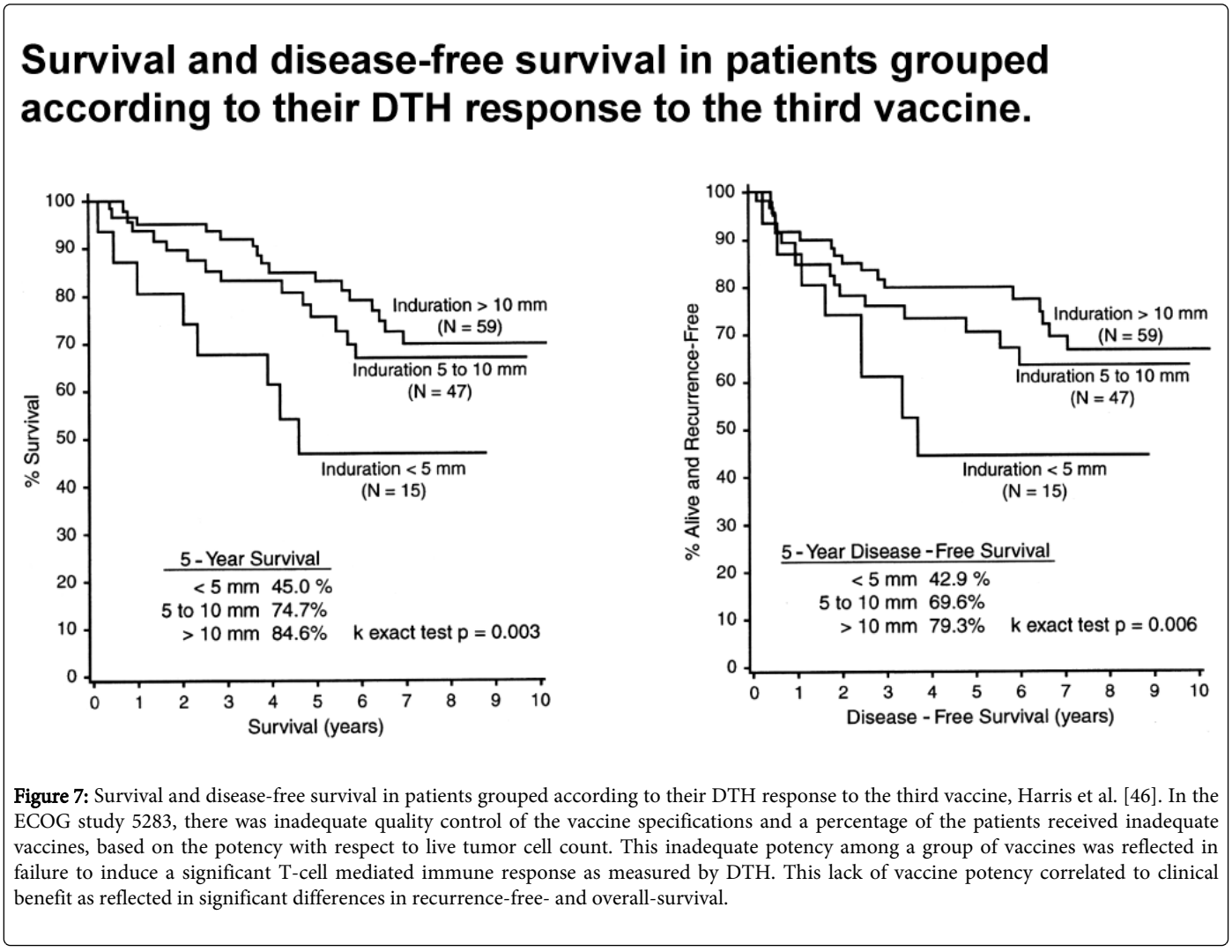


Figure 7: Survival and disease-free survival in patients grouped according to their DTH response to the third vaccine, Harris et al. [46]. In the ECOG study 5283, there was inadequate quality control of the vaccine specifications and a percentage of the patients received inadequate vaccines, based on the potency with respect to live tumor cell count. This inadequate potency among a group of vaccines was reflected in failure to induce a significant T-cell mediated immune response as measured by DTH. This lack of vaccine potency correlated to clinical benefit as reflected in significant differences in recurrence-free- and overall-survival.

