Research Highlights

Highlights from the latest articles in personalized immunotherapy

Evaluation of a personalized peptide vaccine approach in patients with CRPC

Rahul Aggarwal^{†1}
& Andrea Harzstark¹

[†]University of California-San Francisco, Department of Medicine, Division of Hematology/Oncology, 505 Parnassus Avenue, M1286, Box 1270, San Francisco, CA 9413 1270, USA

[†]Author for correspondence:

Tel.: +1 312 315 0127

Fax: +1 415 476 0624

rahul.aggarwal@ucsf.edu

Evaluation of: Noguchi M, Kakuma T, Uemura H *et al.*: A randomized Phase II trial of personalized peptide vaccine plus low dose estramustine phosphate (EMP) versus standard dose EMP in patients with castration resistant prostate cancer. *Cancer Immunol. Immunother.* 59, 1001–1009 (2010).

Immunotherapy, in which the host's immune system is induced to attack prostate cancer cells, is currently under investigation as an alternative or addition to cytotoxic chemotherapy for castration-resistant prostate cancer (CRPC), especially in light of the recent US FDA approval of the dendritic cell-based vaccine, sipuleucel-T, based on an overall survival (OS) benefit demonstrated in several Phase III trials [1,2]. Other immunologic techniques currently being investigated include tumor cell-based vaccines, recombinant viral vectors and CTLA-4 blockade [3–6].

In the present study, Noguchi *et al.* evaluated a personalized peptide-based vaccine (PPV) therapy in HLA-A24+ or HLA-A4+ patients with metastatic CRPC in a randomized Phase II trial, building on previous clinical data [7]. A total of 26 peptide vaccine candidates were tested in each patient prevaccination and every 6 weeks thereafter, using a similar technique, as previously reported [8,9]. The top immune response-inducing peptides were selectively chosen for each patient (up to a total of four peptides). Patients were randomized 1:1 to receive either low-dose estramustine phosphate (EMP; 280 mg/day) and open-label PPV administered subcutaneously weekly versus standard-dose EMP (560 mg/day) (treatment period 1 [TP1]),

with crossover to the other treatment arm at the time of progressive disease (PD; treatment period 2 [TP2]).

With a total of 57 patients enrolled, the study was terminated prematurely on the basis of an improved median progression-free survival from 2.8 to 8.5 months in the experimental arm versus control arm in TP1 (hazard ratio [HR]: 0.28; $p = 0.0012$). At the time of PD, 19 patients and 11 patients crossed over in the control arm and experimental arm, respectively. During TP2, there was no significant difference in progression-free survival (1.8 months vs 2.3 months, PPV + low dose EMP vs standard dose EMP, respectively). Importantly, OS was improved in the experimental arm versus control arm (median OS not reached vs 16.1 months, HR: 0.3, $p = 0.0328$), despite the confounding effect of crossover after PD. The vaccine was well tolerated, with mostly grade 1–2 local skin reactions at the site of peptide injection seen in the majority of patients.

Limitations of this study includes the short median follow-up for OS of approximately 11 months. Furthermore, of the 39 progression events during TP1, 35 were defined on the basis of rising prostate-specific antigen alone, and only four patients had radiographic progression of disease, which may be a more meaningful end point to define disease progression in CRPC.

Nevertheless, these data suggest that PPV therapy may provide clinical benefit to patients with CRPC. Issues to address include the optimal timing of PPV in relation to chemotherapy, effectiveness prior to/in combination with docetaxel as opposed to estramustine (which is not commonly used as first-line chemotherapy

Financial & competing interests disclosure

The authors have no 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. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

for CRPC), and the development of surrogate biomarkers (either clinical or immunological) to select those patients most likely to benefit from peptide-based therapy. Indeed, in the current study, although immune responses were seen in response to peptide-based therapy in the majority of patients (64–72%), there was no association between immune response and clinical outcome. Finally, it is unclear whether selecting specific peptides for individual patients leads to improved efficacy compared with a standardized peptide vaccine. Larger trials are needed to investigate these issues and to confirm the findings observed in this small Phase II trial.

