Editorial

Use of tumor cell lysate to develop peptide vaccine targeting cancer-testis antigens

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Antigenic target vaccines aim to enhance anti-tumor immune response through the administration of immunogenic tumor antigens or cells together with an immunoadjuvant (1). Tumor antigens are grouped into three categories: tumor-associated antigens; cancer-specific antigens (neoantigens) and, cancer-testis antigens (C-TAs) (2). C-TAs are abnormally expressed in many tumors, but they are not expressed in normal tissue, except the testes and placenta. This differential expression pattern, together with their strong immunogenicity, makes C-TAs ideal targets for cancer immunotherapy (3). The human genome encodes more than 200 C-TA genes from 44 gene families (4). New York esophageal antigen-1 (NY-ESO-1) and the melanoma antigen gene (MAGE) family are two immunogenic C-TAs that have been targeted in many completed and ongoing vaccine trials. NY-ESO-1 is highly expressed in bladder cancer, melanoma, and non-small cell lung cancer (NSCLC) and moderately expressed in breast cancer and prostate cancer (5). MAGE-A3, as one of the most immunogenic MAGE proteins, is also aberrantly expressed in a wide variety of cancer types (6). Humoral immune responses and cellular immune responses against NY-ESO-1 and MAGE-A3 have been detected and the restricted epitopes have been identified as the recognition sites for CD8+ cytotoxic T lymphocytes (CTLs) (7,8). A number of preclinical and clinical studies have reported that MAGE-A3 and NY-ESO-1 peptide vaccines trigger anti-tumor immune responses, especially when combines with adjuvants (9-14). One strategy to optimize immunogenicity is to use tumor cell lysate to develop C-TA based peptide vaccine.

In the recently published trial (NCT02054104) (15), Zhang *et al.* reported results from the first-in-human phase 2.5 trial in which 21 patients were treated with H1299 lung cancer cell lysates with Iscomatrix™ adjuvant via deep intradermal injection every 4 weeks (6 doses total). The main objective of the trial was to measure if administration of this cancer cell lysate vaccine could induce immunity to C-TAs as measured by levels of serologic response assessed 1 month after the 6th vaccination. The secondary objective was to measure if metronomic cyclophosphamide and celecoxib enhance vaccine-induced immune responses by reducing immune inhibitory effects of regulatory T-cells (Tregs). Cyclophosphamide was administered at a dose of 50 mg oral twice daily for 7 days prior to the first vaccination, and then on days 8 through 14, and 22 through 28 of each treatment cycle. Celecoxib was administered at a dose of 400 mg oral twice daily for 7 days prior to the first vaccination, and then on day 1 through 28 of each treatment cycle. Of the 21 patients treated, 10 had thoracic malignancies and 11 had extra-thoracic malignancies with thoracic metastases. Patients were required to have completed all cancer treatments and have no evidence of disease. During registration, patients were randomized 1:1 to the vaccine with or without daily oral metronomic cyclophosphamide and celecoxib. The vaccine was well tolerated with local inflammation and flu-like symptoms within 72 to 96 hours of administration being the most common adverse events. There were no dose limiting

toxicities or treatment-related deaths. Fourteen patients (67%) completed all six vaccinations and eight of these patients (57%) exhibited serologic responses to NY-ESO-1. Vaccine therapy decreased the percent of Tregs (P=0.0068), PD-1 expression on Tregs (P=0.0027) and PD-L1 expression on monocytes (P=0.016). However, cyclophosphamide/celecoxib did not increase immune responses or alter vaccine-induced changes in peripheral immune subsets. With a median follow-up of 62.1 months, 12 patients were still alive with no evidence of disease. Unfortunately, the trial was terminated early due to loss of access to Iscomatrix.