Subjects 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), patients received the four vaccine program outlined above. OncoVAX was well-tolerated, with 102 of 128 patients receiving all four vaccinations. To determine the extent of DTH reactivity, injection sites were measured for indurations 48 hours after the third and fourth immunizations. Subjects were defined has having achieved cellular immunity if the average of both measurements were greater than 5 mm. By this criterion, 97% of patients achieved effective cellular immunity after the fourth inoculation.

When patient response in the OncoVAX cohort was determined during follow-up, in an Intent-to-treat analysis, no statistically significant differences in recurrence free survival (RFS), overall survival, or recurrence-free intervals (RFI) were observed. However, when a prospective analysis of patients was analyzed by stage, subjects with stage II disease had clinically meaningful and statistically significant outcomes in both RFI and RFS. Both five-year event-free rates and log rank rates were improved with OncoVAX treatment in stage II patients (Figures 8 and 9). The favorable 16.4% difference between control and OncoVAX patients represents a 41.4% relative risk reduction of disease progression (5-year survival p=0.008; log-rank analysis p=0.018). Overall survival (Figure 10) showed a statistically significant improvement in stage II OncoVAX treated patients (17.5%) over those patients in the control group (27.3%). The favourable 9.8% difference represents a 33.3% relative risk reduction (5-year survival p=0.014; log rank analysis p=0.074).

