

## REVIEW

# Role of Antigen Spread and Distinctive Characteristics of Immunotherapy in Cancer Treatment

James L. Gulley, Ravi A. Madan, Russell Pachynski, Peter Mulders, Nadeem A. Sheikh, James Trager, Charles G. Drake

**Affiliations of authors:** Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD (JLG, RAM); Washington University School of Medicine, St. Louis, MO (RP); Department of Urology, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands (PM); Dendreon Pharmaceuticals Inc., Seattle, WA (NAS, JT); Johns Hopkins Sidney Kimmel Comprehensive Cancer Center and The Brady Urological Institute, Baltimore, MD (CGD).

**Current affiliation:** Nkarta Inc., South San Francisco, CA (JT).

**Correspondence to:** James L. Gulley, MD, PhD, FACP, Center for Cancer Research, National Cancer Institute, National Institutes of Health, 10 Center Drive, 13N240C, MSC-1750, Bethesda, MD 20892 (e-mail: gulleyj@mail.nih.gov).

## Abstract

Immunotherapy is an important breakthrough in cancer. US Food and Drug Administration-approved immunotherapies for cancer treatment (including, but not limited to, sipuleucel-T, ipilimumab, nivolumab, pembrolizumab, and atezolizumab) substantially improve overall survival across multiple malignancies. One mechanism of action of these treatments is to induce an immune response against antigen-bearing tumor cells; the resultant cell death releases secondary (nontargeted) tumor antigens. Secondary antigens prime subsequent immune responses (antigen spread). Immunotherapy-induced antigen spread has been shown in clinical studies. For example, in metastatic castration-resistant prostate cancer patients, sipuleucel-T induced early immune responses to the immunizing antigen (PA2024) and/or the target antigen (prostatic acid phosphatase). Thereafter, most patients developed increased antibody responses to numerous secondary proteins, several of which are expressed in prostate cancer with functional relevance in cancer. The ipilimumab-induced antibody profile in melanoma patients shows that antigen spread also occurs with immune checkpoint blockade. In contrast to chemotherapy, immunotherapy often does not result in short-term changes in conventional disease progression end points (eg, progression-free survival, tumor size), which may be explained, in part, by the time taken for antigen spread to occur. Thus, immune-related response criteria need to be identified to better monitor the effectiveness of immunotherapy. As immunotherapy antitumor effects take time to evolve, immunotherapy in patients with less advanced cancer may have greater clinical benefit vs those with more advanced disease. This concept is supported by prostate cancer clinical studies with sipuleucel-T, PSA-TRICOM, and ipilimumab. We discuss antigen spread with cancer immunotherapy and its implications for clinical outcomes.

Immunotherapy is an important advance in cancer treatment, highlighted as the “breakthrough of the year” by *Science Magazine* in 2013 (1) and the American Society of Clinical Oncology’s “advance of the year” in 2015 (2). Several therapies that enhance immune responses have demonstrated improvements in overall survival (OS) (1). Among the US Food and Drug Administration (FDA)-approved agents used in cancer treatment are ipilimumab for melanoma (3); nivolumab for melanoma (4), non-small cell lung cancer (5,6), renal cell carcinoma

(7), and Hodgkin lymphoma (8); atezolizumab for urothelial cancer (9); pembrolizumab for melanoma (10) and non-small cell lung cancer (11); and sipuleucel-T for prostate cancer (12). Sipuleucel-T is an autologous cellular immunotherapy that targets prostatic acid phosphatase (PAP) and is approved in the United States for the treatment of patients with asymptomatic or minimally symptomatic metastatic, castration-resistant prostate cancer (mCRPC) (13). Additional immunotherapeutic approaches in clinical development include cytokines such as

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interleukin-15, other vaccinations including a poxvirus-based combination regimen, adoptive cell transfer (including chimeric antigen receptor-engineered T-cells), and blockade of immune checkpoints (14–20).

Immunotherapies differ in a number of ways from conventional chemotherapy as they are not directly cytotoxic to the tumor; instead, these therapies aim to engage the immune system to generate antitumor activity (21). Immunotherapies as a class are often associated with statistically significant improvements in OS but not in progression-free survival (PFS) (22), although benefits in reducing tumor progression are often observed (2,23). For example, in mCRPC patients, sipuleucel-T statistically significantly reduced the risk of death compared with control (hazard ratio [HR] = 0.78, 95% confidence interval [CI] = 0.61 to 0.98,  $P = .03$ ), whereas the time to objective disease progression was similar between groups (HR = 0.95, 95% CI = 0.77 to 1.17,  $P = .63$ ) (12). Ipilimumab with or without a glycoprotein 100 (gp100) peptide vaccine statistically significantly reduced the risk of death compared with gp100 alone (comparison of ipilimumab + gp100 vs gp100 alone: HR = 0.68,  $P < .001$ , comparison of ipilimumab alone vs gp100 alone: HR = 0.66,  $P = .003$ , respectively), in patients with metastatic melanoma (3). However, the median PFS was similar across the groups, ie, 2.76 (95% CI = 2.73 to 2.79, ipilimumab with gp100), 2.86 (95% CI = 2.76 to 3.02, ipilimumab alone), and 2.76 months (95% CI = 2.73 to 2.83, gp100 alone) (3).

Limited effect on PFS by immunotherapy may reflect the time required to mount a clinically relevant immune response, in contrast to the immediate action of cytotoxic chemotherapy or targeted agents (eg, tyrosine-kinase inhibitors). However, the immune response can persist long after the completion of treatment (24), and may improve over time (25) and induce development of long-lived memory cells, providing continuous immunologic activity (26). Furthermore, unlike conventional therapy, the immune responses induced or expanded by immunotherapies can spread to include new antigenic targets (27,28).

The onset and broadening of responses with immunotherapy occurs as a result of the tumor immunity cycle (17). Tumor cell death in response to immunotherapy may lead to the release of secondary (ie, nontargeted) tumor antigens that prime subsequent immune responses. Antigen spread (also known as “epitope spread,” “determinant spread,” or “antigen cascade”) is the expansion of an immune response to secondary epitopes that either were not part of the original therapeutic or were not targeted by the therapy (21). This process is dynamic and may continue to expand over time. As discussed below, responses to immunotherapy (both vaccines and immune checkpoint modulation, and likely other immune approaches) may be associated with antigen spread.

This review outlines the concept of antigen spread in the context of cancer immunotherapy. The implications of antigen spread in terms of immunologic and clinical outcomes will be discussed.

