AVAX
TECHNOLOGIES, INC.

AVAX's AUTOLOGOUS CELL (AC) VACCINE® PLATFORM

AVAX has developed a proprietary Autologous Cell (AC) Vaccine® Platform, a therapy that stimulates a patients' immune system to combat their cancer cells. It utilizes a vaccine consisting of patients' own (autologous) cancer cells, which comprise all of the relevant tumor rejection antigens while eliminating the need to identify any of them.

SCIENTIFIC BASIS FOR THE TECHNOLOGY

Advantages of an Autologous Vaccine:

Each patient's own cancer contains all of the antigens to which a destructive immune response could be directed. So, autologous tumor cells are the most logical basis for a therapeutic vaccine. The actual "rejection" antigens do not have to be identified or characterized, and effort that could require vast amounts of time and money, especially if those antigens are different for each cancer patient.

A large and venerable body of experimental work supports the idea that tumor rejection antigens are individually distinct [1-3]. Prehn and Main [1] performed the classic experiments that laid the foundation of modern cancer immunology. They induced fibrosarcomas in multiple syngeneic mice by topical application of the potent carcinogen, methylcholanthrene (MCA). Of the dozens of tumors induced, none were histologically indistinguishable, but all were antigenically distinct in immunological protection experiments. To wit, most of the induced tumors were effective in immunizing mice when the cells were injected and the tumor was excised before it had metastasized: a challenge with live malignant cells derived from the same tumor failed to grow. However, these authors observed little or no cross protection: Mice immunized with Tumor A were protected from subsequent challenge with Tumor A, but not from challenge with Tumors B, C, D, E, etc.

This type of observation has been repeatedly confirmed in tests of intact tumor cells as immunogens [2], and by tests of vaccines composed of extracted antigens. Compelling examples are provided by the work of PK Srivastava with heat shock protein vaccines [4]. Heat shock

proteins act as chaperones, binding a broad range of peptides derived from the cell. Immunotherapy of tumor-bearing mice with heat shock protein preparations derived from the same tumors resulted in marked slowing of the growth of the tumors, a decrease in the number of lung metastases, and prolongation of survival. Treatment with heat shock protein derived from other tumors of the same histology did not confer protection.

The alternative to autologous vaccines are compositions of "common antigens" that are shared by many or all cancers of the same histologic type. Although such antigens have been frequently identified and are easier to manufacture for clinical trials than autologous cell vaccine, their therapeutic relevance in both animal models and humans is doubtful. Ramarathinam *et al* [5] worked with a the plasmocytoma J558 transfected with the costimulatory molecule B7, a construct that activates a cross-reactive cytotoxic T lymphocyte response in vivo. The major antigen recognized by the T lymphocytes is P1A, which is expressed in a number of murine cancers, including, mastocytoma P815, plasmocytoma J558, and fibrosarcoma Meth A. However, immunization with either P1A-expressing or B7-transfected P815 cells did not protect mice from challenge with live cells from any of those tumors. The authors concluded that multiple lineages of tumors are not cross-protected even though they share a tumor antigen that can be recognized by T cells.

A partial list of antigens discovered to be common to human cancers of a certain histological type includes: 1)Melanoma – melanosomal differentiation antigens: tyrosinase, gp100, and MART-1. 2)Melanoma embryonal antigens, MAGE-1 and MAGE-3. 3)Melanoma gangliosides, GM2. GD2, GD3. 4)Non-Small Cell Lung Cancer – MAGE-A3. To date, none of the vaccines comprised of any of these antigens has proven effective in a randomized clinical trial [6-8]. A recent, well-known example is the negative result of the study testing the MAGE-3 antigen vaccine in non-small cell lung cancer, which inspired much disappointment when the results were announced in 2014 [8].

Rationale for *Haptenization*

<u>Discovery of Haptens</u> - Haptens are tiny lights that illuminate the dark recesses of the immune system. They were discovered by Karl Landsteiner (who was the identifier of ABO blood group antigens); he used haptens to explore the breadth and fine sensitivity of antibody responses. Landsteiner worked with a variety of simple chemicals that were incapable of inducing an immune response by themselves, but became immunogenic when they were attached covalently to a protein carrier. He coined the term "hapten" from the Greek "haptein", meaning "to fasten".