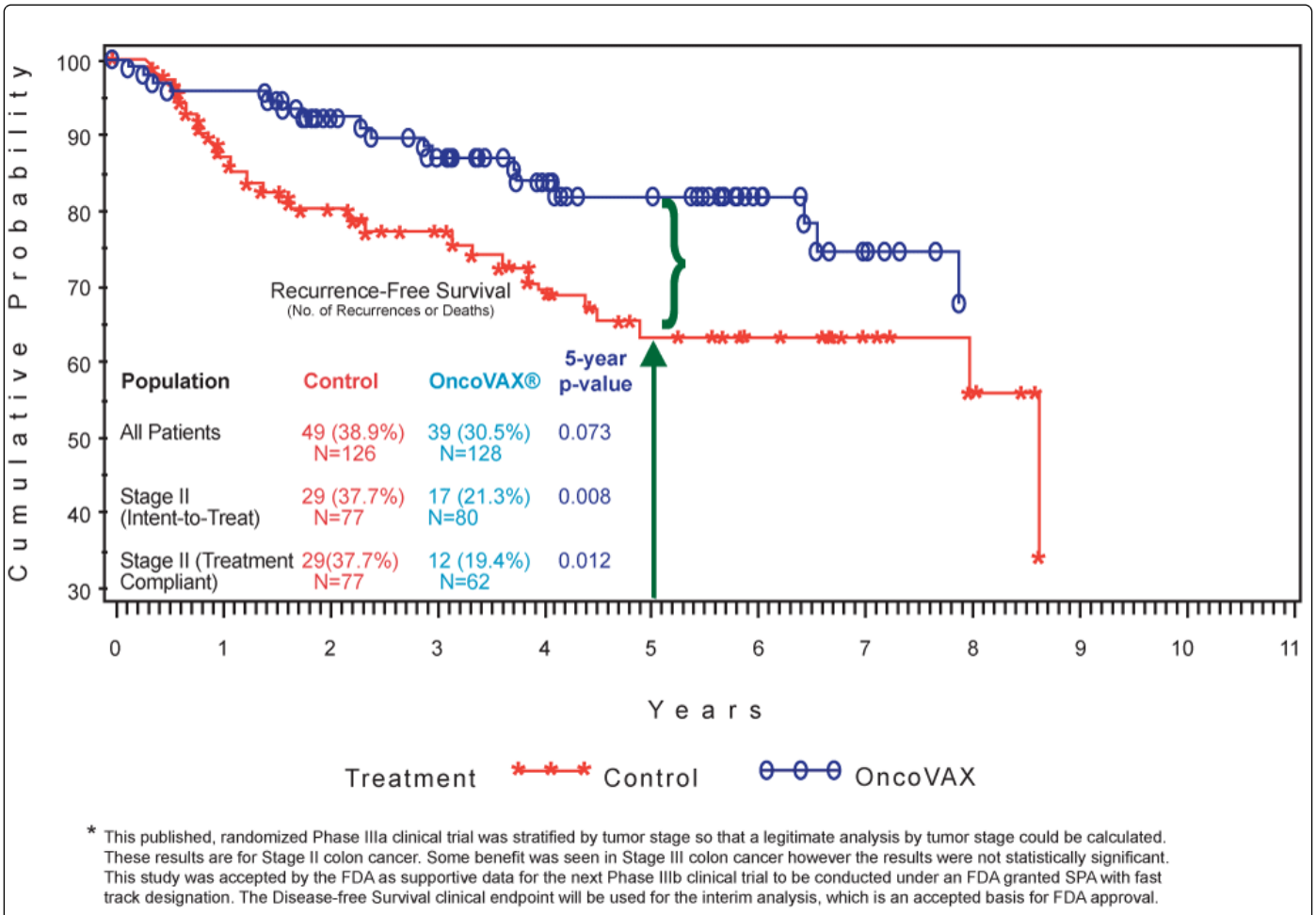


Figure 8: OncoVAX® – Clinical Results. 8701 Study – Recurrence-Free Survival* in Stage II Patients. The results were published in the British Medical Journal The Lancet January 30, 1999; 353: 345-350.

In the intent-to-treat (ITT) population of all randomized stage II patients, there were 43 recurrences. The five-year recurrence free interval p-value (0.01) 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, the control and OncoVAX 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 group (five year survival p=0.026; log-rank analysis p=0.008).

Trends towards efficacy in overall survival was not statistically significant in the full intent-to-treat population. A pre-specified stratification of the trial to analyze by tumor stage demonstrated that

Stage II patients separately reached statistical significance with a p value of 0.014 on a five year analysis.

Since this study was completed, surgical techniques associated with colon cancer treatment have greatly improved. Minimally invasive laparoscopic surgery has become more feasible than open colectomy, especially for patients without locally advanced disease. However, a recent multi-institutional study of 872 patients compared these surgical techniques and determined that while patients preferred the minimally invasive option, time to tumor recurrence was still equivalent after a median follow-up of 4.4 years [49]. These results have also been confirmed in T3 and T4A & B colon adenocarcinoma patients [50]. Thus, the recurrence-free interval curve in the surgical resection only control group is still valid today.