## Antigen Spread

In studies spanning over three decades, antigen spread has been observed in the context of autoimmunity and infectious disease (29–35). Cancer immunotherapy also appears to work, in part, through antigen spread, ie, beginning with an immune response to specific target antigens that broadens over time to additional antigens expressed within the tumor (Figure 1) (21). Mechanistically, this phenomenon likely occurs when an initial antitumor immune response (eg, from a therapeutic anticancer

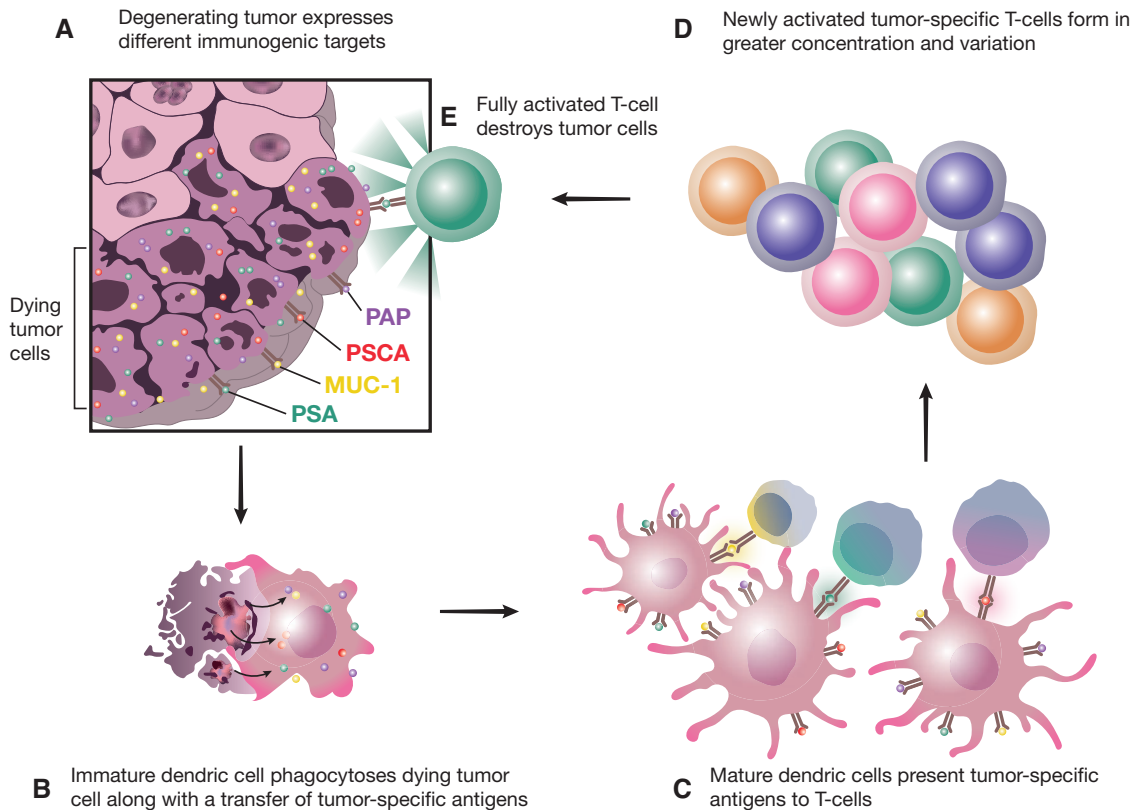
vaccine or from T-cell reactivation by immune checkpoint blockade) induces T-cell-mediated destruction of tumor cells. This destruction leads to the release of additional tumor-associated antigens (TAAs) that may be taken up locally by antigen-presenting cells (APCs) such as macrophages and dendritic cells. The TAAs are processed and presented by APCs to induce B-cell- and T-cell-mediated immune responses against the secondary antigens (21). Figure 2 details the process of antigen spread following immunotherapy. An additional/alternative hypothesis for the generation of secondary immune responses is that a successful immune response against the primary/targeted antigen of an immunotherapy may also overcome suppressive factors (17). Thus, immunotherapy may act to amplify preexisting antibody responses in addition to de novo generation of novel responses. In addition, radiation, chemotherapy, and other commonly used anticancer agents may kill tumor cells in an immunologically relevant manner. This treatment can lead to immunogenic cell death, which induces changes such as translocation of calreticulin to the surface of the dead tumor cells, which mediates the recognition and clearance of these cells by professional and nonprofessional phagocytes, allowing for tumor-antigen processing and presentation to the immune system (37). However, this process may not be as efficient at generating an antitumor immune response as a therapeutic vaccine (38).

Antigens released from the tumor may be more immunogenic than the antigens initially immunologically targeted (eg, from a vaccine), and an ongoing, iterative process of antigen spread can initiate a broader and perhaps more clinically significant immune response (21). Furthermore, antigen spread may lead to an adaptive anticancer immune response that targets new mutations in tumor cell antigens as they occur (21). As the immune response broadens over time, the immune system’s durability and adaptability may mean that the kinetic profile of a clinical response following immunotherapy could differ from that following cytotoxic therapy.

## Evidence for Antigen Spread Mediated by Immunotherapy

To overcome the diversity in both tumor-antigen expression and in immune repertoire, many cancer vaccines are designed to induce responses to multiple antigens, and there is evidence that breadth of response is an important determinant of vaccine efficacy. IMA901 is a novel vaccine containing 10 tumor-associated peptides (TUMAPs) found in renal cell carcinoma (RCC) (39). Among RCC patients immunized with IMA901, those who responded to multiple TUMAPs had better disease control and improved OS compared with patients who had no response or only responded to one TUMAP. While IMA901 comprises multiple antigens, subsequent antigen spread could allow for broadening of the immune response regardless of the complexity of the agent used to generate the primary immune response.

Both preclinical and clinical data support the concept of antigen spread in cancer immunotherapy, and evidence continues to accumulate. One elegant preclinical study involved mice bearing tumors engineered to express carcinoembryonic antigen (CEA). Here, CEA-expressing (CEA+) tumors were implanted in one flank of experimental mice, while the opposite flank was implanted with parental (CEA-) tumors. Mice were vaccinated with a CEA-based vaccine, and substantial decreases in both the CEA+ and CEA- tumors were observed. These responses were associated with induced CD8+ T-cell responses to both CEA and other TAAs not included in the vaccine (40). Interestingly,



**Figure 1.** The process of antigen spread. **A)** An initial immune response is prompted following immunotherapy, releasing other antigens from dying tumor cells. **B)** Dendritic cells act as antigen-presenting cells (APCs), processing the “free” antigens, including neoantigens, and presenting these to T-cells. **C)** The APCs prime T-cells specific to antigens released from the tumor cells, increasing the breadth of the immune response. **D)** The newly activated tumor-specific T-cells form in greater concentration and variation. **E)** The activated T-cells then attack the tumor cells. Thus, while the initial therapy may target one antigen, a broader adaptive antitumor immune response may ensue. MUC-1 = mucin 1; PAP = prostatic acid phosphatase; PSA = prostate-specific antigen; PSCA = prostate stem cell antigen.

