Editorial Commentary

WT1 as an immunotherapy target for thymic epithelial tumors: a novel method to activate anti-tumor immunity

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Thymic epithelial tumors (TETs) are a family of rare cancers that exhibit diverse histology and variable clinical behavior (1). A significant fraction of patients, especially those with thymic carcinoma, have locally-advanced or metastatic disease at diagnosis that is often unresectable (1). Chemotherapy is associated with objective response rates (ORR) of 50–90% in the front-line setting, but limited benefit is observed in patients with recurrent disease (1,2). With few exceptions, biological agents have not demonstrated clinically meaningful activity in relapsed or refractory TETs (3). A low tumor mutation burden and paucity of actionable biological targets creates challenges for the development of targeted therapies for TETs (4,5). Hence, there is a pressing need to develop new treatments, especially for patients with advanced thymic carcinomas.

Wilms' tumor-1 (*WT-1*) is a tumor suppressor gene associated with Wilms' tumor (6), and its protein product, WT1, is overexpressed in various malignancies (7,8). Hence, WT-1 is considered as a tumor-associated antigen and clinical trials targeting WT-1 have shown clinical and immunological activity in hematologic malignancies and solid tumors such as leukemia, glioblastoma multiforme, and pancreatic cancer (7,9-14).

Oji and colleagues have conducted the first study to evaluate WT1 peptide-based vaccine immunotherapy in patients with advanced TETs (15). Participants had histologically-confirmed, locally-advanced or metastatic thymoma or thymic carcinoma and were not eligible for potentially curative therapies. WT1 expression on tumor cells and HLA-A*24:02 positivity was necessary for eligibility. Subjects received 9-mer-WT1-derived peptide emulsified with Montanide ISA51 adjuvant via intradermal administration once a week as monotherapy for 12 consecutive weeks during the 3-month protocol-treatment period. Thereafter, treatment was continued at a 2–4-week interval until disease progression or development of intolerable adverse events. Although there were no objective responses, 6 of 8 (75.0%) patients with thymic carcinoma and 3 of 4 (75.0%) patients with thymoma had stable disease at the end of the protocol-specified 3-month period. The majority of patients with thymic carcinoma tolerated treatment well with no major adverse events except local injection site reactions. However, 2 of 4 patients with thymoma developed immune-related adverse events (irAEs) including pure red cell aplasia and myasthenia gravis after receiving 66 and 38 doses of the vaccine, respectively. Induction of a WT1-specific immune response was observed in most patients based on the positivity of delayed-type hypersensitivity skin test and anti-WT1-peptide IgG antibody production.

These observations suggest that WT-1 peptide-based immunotherapy is a potential treatment option for advanced or recurrent TETs. However, several issues need to be addressed to determine if WT1-based therapy is suitable for treatment of TETs.

First, does WT1 expression level predict the clinical activity of immunotherapies targeting WT1? Most, but not all clinical trials targeting WT1 required WT1 overexpression for eligibility. In a phase II study of WT1 peptide vaccination in acute myeloid leukemia and myelodysplastic syndrome, eligibility was based on WT1 mRNA expression in bone marrow rather than protein

expression in leukemic cells (12). Clinical and immune responses as well as a decrease in WT1 mRNA levels were observed after treatment. If WT1 protein overexpression is considered necessary for activity against solid tumors, more robust data are required to determine the frequency of overexpression in TETs. Based on >10% positivity in the cytoplasm or nucleus of tumor cells when stained with the mouse monoclonal antibody, 6F-H2, Oji and colleagues reported 85% and 80% WT1 overexpression in thymic carcinoma and thymoma, respectively. In contrast, Pan and colleagues interpreted positivity as heterogenous expression in >1% tumor cells using a polyclonal antibody and excluded samples with faint cytoplasmic WT1 positivity (16). Based on these criteria, 1 of 22 (5%) of thymic carcinoma samples and none of the 35 thymoma samples included in their study showed WT1 overexpression. Naitoh and colleagues used the 6F-H2 antibody to score tumor samples based on staining intensity and distribution and found that only 1 of 3 (33%) samples labeled "thymus cancer" showed WT1 overexpression (8). The impact of WT1 mutations on the activity of peptide vaccine-based immunotherapy also appears unclear. Although not common, 3% of advanced and pretreated TETs harbor recurrent *WT1* mutations (4% among recurrent thymic carcinomas) (4). This raises another question: does the presence of *WT1* mutations predict for response to WT1-based immunotherapy?