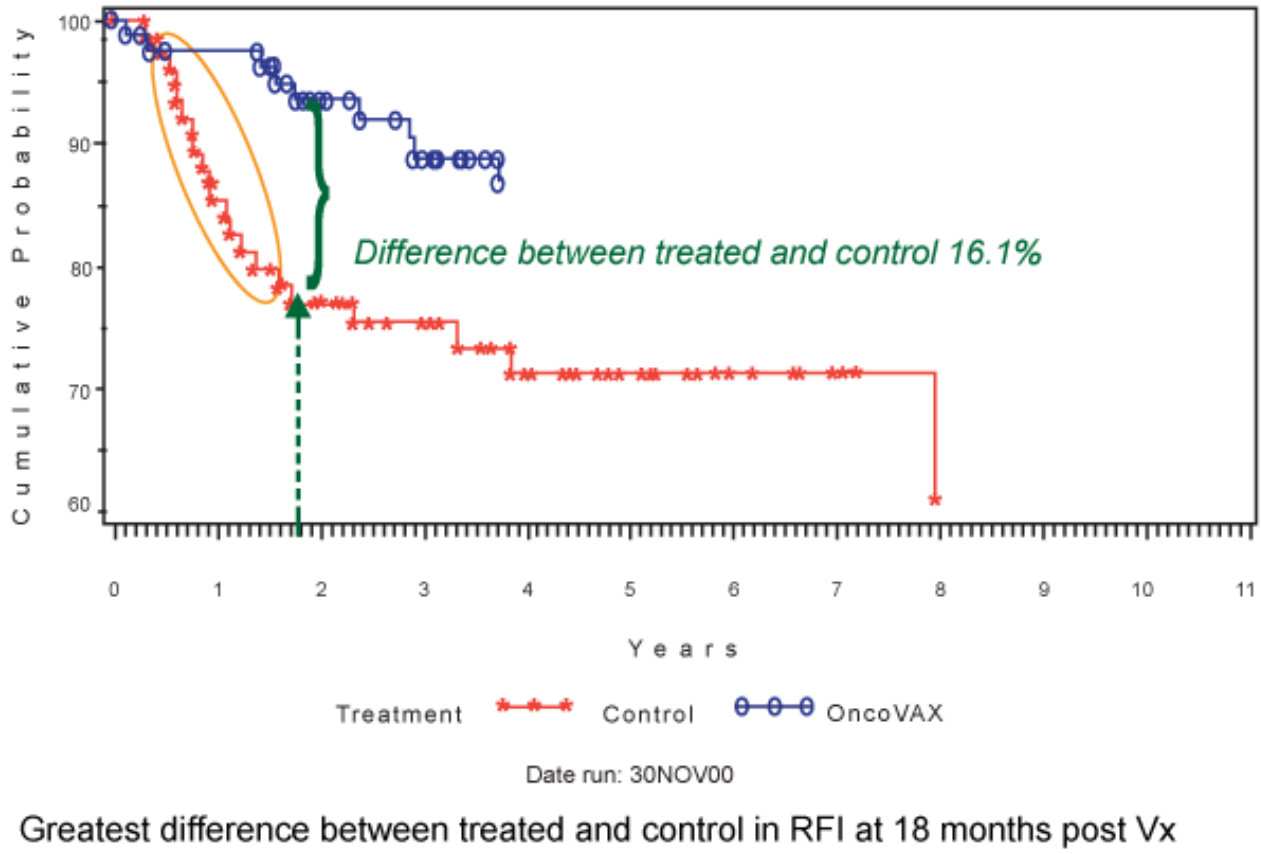
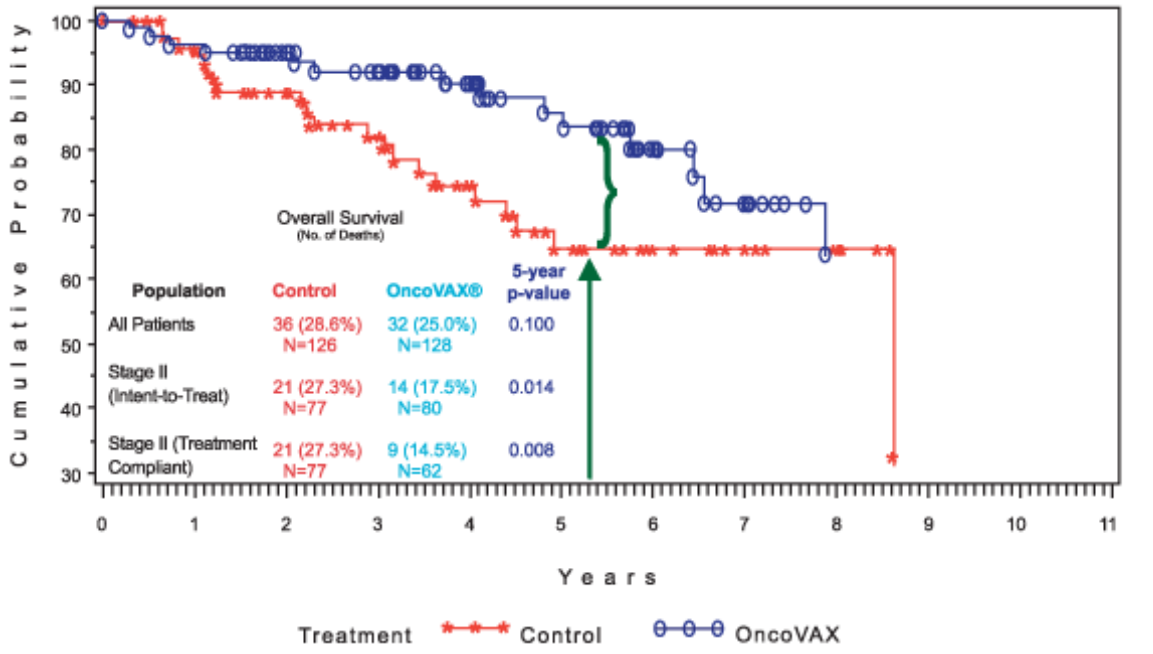


Figure 9: OncoVAX® – Clinical Results. 8701 Study – Recurrence-Free Interval in Stage II Patients.