References

- 1 Small EJ, Shellhammer PF, Higano CS *et al.*: Placebo-controlled Phase III trial of immunologic therapy with sipuleucel-T (APC8015) in patients with metastatic, asymptomatic hormone refractory prostate cancer. *J. Clin. Oncol.* 24, 3089–3094 (2006).
- 2 Schelhammer PF, Higano C, Berger ER *et al.*: A randomized, double-blind, placebo-controlled, multi-center Phase III trial of sipuleucel-T in men with metastatic, androgen independent prostatic adenocarcinoma (AIPC). Presented at: *The Annual Meeting of the American Urological Association*. IL, USA 25–30 (2009).
- 3 Small EJ, Sacks N, Nemunatitis J *et al.*: Granulocyte macrophage colony-stimulating factor-secreting allogeneic cellular immunotherapy for hormone-refractory prostate cancer. *Clin. Cancer Res.* 13, 3883–3891 (2007).
- 4 Kaufman HI, Wang W, Manola J *et al.*: Phase II randomized study of vaccine treatment of advanced prostate cancer (E7897): a trial of the Eastern Cooperative Oncology Group. *J. Clin. Oncol.* 22, 2122–2132 (2003).
- 5 Small EJ, Tchekmedyian NS, Rini BI *et al.*: A pilot study of CTLA-4 blockade with human anti-CTLA-4 in patients with hormone-refractory prostate cancer. *Clin. Cancer Res.* 13, 1810–1815 (2007).
- 6 Noguchi M, Kakuma T, Uemura H *et al.*: A randomized Phase II trial of personalized peptide vaccine plus low dose estramustine phosphate (EMP) versus standard dose EMP in patients with castration resistant prostate cancer. *Cancer Immunol. Immunother.* 59, 1001–1009 (2010).
- 7 Noguchi M, Itoh K, Yao A *et al.*: Immunological evaluation of individualized peptide vaccination and with a low-dose of estramustine for HLA-A24+ HRPC patients. *Prostate* 63, 1–12 (2005).
- 8 Komatsu N, Shichijo S, Nakagawa M *et al.*: New multiplexed flow cytometric assay to measure anti-peptide antibody: a novel tool for monitoring immune responses to peptides used for immunization. *Scand. J. Clin. Lab. Invest.* 64, 1–11 (2004).
- 9 Hida N, Maeda Y, Katagir K *et al.*: A simple culture protocol to detect peptide-specific cytotoxic T lymphocyte precursors in circulation. *Cancer Immunol. Immunother.* 51, 219–228 (2002).

Immunological evaluation of a personalized peptide vaccine for patients with CRPC

Evaluation of: Uemura H, Fujimoto K, Mine T *et al.*: Immunological evaluation of personalized peptide vaccination monotherapy in patients with castration-resistant prostate cancer. *Cancer Sci.* 101, 601–608 (2010).

Personalized peptide vaccination (PPV) therapy is currently being investigated as a novel approach to immunotherapy for men with CRPC [1]. Patients are prescreened prior to treatment with a set of peptides derived from proteins related to cancer

cells, including prostate-specific antigen (PSA) and prostatic acid phosphatase. Only reactive peptides (up to a total of four), based on prevaccination antipeptide serum IgG levels and peptide-specific cytotoxic T lymphocyte (CTL) precursor IFN- γ production, are subsequently administered to the patient. This is based on the hypothesis that immune boosting of peptide-specific CTLs will be more effective than priming of naive T cells in inducing immunologic response. Other studies of PPV therapy, given concurrently

with hormone therapy and low-dose EMP, have shown promising clinical and immunologic activity [1,2].

In the current study by Uemura *et al.* [3], PPV monotherapy was assessed in 23 HLA-A24+ or A2+ patients with CRPC in a single arm study, to evaluate PPV monotherapy without concurrent EMP or hormone therapy. Patients were screened against a set of 32 peptides for HLA-A2+ patients and 25 peptides for HLA-A24+ patients. Reactive peptides (up to four) were subsequently

administered subcutaneously every 2–4 weeks × six doses, followed by the re-evaluation of immunological response. PPV therapy was given until death, intolerance, or significant disease progression. resumption of gonadotropin releasing hormone (GnRH) agonists was allowed at progressive disease or when serum testosterone increased to more than 0.5 ng/ml; addition of EMP was also allowed at the time of progressive disease.

