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Challenges in the development of an autologous heat shock protein based anti-tumor vaccine

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Abbreviations: HSP, heat-shock protein; APCs, antigen presenting cells; MHC, major histocompatibility complex; RCC, renal cell carcinoma; EMA, European Medicines Agency; OS, overall survival; HR, hazard ratio

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Immunotherapy Background and Introduction

In spite of its long history, immunotherapy of cancer has led to only a few regulatory approved treatments, starting with interleukin (IL)-2 in renal cancer and melanoma and interferon in melanoma.[1-4] Sipuleucel-T was approved for castrate-resistant prostate cancer in 2010 and ipilimumab and pegylated interferon-α2b in 2011 for the treatment of metastatic and adjuvant melanoma respectively.[5-7] Evidence of both humoral and cellular immune recognition of human cancer has been found and supports the hypothesis that specific autologous anti-tumor activity exists and may be enhanced with therapeutic activity.[8] Published data from trials with autologous tumor vaccines documented activity although only one autologous-like vaccine has been approved in the US.[5,9] The concept of enhancing specific innate anti-tumor activity is attractive and the approach developed by Srivastava et al., which is designed to present a unique peptide profile of each autologous tumor to the host immune system, using a heat shock protein fraction as both carrier and adjuvant, is conceptually appealing. In syngeneic rat tumors, this approach was shown to lead to anti-tumor activity and as described below evidence of activity was found in clinical trials.

Vitespen (also known as Oncophage or HSPPC-96), is an immunotherapeutic agent derived from the tissue of a patient's own tumor. Vitespen is a heat-shock protein (HSP) (glycoprotein 96)-peptide complex that is purified ex vivo from an individual patient's tumor cells through preparative chromatography.[10] Vitespen failed to show broad activity in randomized clinical trials despite encouraging results in select patients. In this commentary, we highlight the clinical trial experience with vitespen, comment on potential reasons for the limited success and offer suggestions for future tumor vaccine development strategies.

Vitespen Experience

Vitespen consists of antigenic peptides from autologous tumor associated with the heat shock protein carrier and this complex interacts with receptors on the surface of antigen presenting cells (APCs) including clusters of differentiation (CD) CD91, CD36, CD14, CD40, SR-A, Lox-1, TLR2, TLR4 receptors.[11,12] The antigenic peptides are "chaperoned" or internalized by the APC, re-processed and presented via class I and class II major histocompatibility complex (MHC) pathways. This triggers a $CD8^+$ and $CD4^+$ T-cell response in some patients with hypothesized patient-specific anti-tumor activity. Vitespen also interacts with other receptors not fully understood and stimulates innate immune responses.

Phase 3 trials with vitespen have been completed in renal cell carcinoma (RCC) and melanoma. Vitespen was approved in Russia in April 2008 for the treatment of adjuvant RCC under the brand name Oncophage and is in pre-registration status in many other countries. The European Medicines Agency (EMA) recommended the refusal of marketing authorization for Oncophage in 2009 for the treatment of RCC at a high recurrence risk (EMEA/

CHMP/729781/2009). Vitespen was first studied at the University Hospital Charite in Berlin, Germany in a pilot trial in subjects with advanced solid malignancies.[13] The primary objectives of the trial were to assess the feasibility of vitespen preparation and administration, to assess the safety profile and to ascertain if immunological responses could be detected. Tumor material was obtained from each subject at the time of surgery, minced and suspended in sodium bicarbonate and lysed by Dounce homogenization. The suspension was centrifuged and proteins were selectively precipitated from the resultant supernatant. Sequential chromatography was used to isolate vitespen. Only preparations considered to be of acceptable quality were used. Yield, purity, sterility and endotoxin content were tested prior to administration.

Tumor samples ranged between 1 and 22.5 g with an average of 6.5 g. Subjects were required to have 104 μg of vitespen [25 μg x 4 injections + extra for delayed-type hypersensitivity (DTH) tests] in order to participate in the trial. All subjects met this requirement. Yield of vitespen from tumors ranged between 13 and 150 μg/g of tissue. Subjects received 25 μg of vitespen four times at weekly intervals following recovery from surgery when subjects were deemed "immunocompetent" (as judged by recall responses). Administration was typically performed four weeks following surgery. No treatment-related severe adverse reactions were reported. Out of 12 evaluable subjects, 6 exhibited tumor-specific $CD8^+$ T cell responses and eight of 13 subjects had an expansion of NK cell population following immunization. An interesting finding in the trial illustrates the tumor specificity of this autologous approach. One subject in the trial had two primary tumors [hepatocellular carcinoma (HCC) and breast cancer]. Following three vaccinations from her HCC-derived vitespen, the subject showed a response against the HCC tumor but not her breast cancer. Sadly, after the next vaccination with the breast cancer derived vitespen, the subject died due to rapid progression of the breast cancer despite an initial $CD8^+$ response against the breast cancer.