A more recent study by de Weger, et al. [51] updated 8701 patient results with 15-year follow-up data. The event-free survival data are presented as a Kaplan-Meier plot in (Figure 11) for the original study (all 254 patients). OncoVAX patients still demonstrated improved survival compared to surgical patients alone at 15 year follow-up [HR=0.62 (95% CI: 0.40-0.96), p=0.033]. Using formalin-fixed paraffin embedded blocks from 196 of these patients, the authors also determined OncoVAX treatment was particularly effective for patients with microsatellite instability and microsatellite stable Dukes B tumors. The long-term, stable results observed with OncoVAX treatment can only be achieved with a robust immune response employing long-term immunological memory and surveillance. All of these aspects are essential prerequisites for successful and impactful cancer treatment.

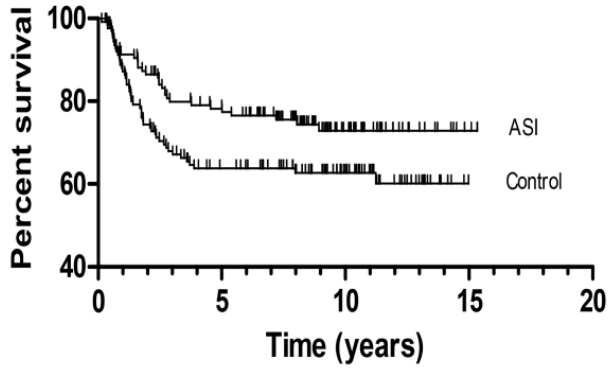
Safety was comparable in the OncoVAX treatment cohort compared to surgery alone. One patient treated with OncoVAX was hospitalized for treatment of a flu-like syndrome and the event resolved nine days later. Another patient required discontinuation of OncoVAX treatment due to a 21 x 32 mm ulceration which developed after the second inoculation (BCG had been omitted due to adverse events after the first inoculation). However, as a group, control patients more commonly experienced non-fatal serious adverse events. 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. Taken together, stage II colon cancer patients had fewer non-fatal serious events and improved recurrence-free and overall survival.

In the adjuvant setting, effective treatments are lacking for stage II colon cancer patients. To address this need, the FDA has requested a second, confirmatory, randomized controlled Phase III trial of OncoVAX in stage II colon cancer patients. 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.



Trends towards efficacy in OS was not statistically significant in the full intent-to-treat population. A pre-specified stratification of the trial to analyze by tumor stage demonstrated that Stage II patients separately reached statistical significance with a p value of 0.014 on a five year analysis.

Figure 10: OncoVAX® – Clinical Results. 8701 Study – Overall Survival in Stage II Patients.



Recurrence Free Interval original study population.

Figure 11: OncoVAX® – Clinical Results 15 year F/U. 8701 Study – Recurrence-Free Interval (RFI) in Stage II Patients. Survival time in years on the X-axis and the percentage Recurrence Free Interval on the Y-axis. Kaplan-Meier curves, comparing ASI with the control group in the original study population (n=254), show a significant better prognosis for patients who received adjuvant ASI therapy. (ASI versus Control at 15 year follow up; HR=0.62 (95% CI: 0.34-0.96) log rank p-value 0.033), de Weger et al. [51].

The primary endpoint of this pivotal Phase III trial is RFS with an interim and final primary analysis with one and three years follow-up, respectively. The study is powered to detect a 50% improvement in RFS with 90% certainty. If a robust statistical significance is achieved during the interim analysis (median follow up of 1.5 years or 70% of the expected events), the Biologic License Application (BLA) can be filed. Past clinical trials using the optimum four immunization regimen (8701) will be accepted as supportive studies during the FDA review of the BLA. This critical and careful approach to the clinical development of OncoVAX should allow for approval in stage II colon cancer patients, which remains a population of true "unmet medical need."