The most common toxicities consisted of grade 1–2 local skin reactions at the site of injection. Two grade 3 adverse events occurred: one patient with a localized subcutaneous abscess and another patient with thrombocytopenia after the second vaccination. Of 17 evaluable patients, only three showed a PSA decline with vaccination monotherapy. Interestingly, in all three patients with a PSA response, there was an initial rise in serum PSA after the first cycle of six vaccinations was followed by a

subsequent decline in PSA and disease control with further vaccination, suggesting a lag time in the benefit of PPV therapy. Peptide-specific serum IgG augmentation after the first six vaccinations was seen in 15 out of 17 patients (88%); augmented peptide-specific CTL responses were seen in six out of 17 patients (35.3%).

The patient with grade 3 thrombocytopenia raised concerns for off-target immune-related side effects, and suggests that carefully monitoring for such adverse events in future studies is warranted. Minimal clinical response was seen with PPV monotherapy, and the discontinuation of GnRH agonist therapy at the start of protocol therapy confounds the evaluation of clinical efficacy by adding another variable that may influence progression of disease. Further studies are needed to clarify the optimal timing of PPV therapy in relation to chemotherapy and/or hormone therapy.

References

- 1 Noguchi M, Kakuma T, Uemura H *et al.*: A randomized Phase II trial of personalized peptide vaccine plus low dose estramustine phosphate (EMP) versus standard dose EMP in patients with castration resistant prostate cancer. *Cancer Immun.* 59(7), 1001–1009 (2010).
- 2 Noguchi M, Itoh K, Yao A *et al.*: Immunological evaluation of individualized peptide vaccination and with a low-dose of estramustine for HLA-A24+ HRPc patients. *Prostate* 63, 1–12 (2005).
- 3 Uemura H, Fujimoto K, Mine T *et al.*: Immunological evaluation of personalized peptide vaccination monotherapy in patients with castration-resistant prostate cancer. *Cancer Sci.* 101, 601–608 (2010).

Evaluation of a randomized Phase II trial of a poxviral-based PSA-targeted immunotherapy in patients with minimally symptomatic metastatic CRPC

Evaluation of: Kantoff PW, Schuetz TJ, Blumenstein BA *et al.*: Overall survival analysis of a Phase II randomized controlled trial of a poxviral-based PSA-targeted immunotherapy in metastatic castration-resistant prostate cancer. *J. Clin. Oncol.* 28, 1099–1105 (2010).

Recombinant vaccinia vector containing the entire PSA transgene with agonist epitope (rV-PSA) is a novel immunotherapy platform that has demonstrated activity in a Phase I trial in men with CRPC [1]. Preclinical studies have shown activity with first the addition of three costimulatory molecules along with the PSA transgene (rV-PSA-TRICOM) and second use of GM-CSF as an adjuvant to recruit dendritic cells [2,3]. A randomized Phase II trial previously demonstrated that a vaccinia vector prime (rV)

followed by multiple fowlpox (rF) boosts had improved progression-free survival (PFS) compared with all-vaccinia or all-fowlpox vaccination [4]. This sequence of rV-PSA-TRICOM prime followed by rF-PSA-TRICOM boosts constitutes poxviral-based PSA-targeted immunotherapy (PROSTVAC-VF) therapy.

In the current study, Kantoff *et al.* evaluated PROSTVAC-VF in a randomized, placebo-controlled Phase II trial of chemotherapy-naïve men with minimally symptomatic, Gleason grade of seven or less, nonvisceral metastatic CRPC [5]. Men were randomized 2:1 to receive PROSTVAC or placebo on days 1, 14, 28, 56, 84, 112 and 140. The PROSTVAC arm received a priming immunization with recombinant rV followed by subsequent boosts with recombinant fowlpox rF; adjuvant GM-CSF was administered for 4 days per treatment cycle, near the site of vaccination. Control

arm patients received placebo injections with empty viral vectors and saline in place of GM-CSF.