In a phase 2, non-randomized trial in subjects with metastatic renal cell carcinoma (mRCC), vitespen failed to provide a clinical benefit in the majority of subjects. Thirty-nine of the 60 evaluable subjects had progressive disease prior to the first evaluation. However, two individuals (of 60 evaluable) had durable complete responses. At the date of publication, both subjects were in continuous complete remission for over seven years.[14]

In a phase 3 randomized trial, vitespen was compared with observation alone in RCC subjects after nephrectomy. Neither recurrence-free survival nor overall survival results were superior for the vitespen arm in the intention to treat full analysis subset. However, in a post-hoc analysis in patients with intermediate risk RCC, there were statistically significant improvements in both recurrence-free survival [28/184 vs. 47/178; HR (95% CI) 0.59 (0.37–0.94), p = 0.026] and in overall survival (OS) [18/184 vs. 32/178; HR (95% CI) 0.54 (0.30–0.97), p = 0.036] according to the survival update published in 2009.[15,16] These observations are consistent with animal data showing superior efficacy of immunotherapy with vitespen in earlier stage disease.[17,18]

In a phase 3 melanoma trial (n = 322) comparing vitespen to physician's choice (PC), there was also a trend toward better efficacy of vitespen over PC in subjects with less advanced disease (M1a and M1b) compared with advanced disease (M1c), who had received a greater number of vaccinations (10+).[19,20] In this exploratory landmark analysis, when combining M1a and M1b subjects who received over 10 immunizations, there was a statistically significant (and clinically significant) improvement in OS [hazard ratio (HR) = 0.45; 95% CI, 0.21–0.96]. Overall, the trial was negative as the survival curves of vitespen and PC virtually overlapped. Limitations of the trial included that many subjects (51%) in the control arm of the trial may not have received adequate amounts of vitespen required to benefit due to various reasons including lack of adequate autologous harvest. The positive exploratory data in this trial (as well as the RCC early stage data) are consistent with the hypothesis that in order for vitespen to be effective, subjects would need to have earlier stage disease to allow adequate duration of treatment to mount an effective response to the vaccine.

Vitespen has been studied in several other indications (with the most advanced stage of development in parentheses) including glioblastoma (phase 2), colorectal cancer (phase 2), Non-Hodgkin's Lymphoma (phase 2), pancreatic cancer (phase 1), non-small cell lung cancer (phase 1), and gastric cancer (phase 1). Data to date shows limited overall activity in these indications with clinical responses in certain patient subsets.

It is of interest to note that another vaccine trial in RCC patients showed a similar trend in that patients with better prognosis had significantly superior response. The phase 3 MVA-5T4 (TroVax) trial evaluated MVA-5T4 compared with placebo when combined with either sunitinib, interleukin (IL)-2 or interferon (IFN)-α in subjects with mRCC. The trial enrolled 733 subjects and showed no difference in overall survival between the treatment arms. However in a subset analysis, subjects with a good prognosis (MSKCC grade 0) and who were treated with TroVax had significantly superior overall survival (HR, 0.54; 95% CI, 0.30–0.98; p = 0.046).[21] Other agents have also demonstrated this same trend with anti-tumor vaccines being more effective in patients with a better prognosis and earlier-stage disease such as sipuleucel-T in prostate cancer, BLP25 liposomal vaccine in non-small-cell lung cancer and autologous tumor cell-BCG vaccine in patients with colon cancer.[22-24]

In summary, the vitespen trial data demonstrated the ability of patient specific autologous tumor vaccine to elicit a tumor specific immunological response. Long-term tumor response was noted post nephrectomy in two of 60 patients with mRCC. A retrospective subset analysis of a phase 3 randomized trials in RCC patients with intermediate risk showed a reduction in the hazard ratio of progression or death which was statistically significant. Interestingly, a similar trend was observed retrospectively in a phase 3 melanoma trial with vitespen. The quantity of the vaccine available for administration depends on the size and quality of the tumor specimen submitted to the laboratory for processing and the duration of administration may be confounded by

the natural history of each subject's cancer. Subjects with an indolent course of disease are more likely to receive more doses of vaccine (if available) than those with rapidly progressive disease. Several other tumor vaccine trials also showed an apparent benefit in patients with relatively favorable prognosis.

