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FOCUSSED RESEARCH REVIEW

Cancer vaccine strategies: translation from mice to human clinical trials

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Abstract We translated two cancer vaccine strategies from mice into human clinical trials. (1) In preclinical studies on TARP, an antigen expressed in most prostate cancers, we mapped epitopes presented by HLA-A*0201, modified them to increase affinity and immunogenicity in HLA transgenic mice, and induced human T cells that killed human cancer cells ("epitope enhancement"). In a clinical trial, HLA-A2$^+$ prostate cancer patients with PSA biochemical recurrence (Stage D0) were vaccinated with two peptides either in Montanide-ISA51 or on autologous dendritic cells (DCs). In stage D0, the Prostate-Specific Antigen (PSA) slope is prognostic of time to radiographic evidence of metastases and death. With no difference between arms, 74% of combined subjects had a decreased PSA slope at 1 year compared to their own baseline slopes ($p = 0.0004$). For patients vaccinated with DCs, response inversely correlated with a tolerogenic DC signature. A randomized placebo-controlled phase II trial is underway. (2) HER2 is a driver surface oncogene product expressed in multiple tumors. We made an adenoviral vector vaccine expressing the extracellular and transmembrane domains of HER2 and cured mice with large established HER2$^+$ tumors, dependent on antibodies to HER2, not T cells. The mechanism differed from that of trastuzumab. We tested a human version in advanced metastatic cancer patients naïve to HER2-directed therapies. At the second and third dose levels, 45% of evaluable patients showed clinical benefit. Circulating tumor cells also declined in some vaccinated patients. Thus, cancer vaccines developed in mice were successfully translated to humans with promising early results.

Keywords Cancer vaccine · TARP · Prostate cancer · PSA slope · HER2 · DC vaccines

Abbreviations

DC	Dendritic cell
GM-CSF	Granulocyte-macrophage colony-stimulating factor
HLA	Human leukocyte antigen
KLH	Keyhole limpet hemocyanin

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MHC	Major histocompatibility complex
PSA	Prostate-specific antigen
TARP	T cell receptor gamma-chain-alternate reading frame protein
TCR	T cell receptor

Cancer vaccines may target either the antibody (humoral) or T cell arm of the immune system (Fig. 1). Antibodies can detect only antigens on the surface of intact tumor cells, whereas T cells can detect any protein antigen made in the cell because the major histocompatibility complex (MHC) molecules (such as HLA in humans) act as an internal surveillance mechanism or spy within the cell to detect fragments of all proteins made in the cell and carry them to the surface where they can be seen by T cell receptors (TCRs) [1, 2]. Thus, most cancer vaccines attempt to induce cytotoxic T cells that can kill tumor cells. Here, we will give examples of both types of cancer vaccines that we have developed based on mouse model preclinical studies and translated to human clinical trials with promising early results. With the increased focus on immunotherapy due to the success of checkpoint inhibitors and adoptive T cell therapy [3–6, 7, 8], as well as with the licensing of the first human therapeutic cancer vaccine, Sipuleucel-T [9, 10], there is a revived interest in cancer vaccines [11–15], especially to induce immune responses in otherwise less-immunogenic tumors. Such cancer vaccines may also be aided by combinations with checkpoint inhibitors, or inhibitors of other negative regulatory cells and molecules, to allow the resulting T cells to have their maximum effect [14–23].

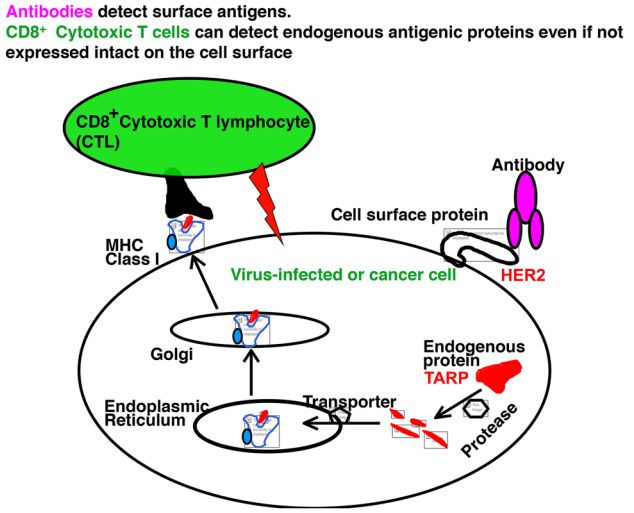

Fig. 1 Two arms of the adaptive immune system that can attack cancer. Antibodies can detect only molecules on the surface of intact cancer cells, such as HER2, whereas T cells can recognize fragments of any protein made in the cell (such as the prostate cancer antigen TARP) after it is processed and the fragments are carried to the surface bound to class I MHC molecules to be recognized by receptors of CD8 cytotoxic T lymphocytes. Thus, most cancer vaccines aim to induce such cytotoxic T cells