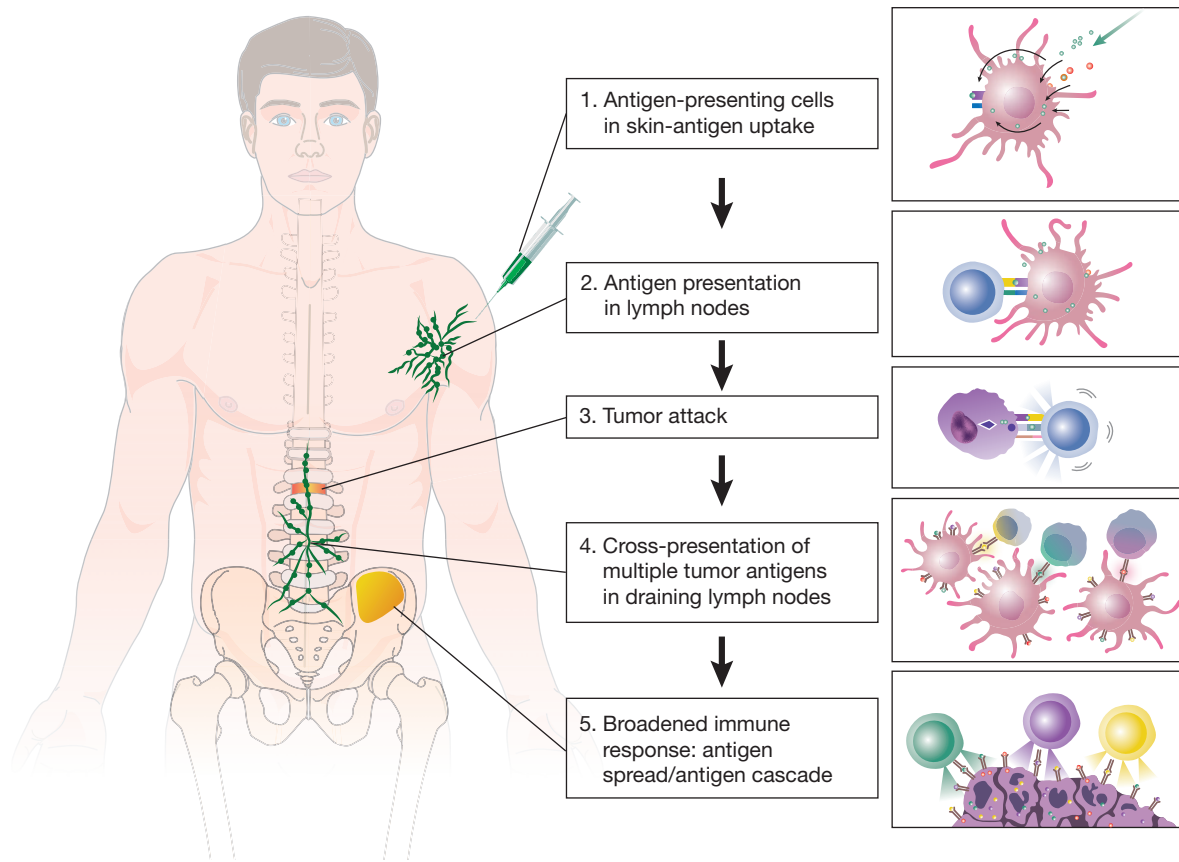
immune responses to wild-type p53 and the endogenous retroviral epitope gp70 were detected, with the immune response to gp70 approximately 15 times greater than the response to CEA, showing that responses to nontargeted antigens might be more important than those to the initial vaccine target (41).

Clinical studies also support the concept of antigen spread. Several of these studies involve the agent sipuleucel-T. In the pivotal phase III study, sipuleucel-T generated peripheral immune responses (either T-cell or humoral) to PA2024 (the immunizing antigen) and/or to PAP (the target antigen) in 79% of patients treated with sipuleucel-T compared with 13% of patients receiving control (42), demonstrating the ability of sipuleucel-T to induce immune responses to the original targeted antigen.

Antigen spread following treatment with sipuleucel-T has been most clearly demonstrated through studies of antibody responses in patients from the phase III pivotal trial, as well as in a phase II study (28). These analyses involved two phases: an initial “discovery” phase followed by a “confirmation” phase. In the initial phase, protein microarrays were used to analyze patients’ sera for immunoglobulin G (IgG) responses. Following treatment with sipuleucel-T, most patients showed increased antibody responses, defined as twofold or greater elevation in antigen-specific IgG after treatment, to a wide variety of nontarget proteins, whereas control patients showed no such responses. Interestingly, the median number of nontarget antigens increased from 56 antigens at two weeks post-treatment to 162 at 10 weeks post-treatment, suggesting a cascading humoral response. In the confirmation phase of

these analyses, an orthogonal assay (Luminex xMAP<sup>®</sup>, Luminex Corporation, Austin, TX) was used with a narrow pool of 10 antigens, five with the greatest fold increases in antibody levels after sipuleucel-T and five with known relevance to tumorigenesis or prostate cancer. The confirmation analyses showed that sipuleucel-T induced antibody increases to the secondary antigens PSA, KLK2, LGALS3, and LGALS8, which have been shown to be expressed at elevated levels in prostate cancer and/or to play a role in prostate cancer development, as well as to K-Ras and E-Ras, which are known to have functional relevance in cancer (43–49). Response rates to individual secondary antigens ranged from 28% to 44% in the phase III study, with 25% of sipuleucel-T-treated patients responding to three or more nontarget antigens. Antigen spread was observed two weeks after sipuleucel-T treatment and persisted for up to six months. Clinically, antibody responses to PSA and LGALS3 were associated with improved OS in sipuleucel-T-treated patients ( $P \leq .05$ ) (28).

Evidence of antigen spread in response to immunotherapy has also been reported in several other tumor types, including metastatic breast cancer (50) and melanoma (26,51,52). Women with metastatic breast cancer were treated with the monoclonal antibody trastuzumab in combination with a vaccine targeting human epidermal growth factor receptor 2 (HER2)/neu. As expected, most patients developed both helper and cytotoxic T-cell responses to the HER2/neu peptides present in the vaccine. However, vaccinated patients also developed new or augmented immunity against two epitopes of HER2/neu that were not included in the vaccine: p98.15 ( $P = .0055$  vs prevaccination)



**Figure 2.** Antigen spread following treatment with immunotherapy (36). Adapted (with permission of Springer) from: Jochems C, Schlom J, Gulley JL. Novel technologies for vaccine development. TRICOM poxviral-based vaccines for the treatment of cancer. In: Lukashevich IS, Shirwan H, eds. Vienna: Springer; 2014:291–328. Original caption: Fig. 10. 1 Schematic overview of TRICOM vaccines showing the tumor antigen gene and the genes for the three costimulatory molecules LFA-3, ICAM-1, and B7-1 that are inserted within the virus. The vaccine is prepared and administered “off the shelf.” 1: Subcutaneous administration leads to antigen uptake by antigen-presenting cells (APC) in the skin. 2: Antigen presentation occurs in the draining lymph nodes, activating antigen-specific T cells. 3: Tumor sites are attacked by antigen-specific cytotoxic (CD8+) T cells. 4: Tumor cell lysis leads to cross-presentation of multiple tumor antigens in the draining lymph nodes (antigen spread/antigen cascade). 5: Antigen cascade leads to activation of additional antigen-specific T-cells, which increases the breadth and quite possibly the clinical activity of the antitumor response.