Considerations for Future Clinical Development of HSP-Based Tumor Vaccines

Since only post-hoc/retrospective data analyses were positive in selected patient subsets, it remains possible that these trials might have demonstrated superiority and thus supported approval if subjects with better prognosis had been identified prospectively and randomized in a separate stratum. Novel adaptive trial designs have been implemented since the development of vitespen was initiated. Trial designs which "learn as you go" (especially in phase 1 and 2 development) may have resulted in a different outcome for vitespen to date. Recent experience suggests that utilizing adaptive randomization methods such as the BATTLE and I-SPY trial designs early in development might have identified patient populations that were likely to respond to vitespen administration.[25,26]

In hindsight, another possible reason for the limited regulatory success thus far despite encouraging results in patient subsets, may be the lack of an optimally defined dose. This is especially relevant given that the activity of vitespen appears to be dose dependent. Very low doses did not immunize, higher doses immunized effectively, yet even higher macro-doses failed to immunize at all.[18] This goldilocks phenomenon is theorized to be an active, antigen-specific effect due to downregulation of the anti-tumor response.[20] Perhaps due to constraints around vaccine yield, few dose levels (i.e., 2.5, 25 and 100 μg) were explored prior to advancing into phases 2 and 3. An adaptive selection of vaccine administration schedules may have identified the most active treatment regimen for phase 3 confirmatory trials. One trial design that could be adapted to address these complexities is the I-SPY design. The I-SPY design is "sophisticated while not mathematically complex" and is designed to identify effective drugs using combinations and unions of combinations of biomarkers.[26] If a promising biomarker is identified, an adaptive test can be used to determine whether the biomarker could select a subgroup of tumors (or patients) likely to respond favorably.[27] Utilizing this type of design early in vitespen's development may have resulted in identifying or enriching the subset of patients most likely to respond.

Since obtainable human tumor tissue can be both limited and heterogeneous in composition, extraction of vitespen is likely to be variable. Human tumors are heterogeneous at the macroscopic, microscopic and molecular level. Large tumors may consist predominantly of connective and vascular tissue, with areas of necrosis with relatively small percentages of viable tumor cells. Tumors may contain infiltrates with immune cells (Jain, ASCO 2012 Plenary Session) which may signify a favorable or poor prognosis.[28] Breast cancers may exhibit many distinctions at the molecular level.[29] Even within one individual, the genetic make-up of tumors may change between the primary and metastatic sites during the natural history of the tumor.[30] Finally, the ability to respond to an immunological stimulus may vary between and within individuals. Again, a Bayesian adaptive approach may offer the best strategy to address the unusually complex development of an autologous anti-tumor vaccine. Finally, since patients are treated with vitespen for varying durations (in part due to different vaccine yields), therapeutic responses are also expected to be variable. Perhaps alternative approaches to vitespen preparation (such as outlined by Randazzo et al.) generate sufficient vitespen allowing for longer treatment periods to be tested in future trials.[31]

Conclusions

Results from preclinical cancer treatment models often translate poorly into outcomes in humans.[32-35] Are the disappointing results with vitespen thus far due to this phenomenon or is there real activity in human cancer that is being obscured by a larger population in the trial in which the treatment has no effect? With so many variables potentially affecting the antigenic profile of a tumor specimen, the vaccine yield and the potential of an individual to respond to an immunological stimulus, it is not likely that an "all comers" approach would be successful.

Using a traditional drug development approach, the patients likely to respond were only identified following a retrospective analysis which is more likely to be seen as hypothesis generating than to support regulatory approval. With the implementation of novel prospectively adaptive clinical trial designs, vitespen and other similar agents may show robust evidence of activity earlier in development. Bayesian adaptive methods such as an I-SPY design may define subgroups of patients and other parameters affecting outcome, thus leading to an earlier definition of the patient population likely to derive benefit from the administration of vitespen and to a more assured path to regulatory approval.

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