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Active specific immunotherapy: Using tumor heterogeneity to successfully fight cancer

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Introduction

Science recently highlighted cancer immunotherapy as the “2013 Breakthrough of the Year.” More to the point, they stated “this year marks a turning point in cancer, as long-sought efforts to unleash the immune system against tumors are paying off—even if the future remains a question mark”.1 Most of the celebration involves the compelling results of targeted agents designed to reactivate the immune system by manipulating the PD-1/PD-L1 and CTLA-4 pathways. By blocking these recently identified suppressor molecules, these targeted therapies are designed to unleash the immune system either as monotherapies or in combination with traditional cytotoxic chemotherapy. The ultimate result of either strategy should improve the treatment of established, late stage disease, a patient population that has yet to be adequately addressed with modern modalities. While these investigations have provided a novel direction for enhancing cancer treatment, additional technologies still need to be developed to specifically identify tumor-associated antigens (TAAs) to harness the full power of the immune system. 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, cell-mediated, cytotoxic immune reaction against tumor cells. This concept is rooted in the reality that patient-derived vaccines can induce a potent and lasting immune response against TAAs capable of halting or even eliminating tumors to prevent recurrence. If immunomodulatory agents are capable of rearming the immune system against cancer, then ASI serves as the guidance system. However, do we have physical evidence to suggest killer T-cells are capable of interacting with malignant cells in a therapeutic manner? There is a considerable amount of literature discussing the quantititative effect of in vitro T-cell mediated cytotoxicity, but there is a paucity of direct evidence. In a previous study, Bucana et al.,2,3 harvested immune lymphocytes and other white blood cells from the peritoneal cavity of guinea pigs that had previously been immunized and cured of a lethal, transplantable, syngeneic hepatocarcinoma. These primed immune cells, with proven in vivo cytotoxic capabilities, were then combined in vitro with the same tumor cell line and followed sequentially with time-lapse cinematography, scanning- and transmission-electron microscopy. In this manner, the authors were able to directly observe lymphocyte and macrophages interact with “familiar” cancer cells in vitro.

During co-culture, considerable migration of lymphocytes and other monocytes (later identified as macrophages), occurred on and around the tumor cells (Fig. 1). Two important observations characterized the interaction of lymphocytes and activated macrophages with the tumor cell surface. First, active extension and contraction of monocyte cytoplasmic processes was observed around and into the tumor cell surface. This “probing” was

Keywords: active specific immunotherapy, Bacillus Calmette Guerin (BCG), colon tumor, tumor heterogeneity, delayed-typed hypersensitivity, human monoclonal antibodies, tumor-associated antigens, T-cell responses, tumor immunity

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sustained for long periods of time without significant lateral movement of the activated monocytes. Thus, considerable clasmatosis of the T-cells and macrophages was observed at the tumor cell surface. Second, exocytosis of osmiophilic organelles from the monocytes to the tumor cells was observed. These organelles had the morphologic and histochemical characteristics of primary and/or secondary lysosomes. Additionally, it was clear that some of the lysosomal organelles were still within segments of monocyte cytoplasm that had detached at the tumor cell surface. Clasmatosis of macrophage or monocyte cytoplasmic extensions with these organelles would allow for tumor cell internalization of toxic proteinases initiating the dramatic cytotoxic events that were observed during the study.

Following cell lysis, the existing macrophages further phagocytized the ruptured tumor cell cytoplasm enabling the identification of secondary or previously “hidden” TAAs. Based on the studies above, the innate immune system already possesses the tools to recognize and destroy malignant cells if appropriately directed.

The early claims of immunotherapy for cancer came from reports of infectious agents reducing or eliminating localized tumors both in animal models and man (for review see Hanna et al., 6). In fact, the first vaccine approved by the US Food and Drug Administration for the treatment of cancer was Bacillus Calmette-Guerin (BCG). In 1976, Morales et al., 7 first reported the use of BCG for treatment of non-muscle invasive superficial bladder tumors. They reported a 12-fold reduction in recurrence rate of superficial bladder tumors following combined intravesical and intradraderal administration of BCG. Subsequently, numerous prospective randomized clinical trials demonstrated the efficacy of intravesical BCG therapy for therapy of Carcinoma-in situ (CIS) and later for preventing the progression and recurrence of superficial papillary bladder cancer. 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, thus categorized as active nonspecific immunotherapy.