Landsteiner [9] made what was at the time an astounding observation: Rabbits immunized with a haptenized protein produced three sets of antibodies: a)to the hapten itself, b)to the carrier protein, and c)to hapten-protein conjugate. Few immunologists believed these results, despite Landsteiner's Nobel Prize, but they were soon shown to be reproducible became one of the foundations of modern immunology. For example, Weigle extended these observations to proteins that were not immunogenic in their native state. Rabbits that had been rendered tolerant to bovine serum albumin (BSA) by neonatal injections of this protein failed to produce anti-BSA antibody even after injection with Freund's adjuvant.   In contrast, unresponsive rabbits injected with BSA conjugated to sulfanilic acid (SA) produced antibody not only to SA-conjugated BSA but to native BSA as well. Thus immunization with a hapten-modified protein could break established immunological tolerance to that protein.

Even more surprising was Weigle's observation [10] that hapten conjugation could break natural tolerance. The injection of rabbits with homologous thyroglobulin in incomplete Freund's adjuvant produced, as expected, little or no antibody to thyroglobulin. However, rabbits injected with thyroglobulin that had been modified with haptens produced precipitating antibody to both *modified and native* thyroglobulin. Moreover, some of the rabbits developed histological evidence of autoimmune thyroiditis. Once the animals had been immunized, the height of their antibody titers and the severity of the thyroiditis could be increased by administering booster injections of native thyroglobulin.

<u>T Cell Response to Haptens</u> - As the physiology and biochemistry of T lymphocytes was elucidated, it became clear that hapten-modified proteins also elicited a T cell response. Guinea pigs that were sensitized by topical application of 1-chloro-2,4-dinitrobenzene (DNFB) developed lymphocytes that proliferated when cultured with DNP-conjugated syngeneic

lymphocytes. Subsequently, Shearer[11] demonstrated that T cell-mediated cytotoxicity could be induced *in vitro* to hapten-modified syngeneic (normal) spleen cells and that the effector T cells were directed against these "new antigenic determinants." The targets could be hapten-modified normal spleen cells or P815 tumor cells.

Chemical Classification of Haptens - The number of synthetic compounds that can function as haptens is limited only by the imagination of the organic chemist. However, most of the experiments published over the past 25 years have utilized six haptens. The reason for focusing on such a small sampling of haptens is that the immunological responses appear to depend less on the structure of the hapten than on the chemistry of its conjugation to protein.

Two of the most intensely studied haptens, DNP and TNP, are attached to proteins by nucleophilic substitution; apparently, "the DNP-NH bond in proteins is even more stable than the peptide bond"[12]. The covalent bond is critical, since treatment with TNP stearoyl dextran, which binds by non-covalent forces, does not result in haptenic modification. Other haptens, such as sulfanilic (SA), must be introduced into proteins by a diazonium reaction; i.e., a diazonium salt is made by treatment with sodium nitrate. It is now clear that DNP (and TNP) couple to the hydrophilic portions of membrane molecules that are rich in lysine and leucine, while the diazonium conjugates, such as sulfanilic acid, have an affinity for tyrosine and histidine. A consequence of this differential chemistry is that conjugation with the hapten, N-iodoacetyl-N'-(5-sulfonic-1-naphthyl) ethylene diamine (AED), which binds to sulfhydryl groups, does not interfere with subsequent modification of the same protein with TNP [13].

Molecular Immunology of Hapten Modification - What are the "new antigenic determinants" produced by hapten conjugation that must have excited and perplexed the early investigators of hapten immunology? An impressive body of work by H.U. Weltzien's group appears to have solved the mystery. By immunizing mice to TNP-modified syngeneic spleen cells, They demonstrated the following: 1)The responding T cells recognized the MHC-associated TNP-modified peptides. 2)The vast majority of cytotoxic T cell clones responded to multiple H2-binding peptides that had in common a TNP-lysine in position 4. Recognition was largely independent of the amino acid sequence. 3)A minor fraction of TNP-specific T cell clones recognized only certain sequences of TNP-modified peptides. Interestingly, these T cell clones

also recognized *unmodified peptides*; i.e. there was associative recognition of unmodified peptides by T cell clones generated by immunization with hapten-modified peptides [14].

