Published in final edited form as:
Expert Rev Vaccines. 2012 December ; 11(12): 1401–1404. doi:10.1586/erv.12.122.

Vaccination using peptides spanning the SYT–SSX tumor-specific translocation

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Abstract

The identification of genetic translocations as key tumor-initiating events has led to the development of novel antigen-specific vaccines targeting these tumor-specific breakpoint regions. Previous studies have evaluated vaccines targeting the breakpoints in the BCR-ABL translocation in patients with chronic myelogenous leukemia and EWS-FLI1 in patients with Ewing sarcoma. In the article under evaluation, the authors evaluated a peptide vaccine targeting the breakpoint in the *SYT–SSX* translocation, the genetic translocation essentially pathognomonic for synovial sarcoma. This is the second small clinical trial reported by this group using HLA-A24-binding peptides as vaccine antigens. In this four-arm trial, using a native or HLA-A24-optimized SYT–SSX peptide with or without adjuvant plus IFN-α, they immunized patients with metastatic synovial sarcoma. Immune responses were evaluated by delayed-type hypersensitivity testing and tetramer analysis. No robust evidence of immune response to the target epitope was detected. Some patients treated with peptide in adjuvant plus IFN-α had stable disease. These results suggest that future similar studies might best evaluate patients with a lower burden of disease, consider alternative immunization approaches to the SYT–SSX target antigen and consider the efficacy of IFN-α alone for the treatment of synovial sarcoma.

Keywords

IFN-α; peptide vaccine; SSX; synovial sarcoma; SYT; SYT–SSX

Synovial sarcoma is a rare, high-grade, soft-tissue cancer that commonly affects adolescents and young adults. Approximately 90% of synovial sarcoma cases are characterized by a specific translocation t(x;18)(p11.2;q11.2). The resultant product is a unique fusion between proteins SYT and either SSX1 or SSX2 [1]. Synovial sarcomas are associated with a high risk of recurrence, with a median survival of 22 months from the onset of the disease [1]. This low survival rate indicates a need for alternative therapies beyond conventional surgery, radiotherapy and chemotherapy.

The presence of a specific translocation event, producing essentially a tumor-specific protein sequence spanning the junction region, led these investigators to study whether the junction

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Financial & competing interests disclosure
This work is supported for JE Bloom, DG McNeel and BM Olson by the Congressionally Directed Medical Research Programs' Prostate Cancer Research Program (W81XWH-11-1-0196) and by the NIH (R01 CA142608). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.
No writing assistance was utilized in the production of this manuscript.

resulted in a peptide epitope that could be presented by HLA-A24 [2]. They identified an epitope, SS393 (GYDQIMPKK) with HLA-A24 binding affinity and found that peptide-specific T cells with cytolytic activity against SYT–SSX-expressing tumor cells could be cultured from synovial sarcoma patients [2]. They further identified that this epitope could be modified at an anchor residue (agretope-modified, K9I substitution – GYDQIMPKI) to increase HLA-A24 binding and generation of CTL responses *in vitro* with cross-reactivity to the native epitope [3]. They previously conducted a clinical trial evaluating the safety and immunological efficacy of the SS393 peptide in HLA-A24$^+$ patients with recurrent synovial sarcoma [4]. In the current study, they evaluate this peptide as well as the K9I variant, alone or with IFN-α, in a trial of HLA-A24$^+$ patients with metastatic synovial sarcoma [5].

Methods

Patients 20–70 years of age were enrolled, all of whom had histologically and genetically confirmed (SSX1-positive or SSX2-positive) synovial sarcoma, were HLA-A*2402 positive and had no prior therapy within 4 weeks of treatment. Patients were treated in one of four groups with either SS393 or modified K9I at a dose of 0.1 or 1 mg, administered alone or with incomplete Freund's adjuvant (IFA) and 3×10^6U IFN-α given on days 1 and 3. All administrations were performed subcutaneously and repeated at 14-day intervals for a goal of six immunizations. Immune responses were measured by delayed-type hypersensitivity (DTH) at the site of immunization after 48 h, and by SS393 tetramer analysis. Computed tomography was used to evaluate tumor size prevaccination after three vaccinations and at the end of the study.

Results

Twentyone patients were enrolled in the study and 13 patients completed the planned 12-week vaccination regimen (five out of nine patients receiving peptide alone, eight out of/12 receiving peptide plus adjuvants); seven patients discontinued owing to rapid disease progression and one discontinued owing to intracerebral hemorrhage. No peptide-specific DTH responses were identified. Low frequencies of tetramer-positive cells were identified in some patients after immunization. Stable disease was observed during the 12-week immunization period in one of nine patients receiving peptide alone and six of 12 patients receiving peptide plus adjuvants.

Discussion

The authors conclude that: the vaccines could be safely administered; more patients receiving peptide with adjuvants had stable disease compared with those receiving peptide alone, indicating 'the adjuvant activity of IFA and IFN-α enhance the anti-tumor effects of the peptide vaccine'; more patients receiving the modified peptide experienced 'greater than twofold increase in the frequency of CTLs' as measured by tetramer staining; increases in CTL frequency had no relation to clinical responses; and response observed in patients receiving peptide plus adjuvant 'is encouraging and warrants further investigation, ideally in an adjuvant setting'.