and p776.15 ( $P = .0006$ ). In a subset of patients, increased T-cell responses were also observed following vaccination against several nontarget antigens, including insulin-like growth factor-binding protein 2 ( $P = .0973$ ), p53 ( $P = .1282$ ), and topoisomerase II- $\alpha$  ( $P = .0111$ ). These results are similar to the preclinical work described previously; both intramolecular and intermolecular antigen spread is evident in treated patients. This study in patients with metastatic breast cancer also included a substantial follow-up phase. During a median follow-up of 36 months, most patients maintained the level of immunity reported at the end of the immunization period for both target and new antigens, and approximately 25% of patients developed further immune responses (50). In a notable case study, a patient with metastatic melanoma had a durable remission after vaccination with melanoma-associated antigen (MAGE), but had only a low-level anti-MAGE cytolytic T-cell (CTL) response (26,51). Using T-cell receptor  $\beta$  (TCR $\beta$ ) cDNA libraries, very few vaccine-specific CTLs

were found in regressing metastases, but several TCR $\beta$  sequences were identified to correspond to nonvaccine tumor antigens. Further TCR $\beta$  analyses of T-cells from tumor-infiltrating lymphocytes led to the identification of a CD8 clone that specifically lysed autologous melanoma cells. The target antigen was caseinolytic protein, a mitochondrial enzyme mutated in the tumor to produce a neoantigen. Thus, in this patient, tumor rejection effectors were CTL responses to nonvaccine tumor-specific antigens, and this antigen spread was involved in tumor regression (26,51). Another recent study of adoptive cellular therapy combined with CTLA-4 blockade demonstrated antigen spread in patients with a durable tumor response (52). Collectively, these data highlight both the persistence of an antitumor immune response and the concept of late, ongoing antigen spread.

Cancer immunotherapy also encompasses agents that function by blocking negative costimulatory molecules in the immunological synapse that inhibit an antitumor response. The

immune response to these agents may also be characterized by the targeting of multiple antigens. Among these agents, collectively referred to as immune checkpoint blockers, the monoclonal antibody ipilimumab was the first-in-class T-cell potentiator for metastatic melanoma (53). Ipilimumab (anti-CTLA-4) blocks the interaction between cytotoxic T-lymphocyte antigen 4 (CTLA-4) and B7 molecules, resulting in T-cell activation and subsequent objective antitumor responses in certain patients. To better understand the antigens targeted in patients treated with ipilimumab, potential cancer neoantigens were identified by DNA sequencing of tissue samples from melanoma patients treated with ipilimumab (54). A high mutational load was statistically significantly correlated with improved OS, suggesting that the breadth of an antitumor immune response has clinical significance. To more closely quantify specific CD8 T-cell responses, two patient-specific neoantigens were assessed. For one of these neoantigens, there was no detectable T-cell response at seven weeks after initiation of ipilimumab; at 24 weeks, however, a strong response was observed (54). These data suggest an evolving response to a neoantigen. While it is unclear if this broader response is due to increased activation of immune cells (direct broadening) or more opportunities for immune cells to kill tumor cells and release antigens in an immunologically relevant manner (tumor immunity cycle-mediated broadening), there are data suggesting that a broader response may be clinically relevant following immune checkpoint blockade as well as after vaccination (55,56).

### Delayed Clinical Response to Immunotherapy

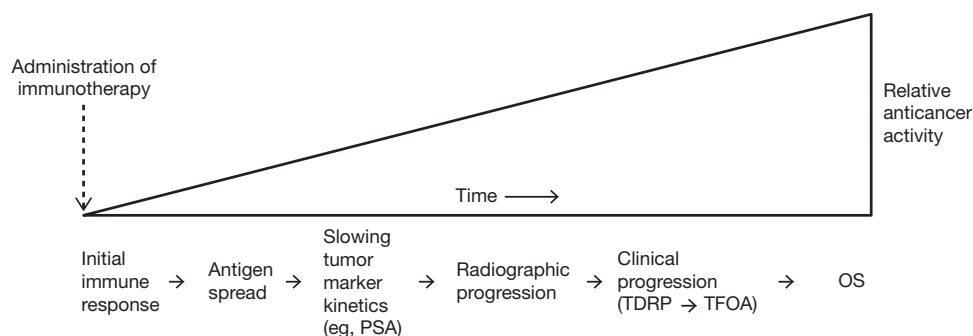
A long-term class effect of immunotherapies is an improvement in OS without short-term changes in conventional measures of disease progression (22). This seemingly paradoxical dissociation between the two end points likely reflects the time it takes to mount an effective immune response and for that response to evolve into a more clinically relevant response, eg, the time it takes for antigen spread to occur (Figure 3).

Although T- and B-cell responses to immunotherapy (ie, immune responses) can be detected in the blood relatively early in treatment, these initial responses may not be sufficient to delay radiographic progression and substantially increase PFS. The association of the kinetics of response to immunotherapy vs conventional therapy was examined using data from several clinical trials with either chemotherapy, androgen-deprivation

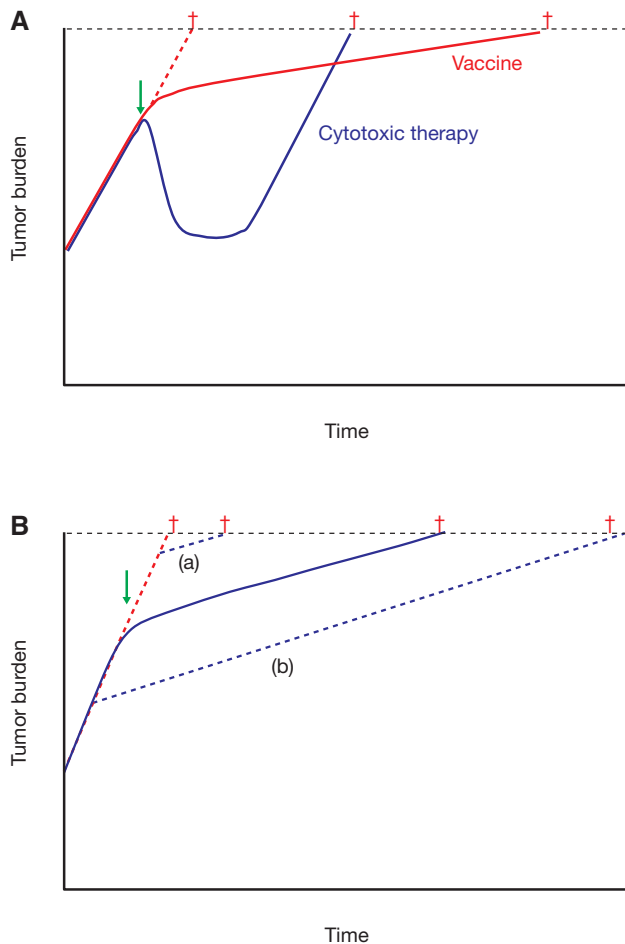
therapy (ADT), or immunotherapy with a vaccine in mCRPC (57–60). These analyses showed that chemotherapy reduced tumor burden quickly after treatment initiation, but effects were frequently short-lived after treatment cessation (Figure 4A). PSA kinetics during chemotherapy indicated tumor growth regression while on treatment; however, upon treatment cessation, the regrowth trajectory reverted back to the pretreatment growth rate (57,58). With immunotherapy, PSA kinetics were not changed immediately by treatment; however, there was an apparent slowing of disease progression as indicated by an OS much greater than predicted by this model (Figure 4A). This modeling confirmed the clinical outcomes reported and suggested that, particularly if started earlier in the course of the disease, immunotherapy may lead to substantially longer OS by slowing the disease trajectory (Figure 4B) (60).