These results 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 (Fig. 3). 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 these trials were conducted in patients with advanced, late stage disease as a primary or salvage treatment to improve overall survival. These patients are often heavily pretreated with extensive disseminated disease. However, we must understand these immune-based treatments are expected to be effective within a well-established, tumor microenvironment that is often immunosuppressive. As mentioned above, we now have considerable evidence that tumor-infiltrating lymphocytes (TILs) demonstrate an “exhausted” phenotype initiated by molecular interactions within the tumor cells. Specifically molecules, such as members of the PD-1/PD-L1 axis, negatively regulate

![Figure 1](image_url)

Figure 1. (A) Time-lapse cinematography of transplantable, L 10 hepatocarcinoma of syngeneic Strain 2 guinea pigs with 3 peritoneal monocytes from L 10 immune animals. Note the extensions from the monocytes (clasmatosis). (B) Implosion of the tumor cell. (C) Cytoplasm from tumor cell being phagocytized by macrophages.
the efficacy of these immune responses.8,9 This critical interaction prevents 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.

The second issue complicating cancer vaccine effectiveness is the staggering degree of heterogeneity observed within established tumors and between patients of a given cancer type. In 2007, a major review of active cancer vaccines outlined the various disappointing results in the field.10 One of the first general considerations of this review highlighted the importance of antigen discovery: “select the most informative targets.” The authors point out that ideal targets should be tumor-specific and “it is important to use the intended study population to assess the proportion of tumors that express the target of choice and the proportion of cells within each tumor that express it.” Thus, it should be a common goal within the field to actively search for a convenient 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.

**Tumor Heterogeneity**

The first significant evidence of phenotypic heterogeneity in tumors was described by Fidler and Kripke.11 They demonstrated that various clones of murine melanoma cells could be derived in vitro which varied greatly in their ability to produce lung metastases in syngeneic mice. This suggested that the parent tumor initially displayed a high degree of heterogeneity and clones with various metastatic potentials preexisted in the parental population. However, a competing view proposed by Peter Nowell12 around this same time, posited that cancer is a disease arising from a surviving mutant clone which progresses into an established tumor with a high degree of homogeneity reflecting its clonal origin. Clearly, the latter hypothesis has been well-represented in the majority of cancer vaccine clinical trials, with the former only recently understood. Fortunately, improvements in DNA sequencing technology have been able to definitively address this debate.

An excellent example of the inter-tumoral heterogeneity inherent to cancer was provided by Wood and Vogelstein.13 To answer this question, Wood et al., asked “how many genes are mutated in a human tumor”? Applying the latest DNA sequencing technology to a cohort of breast and colorectal tumors, the authors 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 remainder 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 (Fig. 4), a troubling view emerges. 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 2 different tumors. Additionally, many of the most common mutations are observed within intracellular signaling molecules (p53, PI3K, 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.

As our knowledge of inter-tumoral heterogeneity has expanded with improved DNA sequencing technology, we have simultaneously gained a greater appreciation for the troubling degree of intra-tumoral heterogeneity inherent to this disease. Recently, 2 definitive studies have proven that individual tumors are comprised of many clonal populations. Yachida et al. were able to demonstrate this in pancreatic tumors (Fig. 5) while Swanton et al. found similar results in renal cancer samples. Undoubtedly, future studies will demonstrate this level of intra-tumoral heterogeneity is a general feature of cancer. While intertumoral heterogeneity calls into question the cancer vaccine trials of the past, intratumoral heterogeneity challenges the promise of “personalized medicine.” The major focus of cancer research today is profiling patient-specific mutations such that appropriate targeted agents can be used in a rational manner to clear primary disease. Given the degree of intratumoral heterogeneity, how can a randomly chosen 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 highly adaptable and exquisitely sensitive utilizing the entire array of parenchymal tumor cells as source material.

Autologous cancer vaccines, or the process of using a patient’s own tumor as source material for an individualized treatment, is not a new endeavor. 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:

1. 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.

2. As immunologists, we are aware of one highly adaptable, exquisitely sensitive tool provided by evolution to address the magnitude of cancer diversity - the immune system.

3. 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 the opportunity of significantly impacting cancer mortality as the majority of cancer patients (~80%) die due to recurrence.

4. 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 which emphasizes this last point is provided in Figure 6.

**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 clinical trial 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. 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. Second, the treatment protocol for this study only involved 3 intradermal vaccine injections, delivered each week beginning 28 to 35 d after tumor resection. The first 2 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 delayed-type hypersensitivity (DTH) reaction visible at the site of injection (Fig. 7). 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

![Figure 4. Genomic Landscape of Colorectal Cancer. Wood, L and B. Vogelstein, Science Vol. 318, November, 2007. 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 2 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.](image)
surgery. Not surprisingly, induration size correlates well with patient outcome (Fig. 8).