Conclusion

The preclinical cancer vaccine immunotherapy results, based on utilization of the inbred Strain 2 guinea pigs using BCG and the progressor L-10 hepatocarcinoma were valuable in defining and performing autologous tumor cell ASI in colon cancer patients. In humans, booster vaccinations were imperative; the three induction vaccinations were necessary but not sufficient for clinical benefit. The requirement for live metabolically active parenchymal cells was important and the cell numbers required for a robust response was comparable. The capability of detecting and measuring DTH 48 hours after the third and fourth vaccination which consists of live tumor cells alone, in both man and guinea pigs was beneficial to quickly determine the potency of the vaccines. These positive *in vivo* immune results compared directly with clinical benefit. In fact, the long lasting RFS

benefit at 5 years and as long as 15 years for occult disease in stage II colon cancer clearly supports a major, long lived immunological memory.

A major consideration we have been challenged with in the development and implementation of this immunotherapeutic approach was the logistics. Actually, for OncoVAX, this is the least concern compared to the FDA approved cancer vaccine, Provenge, and some of the other passive cancer therapies. The preparation of the tumor in surgery is handled as an organ transplant, transported by a specialized courier, centralized cGMP manufacturing process is non-complicated cytology, completed in 6 hours, resulting in a sterile, non-tumorigenic live tumor cell vaccine. This is frozen by controlled-rate freezing which is shipped back to the clinic frozen in LN2 and has one year shelf life.

The compounding of the tumor cells with BCG, the last step of the process is completed at the clinic using standard pharmacy procedures. For the pivotal Phase III clinical trial this step is being performed in a dedicated space. The big issue is the pharmacoeconomics. I headed the team that completed the essential clinical trials and submission of TICE BCG for successful regulatory approval for pre-invasive bladder cancer. We have always been proud that it remains today the most economically viable treatment for any cancer. I speculate that OncoVAX will be the most pharmacoeconomic immunotherapy with major, significant clinical benefit when used initially in an adjuvant setting for prevention of recurrence in stage II colon carcinomas, and in more advanced disease stages in combination with anti-immunosuppressive tools and possibly certain cytotoxic drugs. The cost per life year gained and quality adjusted life year gained will be very acceptable to primary health care insurers.