A total of 125 patients were randomized (84 to PROSTVAC arm; 41 to control arm). There was no difference in the primary end point of centrally reviewed radiographic PFS between arms (HR: 0.88; 95% CI: 0.57–51.38). PSA declines were infrequent and there were no objective radiographic responses. However, the PROSTVAC arm showed a significant OS advantage (median OS: 25.1 vs 16.6 months; HR: 0.58, 95% CI: 0.38–30.88, $p = 0.0095$), despite a crossover rate of approximately 50% in the control arm. The OS benefit was consistent across multiple prognostic subgroups. Unfortunately, there are no data available on whether the use of subsequent therapy after disease progression was balanced between arms. Therapy was well tolerated, with most adverse events related to

injection site reactions of grade 1–2 severity and mild systemic symptoms such as pyrexia and rigors.

The observation of an OS benefit without a gain in PFS, as well as the shape of the OS Kaplan–Meier curve, is similar to the results observed in a Phase III trial of sipuleucel-T, the recently US FDA approved autologous dendritic cell-based vaccine [6]. With both vaccines, the OS curves for vaccine versus placebo arms begin to separate at approximately 1 year, well beyond the median time to disease progression and completion of therapy. This suggests a class effect of immunotherapy, in which immunoreactivity or potentially some other unmeasured factor has the potential

to provide an extended clinical benefit and alter the disease course beyond initial disease progression. Whether PROSTVAC-VF is effective and appropriate in patients with more advanced disease (such as those requiring narcotics for cancer-related pain), given the time required to generate an immune response, remains to be seen. A pivotal Phase III trial of PROSTVAC-VF is being planned, as confirmation of the findings seen in this study.

References

- 1 Eder JP, Kantoff PW, Roper K *et al.*: A Phase I trial of a recombinant vaccinia virus expressing prostate-specific antigen in advanced prostate cancer. *Clin. Cancer Res.* 16, 1632–1638 (2000).
- 2 Hodge JW, Sabzevari H, Yafal AG, Gritz L, Lorenz MG, Schlom J: A triad of costimulatory molecules synergize to amplify T-cell activation. *Cancer Res.* 159, 5800–5807 (1999).
- 3 Kass E, Panicali DL, Mazzara G, Schlom J, Greiner JW: Granulocyte/macrophage-colony stimulating factor produced by recombinant avian poxviruses enriches the regional lymph nodes with antigen-presenting cells and acts as an immunoadjuvant. *Cancer Res.* 161, 206–214 (2001).
- 4 Kaufman HL, Wang W, Manola J *et al.*: Phase II randomized study of vaccine treatment of advanced prostate cancer (E7897): a trial of the Eastern Cooperative Oncology Group. *J. Clin. Oncol.* 122, 2122–2132 (2004).
- 5 Kantoff PW, Schuetz TJ, Blumenstein BA *et al.*: Overall survival analysis of a Phase II randomized controlled trial of a poxviral-based PSA-targeted immunotherapy in metastatic castration-resistant prostate cancer. *J. Clin. Oncol.* 28, 1099–1105 (2010).
- 6 Small EJ, Shellhammer PF, Higano CS *et al.*: Placebo-controlled Phase III trial of immunologic therapy with sipuleucel-T (APC8015) in patients with metastatic, asymptomatic hormone refractory prostate cancer. *J. Clin. Oncol.* 24, 3089–3094 (2006).

Analysis of immunologic and clinical prognostic factors for metastatic CRPC treated with poxviral-based PSA-targeted immunotherapy

Evaluation of: Gulley JL, Arlen PM, Madan RA *et al.*: Immunologic and prognostic factors associated with overall survival employing a poxviral-based PSA vaccine in metastatic castrate-resistant prostate cancer. *Cancer Immunol. Immunother.* 59, 663–674 (2010).

Poxviral-based PSA-targeted immunotherapy (PROSTVAC-VF) is a recombinant vaccinia virus prime/fowlpox virus boost expressing a PSA transgene and three costimulatory molecules (rV-PSA-TRICOM + rF-PSA-TRICOM), designed to induce immune response against cancer cells. It has shown an OS benefit observed in a randomized Phase II trial of minimally symptomatic men with non-visceral metastatic CRPC [1]. The complementary Phase II trial conducted by Gulley *et al.* affords the opportunity to evaluate the immunological response to

PROSTVAC-VF, and activity in a more advanced disease setting including those requiring narcotic use, higher Gleason score, and visceral metastases [2].