Lessons learned from the previous study were incorporated into the next phase III clinical trial (8701). This study 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 4 vaccine regimen: 3 initial weekly treatments (2 with TICE\textsuperscript{R} BCG, one without) and a 6-month follow-up booster inoculation. The follow-up booster was added based on the results of a side phase II trial\textsuperscript{18} that suggested initial immune responses begin to wane 6 mo after the induction vaccinations (Fig. 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 g 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 prospective analysis.

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 4 vaccine program outlined above. OncoVAX was well-tolerated, with 102 of 128 patients receiving all 4 vaccinations. To determine the extent of DTH reactivity, injection sites were measured for indurations 48 h after the third and fourth immunizations. Subjects were defined as having achieved cellular immunity if the average of both measurements was 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 RFS, overall survival, or recurrence-free interval
(RFI) were observed. However, when a prospective analysis of patients were 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 (Fig. 9). The favorable 16.4% difference between control and OncoVAX patients represents a 41.4% relative risk reduction of disease progression (5-y survival $P = 0.008$; log-rank analysis $P = 0.018$). Overall survival showed a statistically significant improvement in stage II OncoVAX treated patients (17.5%) over those patients in the control group (27.3%) (Fig. 10). The favorable 9.8% difference represents a 33.3% relative risk reduction (5-y survival $P = 0.014$; log rank analysis $P = 0.074$).

In the intent-to-treat (ITT) population of all randomized stage II patients, there were 43 recurrences (Fig. 11). 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 with the control group, the favorable 16% difference represents a 57.1% relative risk reduction in the recurrence of colon cancer in the OncoVAX group (5 y survival $P = 0.026$; log-rank analysis $P = 0.008$).

Figure 7. Induction of a DTH response following injection of autogous tumor cells. Hoover HC, Surdyke, M, Dangle, RB, Peters, LC, Hanna, Jr. MG. (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. (A) DTH response specific to tumor, not adjacent mucous. DTH in 27 patients and 11 Controls Responses to Tumor or Colon Mucosa. (B) DTH response to 3rd and 4th vaccine Dose.

Figure 8. Survival and disease-free survival in patients grouped according to their DTH response to the third vaccine. Harris JE, Ryan L, Hoover Jr HC, Stuart RK, Oken MM, Benson AB, Mansour EG, Haller DG, Manola J, Hanna Jr MG (2000) Adjuvant active specific immunotherapy of stage II and III colon cancer with an autologous tumor cell vaccine: ECOG Study E5283. Journal of Clinical Oncology, 18 148–157. 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 with clinical benefit as reflected in significant differences in recurrence-free- and overall-survival.
In a surprising result, safety was actually better in the OncoVAX treatment cohort compared with surgery alone. One patient treated with OncoVAX was hospitalized for treatment of a flu-like syndrome and the event resolved 9 d later. Another patient required discontinuation of OncoVAX treatment due to a 21 × 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 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.

Since this study was originally 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 y.20 These results have also been confirmed in T3 and T4A&B colon adenocarcinoma patients.21 Consequently, effective treatments are lacking for these patients in the adjuvant setting. 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 performed 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 RFS with an interim and final primary analysis with one and 3 y 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, the Biologic License Application (BLA) can be filed. Past clinical trials using the optimum 4 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.”

**Human Monoclonal Antibodies**

Human monoclonal antibody (HuMab) development for cancer treatment, monitoring, and diagnosis is a rapidly evolving field and new sources of cancer-specific HuMabs are in high demand. An ancillary benefit to evaluating OncoVAX in human patients was the isolation of circulating, diploid B-cells which produced an array of cancer-specific HuMabs. In fact, we were able to isolate 36 HuMabs which positively recognized colorectal adenocarcinoma cells and tissues. Furthermore, roughly half of these antibodies appear to recognize cell surface antigens and have immediate potential for cancer diagnosis and treatment.

There are 3 important factors in the development and production of HuMabs for tumor antigen recognition: strategy, specificity, and stability. Tumor-specific B-cell production initiated by OncoVAX inoculation addresses all 3 of these requirements. A number of strategies for producing tumor-specific antibodies have been developed over the last few years, including hybridoma fusion of lymphocytes from tumor-draining lymph nodes to EBV transformation of peripheral blood lymphocytes and splenocytes from cancer patients. The apparent instability of antibody production by EBV-transformed lymphocytes has thus far made them an impractical means of producing HuMabs. Additionally, neither approach has succeeded to reproducibly generate HuMabs reactive with cell surface antigens, presumably due to a lack of immunocompetence in the cancer patient at the time. An inability to recognize cell surface antigens limits the in vivo utility of these agents for diagnosis and therapy.