Because cancers that are detected clinically have already evaded potential immunosurveillance, their antigens may not be optimal immunogens. We developed an approach we call epitope enhancement to modify amino acid sequences of epitopes to make them more immunogenic [24–30] (Fig. 2). This involves identifying amino acid residues critical for binding of the peptide to the MHC molecule without affecting TCR recognition, using a combination of predictions of primary and secondary anchor residues [31–35] and of empirical assays, as well as residues that interfere with binding, and then making substitutions to increase affinity of the peptide for the MHC molecule. Then, the modified sequences have to be tested for binding affinity, immunogenicity in HLA-transgenic mice, and most importantly, for the ability to induce T cells that recognize not only the enhanced sequence but also the wild-type natural sequence

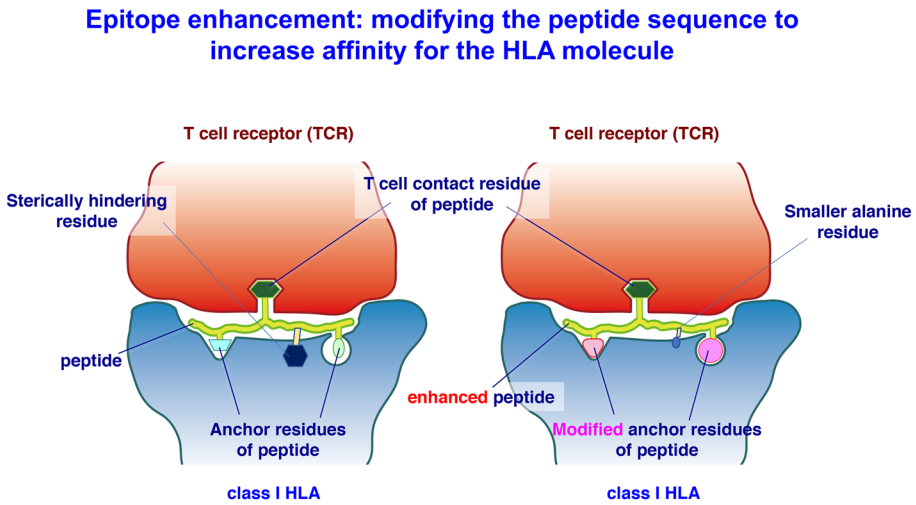

Fig. 2 Epitope enhancement improves affinity for MHC molecules, increasing immunogenicity of antigenic peptides. The natural peptide antigen sequence may have suboptimal anchor residues that can bind in the pockets of the MHC molecules, resulting in suboptimal affinity. The natural peptide may also have side chains that sterically hinder binding to the MHC molecule, so replacing them with a small alanine residue can reduce hindrance and improve binding affinity also

since that is the sequence in the virus or tumor cell. We applied this to both viral and tumor antigens. For the current study, we first mapped epitopes binding to HLA-A*0201, the most common human class I MHC molecule, in the sequence of TARP (T cell receptor gamma chain alternative reading frame protein) originally discovered by Ira Pastan's lab [36]. This protein is encoded by a different reading frame from the TCR gamma chain so the amino acid sequence is unrelated to T cell proteins. TARP was found to be expressed in about 95% of prostate cancers and about half of breast cancers, and at all stages and Gleason types of prostate cancer. We then modified the sequences to increase affinity for HLA-A*0201 and tested these in a binding assay and for immunogenicity in HLA-A2 transgenic mice [37]. One TARP epitope, 27–35, was already high affinity and none of the modifications increased that affinity. However, TARP 29–37, an overlapping but independent epitope, bound only moderately well to HLA-A*0201 but could be improved by replacing the C-terminal residue with valine to make 29-37-9V. That enhanced peptide induced T cells reactive with the wild-type TARP sequence, whereas some others, such as TARP-29-37-3A, did not [37]. Finally, these were tested for induction of human T cells in vitro and found to induce human T cells that could kill human cancer cells (MCF-7) expressing both TARP and HLA-A*0201, and not ones with either alone [37].