In this trial by Gulley *et al.*, 32 chemotherapy-naïve men with CRPC, who were HLA-A2+, were randomized to one of four cohorts:

- No adjuvant GM-CSF
- Recombinant human GM-CSF protein
- 10^7 plaque-forming units (pfu) recombinant fowlpox (rF)-GM-CSF
- 10^8 pfu rF-GM-CSF

All patients were primed with rV-PSA-TRICOM on day 1, followed by monthly boosts of rF-PSA-TRICOM until disease progression. Immunologic response was assessed by several methods: first, changes in T-cell IFN- γ production upon exposure to PSA peptide PSA-3 after 3 months of vaccination therapy. Second, antifowlpox IgG antibody titers

and fowlpox virus neutralization, and third regulatory CD4⁺CD25^{high} T-cell (Treg) number and function [3,4].

Among all 32 patients, the median predicted survival was 17.4 months by the Halabi nomogram [5]. A total of 12 of 32 patients (37.5%) had a decline in serum PSA, with five patients showing a decline of more than 30%. With a median follow-up of 44.6 months, the median OS for all 32 patients was 26.6 months, approximately 9 months longer than predicted by the Halabi nomogram [5]. Intriguingly, for patients with a Halabi predicted survival of less than 18 months ($n = 17$), the observed median OS was 14.6 months (vs predicted median: 12.3 months); by contrast for patients with a predicted survival of less than 18 months ($n = 15$), the observed median OS was 37.3 months or more (vs predicted median OS: 20.9 months). There was no significant difference in OS among the four treatment cohorts evaluating the use of GM-CSF as described earlier.

In terms of immunological response, 13 of 29 (45%) evaluable patients demonstrated a twofold or greater increase in PSA peptide-specific T-cell IFN- γ production. By *post hoc* analysis, patients with a more than sixfold increase in T-cell response, as compared with those with less than sixfold increase, showed a trend towards improved OS ($p = 0.055$). Although antifowlpox antibodies were produced postvaccination, no neutralizing antibody titers were detected. Finally, among patients surviving longer than predicted, 10 out of 13 (77%) patients had a decrease in Treg suppressive function; conversely among patients surviving shorter than predicted, Treg function actually increased in 6/8 (75%) of patients.

These hypothesis-generating results suggest that CRPC patients with a more favorable prognosis based on the Halabi nomogram may derive the most benefit from PROSTVAC-VF therapy. Alternatively,

this could be the result of differences in when chemotherapy is utilized and post-chemotherapy treatments, such that the Halabi nomogram may underestimate OS (a second version of the Halabi nomogram is expected). The study results also suggest that decreases in Treg suppressor function and increased PSA-specific T-cell response may be surrogate biomarkers of OS in patients treated with PROSTVAC-VF. These hypotheses will be evaluated in planned larger randomized trials.

References

- 1 Kantoff PW, Schuetz TJ, Blumenstein BA *et al.*: Overall survival analysis of a Phase II randomized controlled trial of a poxviral-based PSA-targeted immunotherapy in metastatic castration-resistant prostate cancer. *J. Clin. Oncol.* 28, 1099–1105 (2010).
- 2 Gulley JL, Arlen PM, Madan RA *et al.*: Immunologic and prognostic factors associated with overall survival employing a poxviral-based PSA vaccine in metastatic castrate-resistant prostate cancer. *Cancer Immunol. Immunother.* 59, 663–674 (2010).
- 3 Gulley J, Chen AP, Dahut W *et al.*: Phase I study of a vaccine using recombinant vaccinia virus expressing PSA (rV-PSA) in patients with metastatic androgen-independent prostate cancer. *Prostate* 153, 109–117 (2002).
- 4 Yokokawa J, Cereda V, Remondo C *et al.*: Enhanced functionality of CD4⁺CD25(hi)FoxP3⁺ regulatory T cells in the peripheral blood of patients with prostate cancer. *Clin. Cancer Res.* 114, 1032–1040 (2008).
- 5 Halabi S, Small EJ, Kantoff PW *et al.*: Prognostic model for predicting survival in men with hormone-refractory metastatic prostate cancer. *J. Clin. Oncol.* 121, 1232–1237 (2003).