The authors explained these results by postulating self-reactive T cells that survive thymic selection because they have low affinity for self peptides. Haptenization then would increase the binding of TCR to self peptide enough for T cell activation, and, once activated, the T cells could react with unmodified peptide.

Immunotherapy of Experimental Tumors with Hapten-Modified Vaccines - There is considerable evidence that the failure of immunotherapy to eradicate cancers, whether spontaneous human cancers or experimental transplantable tumors, is due to immunological tolerance [15]. However, it is possible to break tolerance against the progressor tumor by haptenization. Thus, mice immunized with hapten-modified regressor tumor rejected a challenge with hapten-modified progressor tumor. Moreover, 28 days later they were able to reject a challenge with *unmodified* progressor tumor [16].

Before and after this work was published, a number of other investigators demonstrated that modification of tumor cells with DNP or TNP increased the efficacy of vaccines. For example, Cavallo and Forni [17] found that mice immunized with DNP-modified mammary adenocarcinoma cells exhibited delayed tumor appearance and slower tumor growth after challenge with unmodified tumor cells. Galili *et al* [18] performed a similar experiment with a virally-induced lymphoma with more impressive results: Immunization with TNP-modified tumor appeared to be effective in increasing the percentage of long-term survivors even in animals in whom the unmodified tumor was non-immunogenic. Roth *et al* [19] were able to demonstrate protective immunity in guinea pigs immunized with a DNP-modified chemically-induced sarcoma. Of course, this approach did not work with all tumors, but published reports showed consistently positive results.

The most recent publication on the use of hapten modification for experimental immunotherapy is from Sojka *et al* [20]. They used the highly metastatic 410.4 tumor that had originated from a spontaneous murine mammary carcinoma. The tumor was injected into the mammary fat pad and was allowed to grow to 6-8 mm diameter and then excised. Following surgery mice were treated with multiple injections of a vaccine consisting of irradiated tumor

cells haptenized with DNP and then mixed with BCG. Control mice received the identical treatment regimen except that the tumor cells in the vaccine were irradiated but *not hapten modified*. These experimental conditions were designed to mimic the post-surgical adjuvant protocols frequently used in clinical vaccine studies and, specifically, to experimentally reproduce our observations in melanoma patients, which are described below.

The result was positive and highly reproducible: Mice that received DNP-modified vaccine had significantly longer relapse-free survival than animals receiving the unmodified vaccine, which, incidentally, was no better than saline. The protective effect of the haptenized vaccine was dependent on both CD4+ and CD8+ T cells.

These observations in animal models provide a strong rationale for an attempt to immunize humans against their autologous tumor cells by modifying their cells with hapten. The hapten that AVAX uses is dinitrophenyl (DNP), because it has been extensively studied in preclinical systems and has been used as test agent in humans without significant toxicity. There are dozens of other haptens that have similar immunopotentiating effects but bind proteins in a chemically different manner. AVAX has in its pipeline a second generation autologous vaccine that is produced by modifying cancer cells with DNP plus a second hapten, sulfanilic acid.

Clinical Studies of AC Vaccines

AVAX's MVax and OVax have orphan drug designations. MVax is positioned to enter a Phase III pivotal registration clinical trial for Stage III or IV melanoma. AVAX is finalizing the results from a Phase I/II clinical trial of its OVax vaccine in patients with advanced ovarian cancer at Cancer Treatment Centers of America ("CTCA").   Clinical results of MVax and OVax are described below.