**Figure 11. OncoVAX® – Clinical Results 8701 Study – Recurrence-Free Interval in Stage II Patients. Greatest difference between treated and control in RFI at 18 mo post Vx.**

**Figure 12. OncoVAX® – Clinical Results 15 y F/U 8701 Study – Recurrence-Free Interval (RFI) in Stage II Patients. Recurrence Free Interval original study population. 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 vs. Control at 15 y follow up; HR = 0.62 (95% CI: 0.34–0.96) log rank p-value 0.033). de Weger, Vincent A, et al., Clinical effects of Adjuvant Active Specific Immunotherapy differ between Patients with Microsatellite Stable and Microsatellite Instable Colon Cancer, Clin Cancer Res 2012Feb1; 18:3882–9.
By comparison, approximately 20% of the cultures tested from our tumor immune patients produced HuMabs, with 15.6% binding colon tumor cell antigens. HuMabs generally reactive with tumors were isolated from 7 of 10 immunized patients. Our results demonstrate that while these tumors may vary widely on a genomic level, a surprising degree of immunogenicity may be shared across patients. Our preliminary HuMab results suggest many colon cancers appear to express multiple TAAs. However, none of the HuMabs isolated thus far detect a single antigen common to all of the tumors (Fig. 13). Therefore, it is quite possible a convenient number of complementary antibodies could be combined to achieve the broad reactivity necessary for cancer diagnosis or therapy in the clinic (Fig. 14).

The HuMabs that we have developed exhibit marked differences in reactivity with normal colonic mucosa. Quantitative differences rather than strict qualitative differences in reactivity were seen in many tests with matched tumor and noninvolved mucosa cells obtained from the same patients. In general antibodies were tested against various normal tissues such as breast, lung, liver, and skin and were found to be negative.

Generating these antibodies from isolated human sources obviates a number of problems associated with developing immunogenic agents for clinical use. First, the expensive process of “humanizing” antibodies from murine sources is not required, although antigenic responses toward these agents still need to be fully explored. Additionally, mouse monoclonal antibodies (MuMabs) very often react with well-known tissue components, particularly carcinoembryonic agent (CEA). To date, none of the HuMabs we have isolated have any reactivity toward CEA, blood group determinants, or histocompatibility antigens, suggesting that HuMab specificity is restricted to those structures recognized as immunogenic in the autologous host. It is very possible this is a reflection of the highly targeted nature of antibody production by autologous vaccination. More specifically, inoculation of mice with human tumor cells is much more likely to identify tissue-specific antigens as foreign rather than autologous vaccination which, in theory, should ignore the majority of self-antigens and focus on unique, cancer-specific epitopes. In fact, many studies comparing MuMab to HuMab cancer recognition has noted a heterogeneous staining pattern. In contrast, HuMabs generated following autologous vaccination demonstrate a homogenous recognition pattern. Thus, it is quite likely MuMabs recognize many more phase- or cell cycle-specific antigens rather than true TAAs, greatly limiting their clinical potential.

Other questions concerning the ultimate in vivo application of HuMabs include their ability to enter the extravascular environment of a tumor, recognize tumor-specific structures or epitopes, and bind with sufficient avidity to be effective couriers for antitumor drugs, radionuclides, or diagnostic reporter molecules. These questions are currently being addressed and preliminary findings in colon, breast, and head and neck cancer indicate that HuMabs generated by autologous vaccination are able to access and bind these tumor cells in vivo. These and additional investigations identifying broadly applicable TAAs will be an exciting complement to the upcoming phase III trial of OncoVAX which will generate a new repertoire of HuMabs for study.

While OncoVAX was originally designed with tumor heterogeneity in mind, it is thrilling this process may ultimately yield a suite of tools which will allow us to standardize this disease. The degree to which these colon cancer-specific tools will be broadly applicable to other cancers remains to be seen; however, autologous cancer vaccines utilizing renal, breast, and lung tumors should be able to produce similar tumor-specific antibodies for their respective cancer subtypes. In the future, it is very possible immunomodulatory agents such as ipilimumab or nivolumab may serve to enhance the efficacy of
ASI therapeutic regimens. In the meantime, novel strategies for ASI and immunomodulation need to be developed in parallel as it is clear these modalities are far from mutually exclusive.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References