Expert commentary

Several groups have evaluated the SSX family as target antigens for anti-tumor vaccines [6]. The use of the breakpoint region of the SYT–SSX fusion protein as a tumor-specific target for synovial sarcomas is highly relevant and was identified as a priority anti-tumor antigen by an NCI consensus panel [7]. Moreover, the breakpoint region of the SYT–SSX fusion protein harbors several potential MHC class I binding epitopes, as identified by algorithm

modeling [8]. In fact, the GYDQIMPKK peptide chosen for the trials conducted by this group has predicted affinity for HLA-A*11:01 and HLA-B*27:05, suggesting that this approach could be broadened to other MHC types as well.

Despite the high relevance of this particular target, the data presented in this report do not demonstrate that a robust immune response was generated by vaccination. No DTH responses were detected, a technique commonly used to detect memory T-cell immune responses elicited by peptide vaccines [9–11]. The absence of DTH responses to the native epitope in the current and previous [4] trials suggests memory-type responses were not elicited. In addition, the tetramer analysis was not uniformly conducted, making it impossible to compare results among treatment groups. The absence of information about the natural variability of tetramer-positive cells in patients, the low frequencies of tetramer-positive cells, and the absence of replicate analyses further suggests that tetramer-positive cells might not have been elicited with vaccination. A similar result and interpretation was found in the previous trial conducted by this group, in which they commented that other measures of immune response should be conducted in the future, such as by IFN-γ ELISPOT [4]. However, no other measures of immune response were employed in the current trial.

The authors conclude that the detection of stable disease in the groups receiving IFA and IFN-α demonstrated that these adjuvants improved the anti-tumor activity of the peptide vaccine. Given the small sample size, it is not clear that there was a difference between these groups with response to outcome, and this could be due to imbalances in treatment assignment as this was not a randomized trial. Assuming that there was an improvement in time to disease progression in the groups receiving IFN-α, this would suggest that IFN-α itself may have had some treatment effect rather than improving the immunogenicity of the peptide vaccine. In support of this, the authors note that IFN-α has demonstrated direct anti-tumor activity on synovial sarcoma cell lines treated *in vitro* [12]. These findings, and the results from the trial of Kawaguchi and colleagues, suggest that IFN-α could itself be investigated as a treatment for synovial sarcoma.

Five-year view

Overall, the investigators should be complimented for applying a rational strategy to a very aggressive cancer with limited treatment options. However, what else can be learned from this trial as we approach the next several years of anti-tumor vaccine efforts? First, the absence of clear immunological response suggests that further investigation should be performed as to why robust responses were not generated. With few exceptions, MHC class I-binding peptide vaccines have shown some immunogenicity but little clinical efficacy, possibly due to the absence of T-cell help necessary for the establishment of $CD8^+$ T-cell memory [13]. Many groups are consequently evaluating different means of peptide delivery and delivery with different adjuvants. IFNα is one such adjuvant that has been suggested to obviate the requirement for T-cell help in $CD8^+$ T-cell priming [14]. Our group has demonstrated that antigen delivery by genetic vaccines can elicit persistent T-cell responses, both $CD4^+$ and $CD8^+$ responses, including genetic vaccines targeting SSX2 [15,16]. We have recently demonstrated that a plasmid DNA vaccine encoding agretope-modified peptides derived from SSX2 can elicit a higher frequency of CTL responses in an animal model [Smith submitted]. Hence, different vaccine strategies and adjuvants may be important to elicit more robust and durable anti-tumor immune responses.

Second, this trial underscores the importance of having robust immunological assays to identify biological activity of vaccines. In the current trial, no significant adverse effects were observed. The absence of safety concerns and more importantly the absence of

objective disease responses makes the identification of biological effect even more important to know how to evaluate this vaccine approach compared with another, or even within a single trial. At present, there is a great emphasis on the use of 'harmonized' methods of immune analysis from anti-tumor vaccines in order for consistent reporting and interpretable comparisons across different trials [17].

Finally, this trial underscores the importance of the appropriate patient population and clinical response evaluation for anti-tumor vaccination trials. The fact that only 13 of 21 patients in the current trial were able to complete a 12-week immunization course suggests that these patients had aggressive, rapidly progressive disease. The time required to elicit adaptive responses may not make vaccination appropriate in the setting of rapid tumor progression. Even in the case of sipuleucel-T, an FDA-approved vaccine for the treatment of metastatic prostate cancer based on the demonstration of improved survival, radiographic progression was observed in nearly half of individuals at 12 weeks, making time to disease progression an untenable end point [18,19]. Results from multiple vaccine trials suggest that other measures of clinical efficacy, and also clinical trial designs able to detect delays in treatment effect, conducted in patients with earlier stage disease, are necessary [20].

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Key issues

- This report describes the results of a Phase I trial evaluating a 9-mer peptide vaccine consisting of the translocation breakpoint region of the SYT–SSX fusion protein expressed in synovial sarcoma.
- Four vaccination protocols were tested using two different peptides, with or without IFN-α adjuvant.
- No clear evidence of robust immune response was detected by delayed-type hypersensitivity analysis or tetramer staining.
- This study underscores the importance of preclinical optimization of vaccine approach, choice of adjuvants, immunological evaluation, and choice of subjects with respect to stage of disease and clinical response evaluation.