MVax in Advanced Metastatic Melanoma - In patients with advanced, metastatic melanoma, not considered to be an ideal population for the testing of immunotherapy, of 97 patients, there were 11 anti-tumor responses: 2 complete, 4 partial, and 5 mixed [21]. Both complete responses and two of the four partial responses occurred in patients with lung metastases. Response durations were as follows: partial responses – 5,6,8, and 47+ months; complete responses – 12, 29 months. Patients who exhibited tumor regression survived longer than those who did not (median survival

times: 21.4 months vs. 8.7 months, p=.010).   These results were the basis for a pivotal marketing granted Special Protocol Assessment by FDA.

MVax in Resectable, Stage III Melanoma - 214 patients with clinical stage III melanoma (117 stage IIIC and 97 stage IIIB) who were melanoma-free after standard lymphadenectomy were treated with multiple intradermal injections of MVax [22]. The five-year overall of the 214
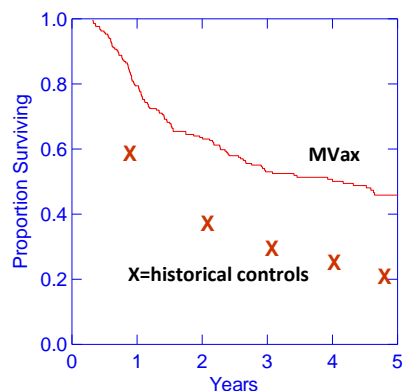

patients was 44%, compared to 22% in historical controls receiving surgery alone.

Discussions with FDA indicate that a phase III trial comparing MVax with an appropriate control treatment could be initiated within 6 months.

Safety of MVax - A phase I/II trial of MVax was conducted with 82 patients evaluable for safety[23]. There were no serious adverse events attributable to MVax. FDA has accepted this study as evidence that MVax is safe.

Immune Response to MVax Correlates with Survival - The development of delayed-type hypersensitivity (DTH) responses to *unmodified* autologous melanoma were induced in 47% of patients. The overall survival of this DTH (+) group was double that of DTH (-) patients (59.3% vs. 29.3%, p<.001) [22]. This result was corroborated in a multivariate analysis.
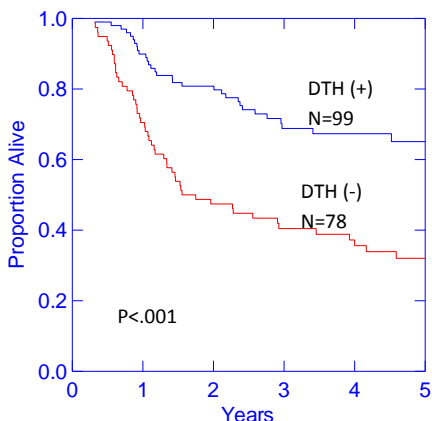
A positive DTH response to unmodified tumor cells remained a statistically significant determinant for both relapse-free survival and overall survival in multivariate analyses that included the previously determined important patient-related variables: sex, number of positive lymph nodes, and presence or absence of extranodal extension. AVAX believes that the development of DTH to unmodified, autologous tumor cells following MVax administration is a surrogate immunologic marker of its effectiveness. Although FDA has not accepted this designation, the agency stated that it could be established in an appropriately designed clinical trial.

OVax Phase I/II Trial with CTCA - Of 26 patients with advanced, chemotherapy-resistant ovarian cancer analyzed to date, the median survival is 25.4 months and the longest survivals are in excess of 5 years.
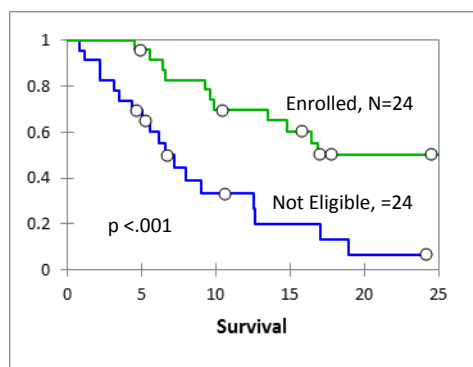
These results are far superior to the expected survivals in this group of patients. OVax appeared to be safe in this study with no serious adverse events attributable to its administration. Accrual to this study is now complete and final analysis is in progress.

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