The pivotal phase III study of sipuleucel-T in mCRPC patients showed similar persistent treatment benefits. Initial results showed a 4.1-month improvement in median survival (25.8 months in the sipuleucel-T group vs 21.7 months in the placebo group, HR = 0.78, 95% CI = 0.61 to 0.98,  $P = .03$ ) (12), and the 36-month survival probability was 31.7% in the sipuleucel-T group vs 23.0% in the placebo group, with differences in survival becoming apparent at six months following the start of treatment. As was the case for the studies above, no statistically significant improvement in time to objective disease progression was observed for sipuleucel-T. Combined post hoc analyses of the three phase III sipuleucel-T trials showed other measures consistent with a delayed treatment effect. While time to disease-related pain (TDRP) was not statistically significantly delayed between treated and control groups (median TDRP = 5.6 months for sipuleucel-T vs 5.3 months for control, HR = 0.82, 95% CI = 0.62 to 1.09,  $P = .170$ ), the 12-month pain-free rates were 39.3% with sipuleucel-T vs 18.9% with control (61). Moreover, time to first use of opioid analgesics (TFOA) was statistically significantly delayed with sipuleucel-T (median TFOA = 12.6 months for sipuleucel-T vs 9.7 months for control, HR = 0.76, 95% CI = 0.58 to 0.99,  $P = .038$ ). These data suggest an increasing impact of sipuleucel-T on end points that occur later in the disease course, as pain develops prior to the use of opioid analgesics (Figure 3).

Studies with other immunotherapies have reported similar delayed treatment effects. Data from 40 patients with mCRPC receiving PSA-TRICOM (a vector-based vaccine) indicated that tumor growth rates, based on PSA doubling time, were not reduced until day 80 relative to baseline (median difference



**Figure 3.** The anticancer activity of immunotherapy increases over time. Schematic representing progression and broadening of response over time following treatment with cancer-targeting immunotherapy. Antigen spread leads to more relevant targets (eg, neoantigens) for a given patient, and this highly individualized precision response could lead to improved clinical activity. Furthermore, with subsequent treatment, the immune response may be further boosted as tumor cells are killed or modulated in an immunogenic manner, which translates into improving clinical activity over time. OS = overall survival; PSA = prostate-specific antigen; TDRP = time to disease-related pain; TFOA = time to first opioid analgesic.



**Figure 4.** Tumor growth rates with immunotherapy (vaccine) and chemotherapy (cytotoxic therapy). **A** Tumor growth rate with no therapy (dotted black line), with cytotoxic therapy (blue line), and with vaccine (red line), demonstrating the slow yet prolonged response with immunotherapy resulting from immune response activation (red line) and a short-term tumor reduction with chemotherapy (blue line). **B** Initiating immunotherapy in early-stage disease may enhance the effects of immunotherapy (line b), whereas in later-stage disease the effects could be minimal (line a). Green arrows denote treatment initiation; crosses denote death (60). Adapted (with permission of Oxford University Press) from: Schlom J. Therapeutic cancer vaccines: Current status and moving forward. *J Natl Cancer Inst.* 2012;104(8):599–613.

=  $-0.04 \log \text{ PSA/month}$ , interquartile range =  $-0.08$  to  $0.01$ ,  $P = .02$ ) (62). A phase II, randomized double-blind study in patients with mCRPC treated with PSA-TRICOM failed to demonstrate an improvement in PFS compared with control (15). However, a statistically significant difference favoring PSA-TRICOM was reported for OS (25.1 vs 16.6 months, respectively, estimated HR = 0.56, 95% CI = 0.37 to 0.85,  $P = .0061$ ). In patients with colorectal cancer, treatment with the PANVAC vaccine (a poxviral-based vaccine targeting CEA and MUC-1) was associated with a similar delayed pattern of response (63). Here, OS improved with PANVAC vaccine after two years compared with controls (median not reached vs 44.1 months, 95% CI = 36.2 to 63.4, respectively,  $P < .0001$ ). However, the two-year recurrence-free survival rate was similar compared with controls (21.9 months, 95% CI = 16.9 to 38.8, vs 25.7 months, 95% CI = 20.0 to 37.2, respectively).

Similar delayed kinetics of benefit likely occur with immune checkpoint-blocking agents. In a phase III, randomized, placebo-

controlled trial comparing ipilimumab plus dacarbazine compared with dacarbazine alone, median PFS was similar in the two groups based on the week 12 assessment. However, a statistically significant difference in OS was observed (11.2 months, 95% CI = 9.4 to 13.6, for ipilimumab + dacarbazine vs 9.1 months, 95% CI = 7.8 to 10.5, for dacarbazine alone; HR for death = 0.72, 95% CI = 0.59 to 0.87,  $P < .001$ ). The higher survival rates for the ipilimumab + dacarbazine group persisted at three years compared with dacarbazine alone (20.8% vs 12.2%) (64). Consistent with this delayed and persistent benefit, a pooled analysis of 12 studies of ipilimumab for the treatment of melanoma reported a median OS of 11.4 months (95% CI = 10.7 to 12.1) and noted that the OS curve plateaus at around year 3 and extends to year 10 (65). Other studies of ipilimumab-treated patients support the notion of delayed benefit. Some patients initially categorized as partial responders ( $n = 10$ ) were later categorized as complete responders ( $n = 5$ ), and the average time to a complete response was 30 months (66). An improvement in OS without improvement in median PFS has been seen with PD1 inhibition, also suggesting that this observation may be a class effect of immunotherapy (5).

These observations highlight the concept that traditional clinical trial end points (tumor response by traditional response evaluation criteria in solid tumors, PFS) may not adequately reflect the survival benefits gained from immunotherapy. Consideration for extending the duration of clinical studies with immunotherapies may be necessary to adequately quantify their effects and/or adapt current response criteria, including immune-related response criteria (67).