Second, does the type of peptide vaccine, nature of the adjuvant, route or frequency of administration used in this trial maximize the stimulatory effects on the immune system? Oji and colleagues use a 9-mer WT-1 derived peptide with a Montanide ISA-51 adjuvant administered intradermally at weekly intervals. Meanwhile, other WT1 vaccines have used full-length WT1 protein or mRNA with adjuvants like keyhole limpet hemocyanin and zoledronate. Granulocyte-monocyte colony stimulating factor has also been used concurrently with WT1 vaccine to increase the immunostimulatory effect. Some therapeutic vaccines are administered subcutaneously, and alternative dosing intervals including biweekly administration with or without booster doses have also been tested (7). These interventions can potentially influence the effectiveness of the WT1 vaccine.

Third, what is the best strategy to assess clinical benefit in patients receiving immunotherapy, including therapeutic vaccines? Although there was no objective radiological response observed in this study, more than half of the treated patients had evidence of WT1-specific immune responses. Therefore, it is unclear if radiological response is the best method to assess the effect of treatment. Other endpoints such as progression-free survival should be considered for evaluating clinical benefit. Also, 3 of 4 patients had stable disease after 3 months. In the absence of a placebo-controlled trial, it is difficult to discern if this observation reflects the natural history of TETs or an effect of vaccination. It is also unclear if these patients had stable disease at study entry. Newer biomarkers need to be developed to evaluate the benefit of therapeutic vaccination. For example, pre- and post-treatment measurement of serum interleukin-8 is being investigated as a marker of benefit from immunotherapy and other therapeutic interventions in patients with various malignancies (17).

Fourth, immune response to WT1 peptide vaccination has been evaluated by measuring WT1 delayed type-hypersensitivity (WT1-DTH) and WT1 IgG production. The authors have previously reported that development of WT1-DTH accompanied by an increase in WT1 IgG production is a better predictor of survival benefit related to WT1 vaccination (18). This scenario was observed in only 3 of 13 evaluable TET patients in the current study and raises the question whether peptide vaccination is a suitable immunotherapeutic strategy for TETs, especially for patients with thymoma who can have concurrent hypogammaglobulinemia and anti-cytokine antibodies (19,20).

Fifth, can a different clinical setting or combination with other therapies be used to maximize the benefits of WT1 peptide vaccine therapy? In the study by Oji and colleagues, WT-1 peptide vaccination was used as monotherapy, and the majority of patients had advanced TETs after prior systemic therapy. In general, patients with minimal residual disease after frontline therapy appear to derive substantial benefit from WT1 peptide vaccination (9,21). Therefore, can WT1-based vaccination strategies for TETs achieve the greatest potential benefit when used as adjuvant therapy after surgical resection in patients with locally-advanced disease, rather than in patients with advanced disease and a greater tumor burden? Previous studies have also shown that depletion of regulatory T-cells (Tregs) is associated with increased activity of peptide vaccines (22). These observations provide justification for combination of WT1 peptide vaccination with drugs such as sunitinib, which are known to decrease the population of Tregs (23).

Finally, this study shows once again that immunotherapy in TET patients can be associated with unique and potentially life-threatening irAEs. Two out of 4 patients with advanced thymoma in the current study developed irAEs, 2.8 and 2.2 years after starting treatment. We have previously

reported development of myositis and myocarditis in thymoma patients treated with avelumab (an anti-PD-L1 antibody) (24). A high frequency of musculoskeletal, cardiac and neuromuscular irAEs, especially in patients with thymoma, has also been observed after treatment with pembrolizumab (an anti-PD-1 antibody) (25,26).

These observations are in stark contrast to the low frequency of severe musculoskeletal and cardiac irAEs associated with immune checkpoint inhibitors in other solid tumors (27). Furthermore, the occurrence of myositis, myocarditis and myasthenia gravis in TET patients after chemotherapy or targeted therapies, highlights the unique biology of TETs and the need for caution when considering immunotherapeutic interventions (28,29). Therefore, careful patient selection by excluding patients with a history of autoimmune or connective tissue disorders is extremely important before offering immunotherapy to patients with thymoma and thymic carcinoma.

Our group recently identified pre-existing acetylcholine receptor and striational autoantibodies and severe B cell lymphopenia in thymoma patients with no previous history of autoimmunity as risk factors for the development of neuromuscular complications after treatment with an immune checkpoint inhibitor (30). These results highlight the need to identify TET patients with no clinical history of autoimmune disorders who might be predisposed to irAEs when treated with immunotherapy.

In conclusion, WT1 peptide vaccination offers a new avenue for treatment of TETs. Further studies are needed to identify the subset of TET patients most likely to benefit from treatment. Combinatorial strategies need to be evaluated as well to increase activity of the WT1 peptide vaccine, since monotherapy is associated with limited clinical benefit, if any. Lastly, as with other forms of immunotherapy, careful patient selection is of paramount importance to prevent catastrophic complications related to immune-activation in this patient population.

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Footnote

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