References

1. Leach DR, Krummel MF, Allison JP (1996) Enhancement of antitumor immunity by CTLA-4 blockade. Science 271: 1734-1736.
2. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, et al. (2010) Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 363: 711-723.
3. Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, et al. (2012) Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med 366: 2455-2465.
4. Ostrand-Rosenberg S, Sinha P, Beury DW, Clements VK (2012) Cross-talk between myeloid-derived suppressor cells (MDSC), macrophages, and dendritic cells enhances tumor-induced immune suppression. Semin Cancer Biol 22: 275-281.
5. Möller E (1965) Interaction between tumor and host during progressive neoplastic growth in histoincompatible recipients. J Natl Cancer Inst 35: 1053-1059.
6. Pollack SB (1971) Effect of host sex and splenectomy on moloney virus-induced sarcomas. Int J Cancer 8: 264-271.
7. Rumi L, Pasqualini CD, Rabasa SL (1971) Growth of sarcoma 180 in splenectomized mice bearing diffusion chambers containing spleen or tumor cells. Eur J Cancer 7: 551-555.
8. Hawrylko E (1975) Immunopotentiation with BCG: dimensions of a specific antitumor response. J Natl Cancer Inst 54: 1189-1197.
9. Hoption Cann SA, van Netten JP, van Netten C (2003) Dr William Coley and tumour regression: a place in history or in the future. Postgrad Med J 79: 672-680.
10. Morales A, Eidinger D, Bruce AW (1976) Intracavitary Bacillus Calmette-Guerin in the treatment of superficial bladder tumors. J Urol 116: 180-183.
11. Hanna Jr MG, DeJager R, Guinan P, Crispen R, Lamm, et al. (1992) Bacillus Calmette-Guerin (BCG) Vaccine for Tuberculosis: Antitumor Effect in Experimental Animals and Humans. Vaccine Research 1: 69-91.
12. Crispen R (1976) Action of immunotherapy agents. ITR Publishers, Chicago, USA: 185-192.
13. Bast RC, Bast BS (1976) Critical review of previously reported animal studies of tumor immunotherapy with nonspecific immunostimulants. Ann N Y Acad Sci 277: 60-93.
14. Davies M (1982) Bacillus Calmette-Guérin as an anti-tumor agent. The interaction with cells of the mammalian immune system. Biochim Biophys Acta 651: 143-174.
15. Mitchell MS, Murahata RI (1979) Modulation of immunity by bacillus Calmette-Guérin (BCG). Pharmacol Ther 4: 329-353.
16. Howard JG, Biozzi G, Halpern BN, Stiffel C, Mouton D (1959) The effect of Mycobacterium tuberculosis (BCG) infection on the resistance of mice to bacterial endotoxin and Salmonella enteritidis infection. Br J Exp Pathol 40: 281-290.
17. Sher NA, Chaparas SD, Greenberg LD, Bernard S (1975) Effects of BCG, Corynebacterium parvum and methanol extractions residue in reduction in mortality for Staphylococcus aureus and Candida albicans infection in immunosuppressed mice. Infect Immunol 12: 1325-1330.
18. Clark IA, Allison AC, Cox FE (1976) Protection of mice against Babesia and Plasmodium with BCG. Nature 259: 309-311.
19. Floc'h F, Werner GH (1976) Increased resistance to virus infections of mice inoculated with BCG (Bacillus calmette-guérin). Ann Immunol (Paris) 127: 173-186.
20. Spencer JC, Ganguly R, Waldman RH (1977) Nonspecific protection of mice against influenza virus infection by local or systemic immunization with Bacille Calmette-Guérin. J Infect Dis 136: 171-175.
21. Hibbs JB Jr (1974) Discrimination between neoplastic and non-neoplastic cells in vitro by activated macrophages. J Natl Cancer Inst 53: 1487-1492.
22. Meltzer MS, Jones EE, Boetcher DA (1975) Increased chemotactic responses of macrophages from BCG-infected mice. Cell Immunol 17: 268-276.
23. North RJ (1974) T cell dependence of macrophage activation and mobilization during infection with Mycobacterium tuberculosis. Infect Immun 10: 66-71.
24. Matsuo K, Takeya K, Nomoto K, Shimotori S, Terasaka R (1981) T-cell-independent activation of macrophages by viable BCG in tumor-bearing mice. Cell Immunol 57: 293-306.
25. OLD LJ, CLARKE DA, BENACERRAF B (1959) Effect of Bacillus Calmette-Guerin infection on transplanted tumours in the mouse. Nature 184: 291-292.
26. Biozzi G, Stiffel C, Hallpern BN, Mouton D (1959) Effect de l'inoculation du bacille de Calmette-Guérin sur le development de tumeur ascitique d'Ehrlich chez le souris. CR Seanc Soc Biol 153: 987.
27. Hallpern BN, Biozzi G, Stiffel C, Mouton D (1959) Effect de la stimulation du systeme reticuloendothelial par l'inoculation du bacillue de Calmette-Guerin sur le development de l'epithelioma atypique T-8 de Guérin chez le rat. CR Seanc Soc Biol 1953: 919.
28. Bast RC, Zbar B, Borsos T, Rapp HJ (1974) BCG and cancer. N Engl J Med 290: 1413-1458.
29. Zbar B, Bernstein I, Tanaka T, Rapp HJ (1970) Tumor immunity produced by the intradermal inoculation of living tumor cells and living Mycobacterium bovis (strain BCG). Science 170: 1217-1218.
30. Hanna MG Jr, Zbar B, Rapp HJ (1972) Histopathology of tumor regression after intralesional injection of Mycobacterium bovis. I. Tumor growth and metastasis. J Natl Cancer Inst 48: 1441-1455.
31. Hanna MG Jr, Zbar B, Rapp HJ (1972) Histopathology of tumor regression after intralesional injection of Mycobacterium bovis., 2. Comparative effects of vaccinia virus, oxazolone, and turpentine. J Natl Cancer Inst 48: 1697-1707.
32. Tanaka T, Tokunaga T (1971) Suppression of tumor growth and induction of specific tumor immunity by intradermal inoculation of a

mixture of living tumor cells and live Mycobacterium bovis in syngeneic mice. Gan 62: 433-434.