This trial by Zhang *et al.* was well conducted and adequate measures were taken to ensure the quality and integrity of the vaccine. Immunoblot techniques using primary and secondary antibodies were used to confirm levels of NY-ESO-1, MAGE-A1, and MAGE-A3 prior to certification and release of the final product. As H1299 cells express class I and class II MHC antigens, freeze-thaw lysate techniques were used to deplete HLA proteins while preserving C-TA antigen levels. Extensive immune phenotyping was performed to measure vaccine-induced changes in immune subsets. Adjuvant used was Iscomatrix which has been tested previously for its ability to enhance cytotoxic CD8+ T lymphocyte activity (16). The study did identify serological responses to NY-ESO-1 in majority of the patients who completed the 6 vaccine doses (12 out of 14). Interestingly, significant responses to MAGE were not observed. These differential responses to NY-ESO-1 and MAGE may be explained by the low sample size or by the difference in the amount and immunogenicity of C-TA antigens in the vaccine lysate. As the trial closed to enrolment before the accrual goal was reached due to loss of supply to adjuvant, the trial was underpowered for the primary outcomes. The lack of primary tumor tissues from most of the patients prevented analysis of baseline C-TA expression and correlation with serum antibody titers. Twelve of 21 patients showed no evidence of disease on long-term follow-up and majority of majority of tumor recurrences occurred before the 6 vaccine doses were completed. The trial only enrolled and treated 21 patients with a variety of tumors with only half being primary thoracic malignancy. Although small size of study and heterogeneous patient population, the long-term disease control appears promising.

Developing peptide vaccines targeting C-TAs does not require the use of autologous cells, which is a strength of this approach. However, heterogeneity in the amount and immunogenicity of C-TA antigens in vaccine lysate is a limitation and this needs to be addressed in future trials investigating this approach. This heterogeneity is likely because the frequency of C-TA expression is highly dependent on tumor type, degree of differentiation, and stage of progression (17). In-depth studies on C-TA expression and function are needed to guide future research. Another limitation of using peptide vaccines targeting C-TAs is the inhibitory effect of immunosuppressive cells in the tumor microenvironment such as Tregs. In a previous phase 1 trial that investigated a five-peptide cancer vaccine (KOC1, TTK, URLC10, DEPDC1 and MPHOSPH1), escalating doses of cyclophosphamide were associated with drop in levels of Tregs (18). However, in the trial by Zhang *et al.*, use of cyclophosphamide/celecoxib did not increase immune responses or alter vaccine-induced changes in peripheral immune subsets. This finding may have been impacted by early termination and small size of the study.

While many of the C-TA peptide vaccine trials have demonstrated a favorable toxicity profile and an immune response after vaccination (19,20), these have not translated into significant survival advantages in the phase II/III trials to date. MAGRIT trial (NCT00480025) (21) was a randomized international trial in 2,312 patients with completely resected stage IB to IIIA MAGE-A3-positive NSCLC based on promising data from early-phase trials. Patients were randomly assigned (2:1) to receive 13 intramuscular injections of recMAGE-A3 with AS15 immunostimulant or placebo. Although the vaccine was well-tolerated, there was no difference in median disease-free survival (DFS) between the two groups (60.5 *vs.* 57.9 months; P=0.74). This may be explained by the ability of these vaccines to elicit immune responses but not strong enough to result in clinical benefit. To overcome this limitation, a number of ongoing trials are now investigating vaccine therapy in combination with checkpoint inhibitors such as PD-1/PD-L1 antibodies (NCT02775292, NCT04639245). This is supported but preclinical evidence as a study investigating the combination of anti-PD-1 antibody and a multipeptide vaccine (consisting of immunogenic peptides derived from breast cancer antigens, neu, legumain, and β-catenin) in breast cancer-bearing mice reported increase in vaccine induced progression-free survival (PFS) with the addition of the PD1 agent (22). This prolonged survival was associated with increase in number of CD8 T cells, and decrease in number of PD-1+ dendritic cells (DC). Another approach is to use C-TA based engineered T-cell therapy. However, this approach does require autologous

cells from patients and is associated with higher rate of toxicity. A previous study for instance, reported that anti-MAGE-A3 TCR gene therapy is associated with neurologic toxicity (23).

In conclusion, the recently reported trial by Zhang *et al.* adds to the existing literature on the ability of peptide vaccines targeting C-TAs in eliciting serological immune response. However, due to the early termination, small study size and heterogeneous patient population, clinical benefit of this approach is unclear. The trial did report a significantly prolonged DFS in patients who completed all 6 vaccine doses in the adjuvant setting. Heterogeneity in the amount and immunogenicity of C-TA antigens in vaccine lysate remains a challenge and future research should focus on novel combinations of C-TA peptide vaccines with checkpoint inhibitors or oncolytic viruses.

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