Based on these results, we initiated a phase I clinical trial (NCT00972309) to test safety and immunogenicity of these two peptides in HLA-A*0201^{+} prostate cancer patients. We divided the subjects into two arms, one receiving the peptides emulsified together with GM-CSF in Montanide-ISA51 given subcutaneously, and one receiving the same peptides pulsed together with keyhole limpet hemocyanin (KLH) as a source of help onto autologous dendritic cells (DCs), given intradermally [38]. Autologous DCs were prepared monocytes from an apheresis pack cultured for 4 days with GM-CSF and IL-4 and then matured with interferon-gamma and lipopolysaccharide. We elected to treat stage D0 prostate cancer, the stage in which primary tumor had been definitively eradicated with surgery or radiation, but now a rising PSA in the absence of radiographically evident metastases is a biochemical indication of micro-metastatic tumor recurrence. This has the advantage that the tumor burden is small, such as in an adjuvant setting, but there is a parameter (PSA) one can measure to monitor effects on tumor growth without waiting for tumor to be detectable radiographically. In this setting, it has been shown that the rate of rise (slope or doubling time) of the PSA is a valid predictor of clinical outcome measured as time to radiographic progression or to death and response to therapy [39–44]. The practical problem with the widely used doubling time is that if the rate of PSA rise goes to 0, a desirable outcome, the doubling time becomes infinite, not a useful number for statistics, and if the PSA starts actually decreasing, an even better outcome, the doubling time becomes negative, not a meaningful number (Fig. 3). On the other hand, the slope, proportional to the reciprocal of the doubling time, is a continuous variable, whether positive, 0, or negative. Thus, we used the slope (log (PSA)) as our measure, compared to the patient's own pretreatment slope measured at ≥ 4 time points over > 3 months during the 12 months prior to vaccination. Patients were immunized five times at 3-week intervals from week 3-week 15, and PSA values followed for at least 1 year. Slope (log(PSA)) was determined at each time point from week 3 to that time point, and compared with that person's pre-treatment slope to determine the change in slope. Because the two arms were not statistically different, we were advised to combine them to increase statistical power. Of 40 patients treated on both arms, 71.8% had a decreased slope (log(PSA)) at 24 weeks ($p = 0.0012$) and 74.2% had a decreased slope at 48 weeks ($p = 0.0004$) [38]. 15% of the patients actually developed a negative slope, that is a decreasing PSA. This decreased tumor growth rate was corroborated by an independent analysis of tumor growth rate constant obtained by fitting the PSA values to an exponential growth curve [45], which found that the tumor growth rate constant fell in half ($p = 0.003$). Thus, the vaccine appeared to slow tumor growth in nearly ¾ of patients [38].

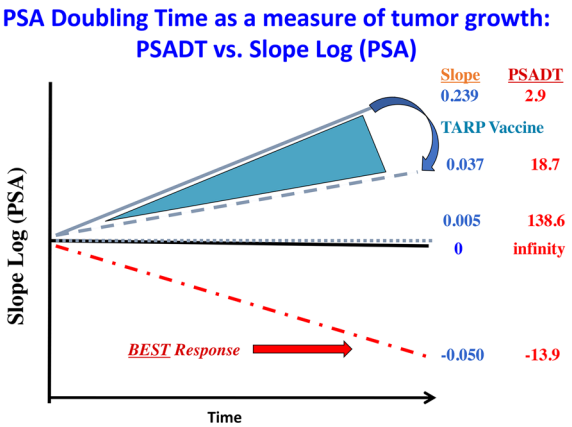

Fig. 3 Advantage of PSA slope instead of doubling time. Doubling time, which is proportional to the reciprocal of the slope, has the disadvantage that when the slope falls to 0, the doubling time goes to infinity, which is not a useful number even though it is clinically beneficial. When the slope becomes negative, the doubling time formula gives a negative value, which is meaningless. However, the slope itself is a continuous variable whether positive, 0, or negative. Doubling time or slope is a validated predictor of clinical outcome and response to therapy (see text). The shaded area shows the range of doubling times for eligibility for the clinical trial, between 3 months which implies rapid progression to 15 months, which corresponds with very slow progression. Modified from [38] with permission