## Immunotherapy in Early Disease States

Because the clinical antitumor effects of immunotherapy may take time to evolve, it follows naturally that treatment with immunotherapy earlier in the disease course should yield greater clinical benefit. Indeed, a subset analysis of the pivotal phase III trial of sipuleucel-T demonstrated that with decreasing baseline PSA (used as a surrogate for disease severity), both OS and OS benefit relative to control improved (68). The median OS in patients in the lowest baseline PSA quartile ( $\leq 22.1 \text{ ng/mL}$ ) was 41.3 months (sipuleucel-T) vs 28.3 months (placebo; difference = 13.0 months, HR = 0.51, 95% CI = 0.31 to 0.85). For patients in the highest baseline PSA quartile ( $> 134 \text{ ng/mL}$ ), median OS was 18.4 vs 15.6 months for sipuleucel-T and placebo, respectively (difference = 2.8 months, HR = 0.84, 95% CI = 0.55 to 1.29) (68). Therefore, patients in the lowest baseline PSA quartile appeared to derive the greatest benefit from treatment with sipuleucel-T vs control. Consistent with these observations, patients with lower lactate dehydrogenase values and better performance status also experienced greater benefit from sipuleucel-T compared with patients with more advanced disease. Furthermore, patients with low tumor burden are generally less immunosuppressed both systemically and in the tumor microenvironment (69). This concept is supported by a recent study of ipilimumab in prostate cancer (70). In a post hoc analysis of this study, patients with favorable prognostic factors at baseline (ie, alkaline phosphatase concentration  $< 1.5 \times$  upper limit of normal, hemoglobin levels  $\geq 11.0 \text{ g/mL}$ , no visceral metastases) had statistically significantly prolonged OS with ipilimumab compared with placebo, whereas in patients with at least one adverse prognostic factor, ipilimumab did not prolong OS (70). Taken together, these data support the concept that patients with less advanced disease may have a more robust and effective

immunologic response to therapy, allowing more time for antigen spread and associated survival benefits to be observed.

## Future Considerations

Further studies examining antigen spread following treatment with immunotherapy may be useful in not only understanding the full benefits to be gained from current treatments, but also in identifying other TAAs that may be appropriate targets for the development of immunotherapies. In addition, a detailed exploration of the clinical kinetics of immunotherapy should provide information of potential value in assisting clinical trial design and decision-making. If the hypothesis that immunotherapy has a delayed effect on the tumor growth curve is validated, treating earlier with immunotherapies may result in improved outcomes (60,71). This observation is supported by analyses that indicate that patients with a lower tumor burden have a differentially better clinical response to immunotherapy than those with a high tumor burden (68,70,72,73).

As our understanding of the mechanisms associated with cancer immunotherapies expands, future research will likely focus on four key concepts: 1) Combination regimens: The disparate properties of immunotherapy and conventional chemotherapy (targeting the tumor vs the immune system; timing of response and potential for resistance) provide a strong rationale for combining immunotherapy with cytoreductive therapy. In addition, conventional therapy can lead to tumor cell death, which weakens the mechanisms of tumor immune evasion and can enhance the ability of an activated immune system to recognize and control subsequent tumor growth (37,74). 2) Sequence of agents: The concept that the relative sequence of therapy may have immunological implications was examined in a randomized phase II study evaluating the optimal sequencing of sipuleucel-T and ADT in men with biochemically recurrent prostate cancer at high risk of developing metastases (sipuleucel-T combined with androgen deprivation therapy [STAND], NCT01431391) (24). Data from STAND indicate that cellular and humoral responses to PA2024 statistically significantly increased following sipuleucel-T treatment compared with baseline, and that these responses were sustained at all postdosing time points through 24 months ( $P < .001$ ). Sipuleucel-T given before, rather than after, ADT initiation resulted in greater PA2024-specific T-cell proliferation. Interestingly, sipuleucel-T-mediated antigen spread was observed with both treatments and was maintained through 12 months (24). 3) Combining vaccination with immune checkpoint blockade: While immune checkpoint blockers have shown promise, patients with no underlying immune response appear to be much less likely to benefit (75). Thus, vaccines may possibly convert a patient who is unlikely to respond into one more likely to respond to an immune checkpoint inhibitor (76–78). 4) Biomarkers: Identifying and validating biomarkers of response are important factors in early confirmation that patients are responding to treatment. Such biomarkers may be useful both in guiding the timing and selection of subsequent therapy and in making more reliable determinations of the success or failure of novel immunotherapy agents in early-stage clinical trials. Some progress has been made in identifying potential biomarkers. The trials with sipuleucel-T have identified antibody responses to the immunogen, and transient increases in serum eosinophil count and IgG responses to PSA and LGALS3 have been linked to OS (12,28,79). In vitro measures of immune activation in the sipuleucel-T product (cumulative APC activation, APC number, and total nucleated cell count) and antigen-specific

immune responses to sipuleucel-T have also been shown to correlate with OS in patients with mCRPC (42). Similarly, an antibody response to a glycan is associated with improved OS in patients treated with PSA-TRICOM (80).

## Conclusion

Antigen spread following treatment with immunotherapy can evoke robust, durable, and adaptable immune responses against tumors over an extended time period (3,12). Understanding the process of antigen spread, and thus the mode of action of immunotherapies, may help explain the greater improvements in efficacy observed in longer-term clinical study outcomes compared with short-term studies (eg, OS vs PFS) (3,12,42,70). Although immunotherapy has led to major clinical advances in the past several years, we continue to learn more about antigen spread and the mechanism of these treatments. In addition, we are exploring how to fully integrate and optimize their use with our current and emerging armory against cancer. Thus, the coming years promise to accelerate our understanding of how to further optimize patient outcomes.

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## References

1. Science's Top 10 Breakthroughs of 2013. Science AAAS. <http://www.science.org/news/2013/12/sciences-top-10-breakthroughs-2013-0>. Accessed July 21, 2016.
2. Dizon DS, Krilov L, Cohen E, et al. Clinical Cancer Advances 2016: Annual report on progress against cancer from the American Society of Clinical Oncology. *J Clin Oncol*. 2016;34(9):987–1011.
3. Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med*. 2010;363(8):711–723.
4. Wolchok JD, Kluger H, Callahan MK, et al. Nivolumab plus ipilimumab in advanced melanoma. *N Engl J Med*. 2013;369(2):122–133.
5. Borghaei H, Paz-Ares L, Horn L, et al. Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. *N Engl J Med*. 2015;373(17):1627–1639.
6. Brahmer J, Reckamp KL, Baas P, et al. Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer. *N Engl J Med*. 2015;373(2):123–135.
7. Motzer RJ, Escudier B, McDermott DF, et al. Nivolumab versus everolimus in advanced renal-cell carcinoma. *N Engl J Med*. 2015;373(19):1803–1813.
8. Ansell SM, Lesokhin AM, Borrello I, et al. PD-1 blockade with nivolumab in relapsed or refractory Hodgkin's lymphoma. *N Engl J Med*. 2015;372(4):311–319.
9. Rosenberg JE, Hoffman-Censits J, Powles T, et al. Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: A single-arm, multicentre, phase 2 trial. *Lancet*. 2016;387(10031):1909–1920.
10. Robert C, Schachter R, Long GV, et al. Pembrolizumab versus ipilimumab in advanced melanoma. *N Engl J Med*. 2015;372(26):2521–2532.
11. Garon EB, Rizvi NA, Hui R, et al. Pembrolizumab for the treatment of non-small-cell lung cancer. *N Engl J Med*. 2015;372(21):2018–2028.
12. Kantoff PW, Higano CS, Shore ND, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. *N Engl J Med*. 2010;363(5):411–422.