33. Chung EB, Zbar B, Rapp HJ (1973) Tumor regression mediated by Mycobacterium bovis (strain BCG). Effects of isonicotinic acid hydrazide, cortisone acetate, and antithymocyte serum. J Natl Cancer Inst 51: 241-250.
34. Baldwin RW, Pimm MV (1973) BCG immunotherapy of a rat sarcoma. Br J Cancer 28: 281-287.
35. Kreider JW, Bartlett GL, Purnell DM (1976) Suitability of rat mammary adenocarcinoma 13762 as a model for BCG immunotherapy. J Natl Cancer Inst 56: 797-802.
36. Zbar B, Bernstein ID, Bartlett GL, Hanna MG Jr, Rapp HJ (1972) Immunotherapy of cancer: regression of intradermal tumors and prevention of growth of lymph node metastases after intralesional injection of living Mycobacterium bovis. J Natl Cancer Inst 49: 119-130.
37. Hanna MG Jr, Snodgrass MJ, Zbar B, Rapp HJ (1973) Histopathology of tumor regression after intralesional injection of Mycobacterium bovis. IV. Development of immunity to tumor cells and BCG. J Natl Cancer Inst 51: 1897-1908.
38. Hanna MG Jr, Peters LC (1981) Morphological and functional aspects of active specific immunotherapy of established pulmonary metastases in guinea pigs. Cancer Res 41: 4001-4009.
39. Izak G, Stupp Y, Manny N, Wiess D (1977) The immune response in acute myelocytic leukemia, effect of methanol extraction residue function of tubercle bacilli (MER) on T- and B-cell functions and their relation to the course of the disease. Isr J Med Sci 13: 71-87.
40. Hanna MG Jr, Hoover HC Jr (1985) Human Tumor Antigens and Specific Tumor Therapy, Metzger R, Mitchell M, eds Alan R. Liss, New York 335-344.
41. Lamm DL, Reichert DF, Harris SC, Lucio RM (1982) Immunotherapy of murine transitional cell carcinoma. J Urol 128: 1104-1108.
42. Kudrin A, Hanna MG Jr (2012) Overview of the cancer vaccine field: are we moving forward? Hum Vaccin Immunother 8: 1135-1140.
43. Wood LD, Parsons DW, Jones S, Lin J, Sjöblom T, et al. (2007) The genomic landscapes of human breast and colorectal cancers. Science 318: 1108-1113.
44. Yachida S, Jones S, Bozic I, Antal T, Leary R, et al. (2010) Distant metastasis occurs late during the genetic evolution of pancreatic cancer. Nature 467: 1114-1117.
45. Swanton C (2012) Intratumor heterogeneity: evolution through space and time. Cancer Res 72: 4875-4882.
46. Harris JE, Ryan L, Hoover HC Jr, Stuart RK, Oken MM, et al. (2000) Adjuvant active specific immunotherapy for stage II and III colon cancer with an autologous tumor cell vaccine: Eastern Cooperative Oncology Group Study E5283. J Clin Oncol 18: 148-157.
47. Vermorken JB, Claessen AM, van Tinteren H, Gall HE, Ezinga R, et al. (1999) Active specific immunotherapy for stage II and stage III human colon cancer: a randomised trial. Lancet 353: 345-350.
48. Hoover HC Jr, Surdyke M, Dangle RB, Peters LC, Hanna MG Jr (1984) Delayed cutaneous hypersensitivity to autologous tumor cells in colorectal cancer patients immunized with an autologous tumor cell: Bacillus Calmette-Guerin vaccine. Cancer Res 44: 1671-1676.
49. Nelson H, Sargent DJ, Wieand HS, Fleshman J, Anvari M, et al (2004) A comparison of laparoscopically assisted and open colectomy for colon cancer. N Engl J Med 350: 2050-2059.
50. Benson AB 3rd, Schrag D, Somerfield MR, Cohen AM, Figueredo AT, et al. (2004) American Society of Clinical Oncology recommendations on adjuvant chemotherapy for stage II colon cancer. J Clin Oncol 22: 3408-3419.
51. De Weger VA, Turksma AW, Voorham QJ, Euler Z, Bril H, et al. (2012) Clinical effects of adjuvant active specific immunotherapy differ between patients with microsatellite stable and microsatellite instable colon cancer. Clin Cancer Res 18: 882-889.

This article was originally published in a special issue, entitled: **"Tumor Immunology"**, Edited by David J Vigerust, Vanderbilt University School of Medicine, USA