When T cell responses were measured as interferon-gamma ELISPOT responses to the two vaccine peptides and the wild-type version of 29–37, 77.5% of patients made a new response not present at baseline (considered positive if ≥ 3-fold over background at at least two time points and statistically significant), but the magnitude of response did not correlate with clinical response [38]. Thus, the vaccine was safe and immunogenic, and showed preliminary evidence of clinical benefit, but we did not have an immune correlate of clinical activity. We hypothesized that differences in T cell function or avidity might correlate better, but a mechanistic correlate has not yet been identified. We had found earlier that human T cells raised against the TARP peptides could kill human tumor cells expressing TARP and HLA-A*0201, indicating that the epitopes were endogenously processed and presented in the tumor cells [37], but T cells from TARP-immunized patients in this study have not yet been tested for lytic activity. Nevertheless, based on the phase I results, a randomized placebo-controlled phase II trial (NCT02362451) has been opened. This trial extends the vaccine peptides to cover all of the TARP sequence (so called multi-epitope TARP), in addition to the original 2 peptides, to avoid limitations to HLA-A*0201. The phase II trial is being conducted with autologous DCs because this arm showed a more significant change in slope on its own that the Montanide arm in the phase I study and because making seven emulsions would have been impractical.

However, another parameter was discovered that might help explain which patients responded, at least in the arm that received autologous monocyte-derived DCs [46]. It was found that a combination of genes that correlated with a tolerogenic DC phenotype was inversely associated with a greater decrease in PSA slope and with poor immunological response [46]. By ROC curves, this gave 85% power to discriminate among PSA responders vs non-responders, and 98% power to discriminate among immunological responders and non-responders. Moreover, a simplified combination of four parameters, increased CD14 levels, increased IL-10 and CCL2 secretion, and decreased CCL22 secretion, correlated with similar predictive power [46]. This ability of the DC properties to predict DC vaccine effectiveness has important implications for optimizing DC-based vaccines.

We now turn to a vaccine that functions through an antibody response. In this case, it is fortunate that the driver oncogene product, HER2, is expressed on the surface of cancer cells where antibodies can detect it on intact cells [47]. One can think of it as a receptor that is constitutively driving the cells to proliferate. We know that antibodies to HER2 can be effective at least in some breast tumors because trastuzumab and other anti-HER2 antibodies are approved for this use. However, no vaccine has been developed to induce a patient to make her/his own antibodies to HER2 that are effective. However, there are peptide-based and other vaccines under study to induce T cell responses to HER2 [48, 49]. For mouse preclinical studies, we made an adenovirus vector expressing the extracellular (EC) and transmembrane (TM) domains of rodent HER2 [50], and immunized HER2-transgenic mice [50, 51] and mice bearing large established TUBO tumors [52]. The TUBO tumors derive from a BALB/c mouse transgenic for the rodent HER2 oncogene, and express high levels of HER2. We were gratified to see that even tumors 2 cm in diameter regressed completely within about 3 weeks after one dose of the vaccine [52]. Large established lung metastases also completely regressed. Thus, this vaccine fulfilled the key requirement that it could treat large established tumors, not just prevent ones injected after the vaccination. We had intended to make a vaccine to induce a T cell response, but it turned out to our surprise that the mechanism was completely dependent on antibodies. CD8 T cells were not necessary, as they could be depleted prior to vaccination, and as beta-2 microglobulin knockout mice (without MHC class I molecules), which lack CD8 T cells, were protected as well as wild-type mice. CD4 T cells could be depleted after the first 2 days, when they were needed to provide help for an antibody response, without affecting tumor rejection, so effector CD4 T cells were also not required. However, the vaccine did not work in J_H knockout B-cell deficient mice. In addition, serum from immunized mice could transfer the protection [52]. Thus, the protection was purely antibody-mediated. However, unlike trastuzumab, which was shown to require Fc receptors [53], the protection was just as effective in FcR deficient mice. Moreover, serum from the immunized mice could kill a pure population of TUBO tumor cells in vitro (in the absence of cells that could mediate antibody-dependent cellular cytotoxicity), and at 1:100 or 1:20 dilution, immune serum could inhibit phosphorylation of HER2 on the cell surface, suggesting that it worked by inhibiting oncogene function, not by cytotoxicity. Thus, the mechanism was different from that of trastuzumab, and the vaccine might work in patients who had failed trastuzumab. The vaccine also had the advantage that it did not require multiple expensive intravenous infusions of immunoglobulin every few weeks for the life of the patient. Polyclonal antibodies induced by the vaccine might also be more resistant to escape mutations than a monoclonal antibody.