13. PROVENGE® (sipuleucel-T) prescribing information. Dendreon Corporation, Seattle, WA. <http://www.valeant.com/Portals/25/Pdf/PI/Provenge-PI.pdf>. Accessed June 09, 2016.
14. Amos SM, Duong CPM, Westwood JA, et al. Autoimmunity associated with immunotherapy of cancer. *Blood*. 2011;118(3):499–509.
15. Kantoff PW, Schuetz TJ, Blumenstein BA, et al. Overall survival analysis of a phase II randomized controlled trial of a Poxvirus-based targeted immunotherapy in metastatic castration-resistant prostate cancer. *J Clin Oncol*. 2010; 28(7):1099–1105.
16. Pardoll MD. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer*. 2012;12(4):252–264.
17. Chen DS, Mellman I. Oncology meets immunology: The cancer-immunity cycle. *Immunity*. 2013;39(1):1–10.
18. Waldmann TA. The biology of interleukin-2 and interleukin-15: Implications for cancer therapy and vaccine design. *Nat Rev Immunol*. 2006;6(8):595–601.
19. Gill S, June CH. Going viral: Chimeric antigen receptor T-cell therapy for hematological malignancies. *Immunol Rev*. 2015;263(1):68–89.
20. Hillerdal V, Essand M. Chimeric antigen receptor-engineered T-cells for the treatment of metastatic prostate cancer. *BioDrugs*. 2015;29(2):75–89.
21. Gulley JL. Therapeutic vaccines: The ultimate personalized therapy. *Hum Vaccin Immunother*. 2013;9(1):219–221.
22. Singh HB, Gulley JL. Immunotherapy and therapeutic vaccines in prostate cancer: An update on current strategies and clinical implications. *Asian J Androl*. 2014;16(3):364–371.
23. Rothschild SI, Thommen DS, Moersig W, Müller P, Zippelius A. Cancer immunology – development of novel anti-cancer therapies. *Swiss Med Wkly*. 2015; 145:w14066.
24. Antonarakis ES, Kibel AS, Adams GW, et al. Immune responses and clinical outcomes in STAND, a randomized phase 2 study evaluating optimal sequencing of sipuleucel-T (sip-T) and androgen deprivation therapy (ADT) in biochemically-recurrent prostate cancer (BRPC) after local therapy failure. *J Clin Oncol*. 2015;33(suppl 15):5030.
25. Taylor C, Hershman D, Shah N, et al. Augmented HER-2 specific immunity during treatment with trastuzumab and chemotherapy. *Clin Cancer Res*. 2007; 13(17):5133–5143.
26. Hardwick N, Chabin B. Epitope spreading contributes to effective immunotherapy in metastatic melanoma patients. *Immunotherapy*. 2011;3(6):731–733.
27. Kreiter S, Vormehr M, van de Roemer N, et al. Mutant MHC class II epitopes drive therapeutic immune responses to cancer. *Nature*. 2015;520(7549): 692–696.
28. GuhaThakurta D, Sheikh NA, Fan LQ, et al. Humoral immune response against non-targeted tumor antigens after treatment with sipuleucel-T and its association with improved clinical outcome. *Clin Cancer Res*. 2015;21(16): 3619–3630.
29. Lehmann PV, Forsthuber T, Miller A, Sercarz EE. Spreading of T-cell autoimmunity to cryptic determinants of an autoantigen. *Nature*. 1992;358(6382): 155–157.
30. Kaufman DL, Clare-Salzler M, Tian J, et al. Spontaneous loss of T-cell tolerance to glutamic acid decarboxylase in murine insulin-dependent diabetes. *Nature*. 1993;366(6450):69–72.
31. Bothamley GH. Epitope-specific antibody levels in tuberculosis: Biomarkers of protection, disease, and response to treatment. *Front Immunol*. 2014;5:243.
32. Dai YD, Carayanniotis G, Sercarz E. Antigen processing by autoreactive B cells promotes determinant spreading. *Cell Mol Immunol*. 2005;2(3):169–175.
33. Milich DR, McLachlan A, Thornton GB, Hughes JL. Antibody production to the nucleocapsid and envelope of the hepatitis B virus primed by a single synthetic T cell site. *Nature*. 1987;329(6139):547–549.
34. Bartholdy C, Stryhan A, Christensen JP, Thomsen AR. Single-epitope DNA vaccination prevents exhaustion and facilitates a broad antiviral CD8+ T cell response during chronic viral infection. *J Immunol*. 2004;173(10):6284–6293.
35. Nakagawa M, Greenfield W, Moerman-Herzog A, Coleman HN. Cross-reactivity, epitope spreading, and de novo immune stimulation are possible mechanisms of cross-protection of nonvaccine human papillomavirus (HPV) types in recipients of HPV therapeutic vaccines. *Clin Vaccine Immunol*. 2015;22(7): 679–687.
36. Jochems C, Schlom J, Gulley JL. TRICOM poxviral-based vaccines for the treatment of cancer. In: IS Lukashevich, H Shirwan, eds. *Novel Technologies for Vaccine Development*. Vienna: Springer; 2014:291–328.
37. Kroemer G, Galluzzi L, Kepp O, Zitvogel L. Immunogenic cell death in cancer therapy. *Annu Rev Immunol*. 2013;31:51–72.
38. Nesslinger NJ, Ng A, Tsang KY, et al. A viral vaccine encoding prostate-specific antigen induces antigen spreading to a common set of self-proteins in prostate cancer patients. *Clin Cancer Res*. 2010;16(15):4046–4056.
39. Walter S, Weinschenk T, Stenzl A, et al. Multipptide immune response to cancer vaccine IMA901 after single-dose cyclophosphamide associates with longer patient survival. *Nat Med*. 2012;18(8):1254–1261.
40. Hodge JW, Sharp HJ, Gameiro SR. Abscopal regression of antigen disparate tumors by antigen cascade after systemic tumor vaccination in combination with local tumor radiation. *Cancer Biother Radiopharm*. 2012;27(1):12–22.
41. Kudo-Saito C, Schlom J, Hodge JW. Induction of an antigen cascade by diversified subcutaneous/intratumor vaccination is associated with antitumor responses. *Clin Cancer Res*. 2005;11(6):2416–2426.
42. Sheikh NA, Petrylak DP, Kantoff P, et al. Sipuleucel-T product and immune parameters correlate with overall survival in men with castrate-resistant prostate cancer. *Cancer Immunol Immunother*. 2013;62(1):137–147.
43. Taylor BS, Schultz N, Hieronymus H, et al. Integrative genomic profiling of human prostate cancer. *Cancer Cell*. 2010;18(1):11–22.
44. Laderach DJ, Gentilini LD, Giribaldi L, et al. A unique galectin signature in human prostate cancer progression suggests galectin-1 as a key target for treatment of advanced disease. *Cancer Res*. 2013;73(1):86–96.