Based on these preclinical results, we translated this vaccine to humans, making a cGMP version of the adenovirus expressing the EC and TM domains of human HER2. By omitting the intracellular domain, we avoided any chance of oncogenicity or reversion to an oncogenic phenotype. To avoid neutralization of the adenovirus in adenovirus-seropositive people, we used the adenovirus to transduce autologous DCs as the vaccine, and showed that this approach also worked in mice [50]. Patients with advanced $HER2^+$ metastatic cancers who had failed all standard therapies available

were immunized at 4–8 week intervals from week 0 to week 24 with escalating doses of autologous transduced DCs, and followed for 2 years after the last dose of vaccine for safety assessment. Because of the approximately 7% cardiotoxicity rate in patients receiving long-term trastuzumab therapy, we wanted to avoid testing safety of the adenovirus vaccine in patients previously exposed to trastuzumab or other HER2-directed therapy. Thus, part I of the trial (NCT01730118) was designed to treat patients naïve to these agents, mostly patients with non-breast tumors that expressed 1+ to 3+ levels of HER2 who were not eligible for trastuzumab. If safety was shown in these, then in Part II, we would proceed to treat breast cancer patients with 3+ levels of HER2 who had failed other HER2-directed therapies. Enrollment in Part I of the trial has been completed and we have seen no evidence of cardiotoxicity, despite frequent monitoring of left ventricular ejection fraction. At the lowest dose of 5 million autologous DCs, we saw no clinical responses (and no antibody responses), but at 10 and 20 million DCs, 5/11 evaluable patients, with metastatic cancers that had failed all standard therapies, showed evidence of clinical benefit (either complete response, partial response, or stable disease lasting ≥ 6 months) (Wood et al., manuscript in preparation). Several patients have also shown significant decreases in the number of circulating tumor cells, often almost complete disappearance. Antibody responses for later dose groups are pending, as are T cell responses. Based on this evidence and the safety profile, we have received approval to extend the treatment to 40 million DCs and to start enrollment in Part II of the study involving treatment of breast and other cancer patients who have progressed on licensed HER2-targeted therapies. If these promising results are borne out, then the next step would be a phase II efficacy trial.

In conclusion, we have translated two types of cancer vaccines from mice to human clinical trials. One vaccine is to induce T cells to a cell-internal prostate antigen, TARP, using an epitope-enhanced cancer vaccine and demonstrating the utility of the concept of epitope enhancement. This vaccine appears to slow tumor growth in nearly three quarters of stage D0 prostate cancer patients, and is now in phase II trials. The second vaccine is to induce antibodies to a cell-surface tumor antigen, HER2, that is a driver oncogene product accessible to antibodies. This vaccine has shown preliminary evidence of clinical benefit in patients with advanced metastatic HER2+ cancers that have failed all other therapies, including complete response, partial response and stable disease lasting ≥ 6 months, and decrease in circulating tumor cells. Both vaccines make use of autologous DCs, and we have seen that certain qualities of such DCs are critical for the success of such cancer vaccines. We conclude that both categories of cancer vaccines (targeting both arms of the adaptive immune system) can be translated from preclinical murine models to human clinical trials with promising early results.

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Author contributions Jay A. Berzofsky—planned and supervised all the preclinical and clinical projects and wrote the manuscript. Masaki Terabe—oversaw and supervised the preclinical research. Jane Trepel—planned and supervised the testing of circulating tumor cells. Ira Pastan—discovered the TARP antigen, provided unpublished information, and helped plan the clinical trials. David F. Stroncek—supervised the preparation of DCs for the clinical trials and planned and supervised the study of DC phenotype as a correlate of DC vaccine efficacy. John C. Morris—Prepared the original Adeno-HER2 vaccine and planned and supervised some of the preclinical studies of that vaccine, as well as helping to plan the HER2 clinical protocol. Lauren V. Wood—wrote both clinical protocols and carried out the clinical trials.

Compliance with ethical standards

Conflict of interest The authors declare they have no conflict of interest.

Ethical approval and ethical standards The animal protocols were approved by the NCI Animal Care and Use Committee accredited by the AAALAC and followed all the AAALAC regulations for animal care and use. The human protocols (NCI 09-C-0139 and 13-C-0016) were all approved by the NCI-NIH Institutional Review Board and the US Food and Drug Administration, and met all the United States ethical standards required for human studies.

Informed consent After appropriate explanation provided by the protocol principal investigator, all human subjects signed informed consent documents approved by the NCI-NIH Institutional Review Board.

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