45. Califice S, Castronovo V, Bracke M, van den Brule F. Dual activities of galectin-3 in human prostate cancer: Tumor suppression of nuclear galectin-3 vs tumor promotion of cytoplasmic galectin-3. *Oncogene*. 2004;23(45): 7527–7536.
46. Williams SA, Xu Y, De Marzo AM, Isaacs JT, Denmeade SR. Prostate-specific antigen (PSA) is activated by KLK2 in prostate cancer ex vivo models and in prostate-targeted PSA/CLK2 double transgenic mice. *Prostate*. 2010;70(7): 788–796.
47. Rittenhouse HG, Finlay JA, Mikolajczyk SD, Partin AW. Human kallikrein 2 (hK2) and prostate-specific antigen (PSA): Two closely related, but distinct, kallikreins in the prostate. *Crit Rev Clin Lab Sci*. 1998;35(4):275–368.
48. Kubota E, Kataoka H, Aoyama M, et al. Role of ES cell-expressed Ras (ERas) in tumorigenicity of gastric cancer. *Am J Pathol*. 2010;177(2):955–963.
49. Liu Y, Wang Z, Li H, Wu Z, Wei F, Wang H. Role of ERas gene in gastric cancer cells. *Oncol Rep*. 2013;30(1):50–56.
50. Disis ML, Wallace DR, Gooley TA, et al. Concurrent trastuzumab and HER2/neu-specific vaccination in patients with metastatic breast cancer. *J Clin Oncol*. 2009;27(28):4685–4692.
51. Corbière V, Chapiro J, Stroobant V, et al. Antigen spreading contributes to MAGE vaccination-induced regression of melanoma metastases. *Cancer Res*. 2011;71(4):1253–1262.
52. Chapuis AG, Roberts IM, Thompson JA, et al. T-cell therapy using interleukin-21-primed cytotoxic T-cell lymphocytes combined with cytotoxic T-cell lymphocyte antigen-4 blockade results in long-term cell persistence and durable tumor regression. *J Clin Oncol*. 2016; in press.
53. Lipson EJ, Drake CG. Ipilimumab: An anti-CTLA-4 antibody for metastatic melanoma. *Clin Cancer Res*. 2011;17(22):6958–6962.
54. Snyder A, Makarov V, Merghoub T, et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. *N Engl J Med*. 2014;371(23):2189–2199.
55. Kvistborg P, Phillips D, Kelderman S, et al. Anti-CTLA-4 therapy broadens the melanoma-reactive CD8+ T-cell response. *Sci Transl Med*. 2014;6(254): 254ra128.
56. Kwek SS, Dao V, Roy R, et al. Diversity of antigen-specific responses induced in vivo with CTLA-4 blockade in prostate cancer patients. *J Immunol*. 2012; 189(7):3759–3766.
57. Stein WD, Figg WD, Dahut W, et al. Tumor growth rates derived from data for patients in a clinical trial correlate strongly with patient survival: A novel strategy for evaluation of clinical trial data. *Oncologist*. 2008;13(10):1046–1054.
58. Stein WD, Gulley JL, Schlom J, et al. Tumor regression and growth rates determined in five intratumoral NCI prostate cancer trials: The growth rate constant as an indicator of therapeutic efficacy. *Clin Cancer Res*. 2011;17(4):907–917.
59. Madan RA, Gulley JL, Fojo T, Dahut WL. Therapeutic cancer vaccines in prostate cancer: The paradox of improved survival without changes in time to progression. *Oncologist*. 2010;15(9):969–975.
60. Schlom J. Therapeutic cancer vaccines: Current status and moving forward. *J Natl Cancer Inst*. 2012;104(8):599–613.
61. Small EJ, Higano CS, Kantoff PW, Whitmore JB, Frohlich MW, Petrylak DP. Time to disease-related pain and first opioid use in patients with metastatic castration-resistant prostate cancer treated with sipuleucel-T. *Prostate Cancer Prostatic Dis*. 2014;17(3):259–264.
62. DiPaola RS, Chen YH, Buble G, et al. A national multicenter phase 2 study of prostate-specific antigen (PSA) pox virus vaccine with sequential androgen ablation therapy in patients with PSA progression. *Eur Urol*. 2015;68(3): 365–371.
63. Morse MA, Niedzwiecki D, Marshall JL, et al. A randomized phase II study of immunization with dendritic cells modified with poxvectors encoding CEA and MUC1 compared with the same poxvectors plus GM-CSF for resected metastatic colorectal cancer. *Ann Surg*. 2013;258(6):879–886.
64. Robert C, Thomas L, Bondarenko I, et al. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. *N Engl J Med*. 2011;364(26): 2517–2526.
65. Schadendorf D, Hodi FS, Robert C, et al. Pooled analysis of long-term survival data from phase II and phase III trials of ipilimumab in unresectable or metastatic melanoma. *J Clin Oncol*. 2015;33(17):1889–1894.
66. Prieto PA, Yang JC, Sherry RM, et al. CTLA-4 blockade with ipilimumab: Long-term follow-up of 177 patients with metastatic melanoma. *Clin Cancer Res*. 2012;18(7):2039–2047.
67. Bohnsack O, Hoos A, Ludajic K. Adaptation and modification of the immune related response criteria (IRRC): IrRECIST. *J Clin Oncol*. 2014;32(suppl);abstr e22121.
68. Schellhammer PF, Chodak G, Whitmore JB, et al. Lower baseline prostate-specific antigen is associated with a greater overall survival benefit from sipuleucel-T in the Immunotherapy for Prostate Adenocarcinoma Treatment (IMPACT) trial. *Urology*. 2013;81(6):1297–1302.



69. Escors D. Tumour immunogenicity, antigen presentation and immunological barriers in cancer immunotherapy. *New J Sci*. 2014;pii:734515.
70. Kwon ED, Drake CG, Scher HI, et al. Ipilimumab versus placebo after radiotherapy in patients with metastatic castration-resistant prostate cancer that had progressed after docetaxel chemotherapy (CA184-043): A multicentre, randomised, double-blind, phase 3 trial. *Lancet Oncol*. 2014;15(7):700-712.
71. Gulley JL, Madan RA, Heery CR. Therapeutic vaccines and immunotherapy in castration-resistant prostate cancer: Current progress and clinical applications. *Am Soc Clin Oncol Educ Book*. 2013;doi:10.1200/EdBook\_AM.2013.33.e166.
72. 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*. 2010;59(5):663-674.
73. Gulley JL, Madan RA, Schlom J. Impact of tumour volume on the potential efficacy of therapeutic vaccines. *Curr Oncol*. 2011;18(3):e150-157.
74. Hodge JW, Garnett CT, Farsaci B, et al. Chemotherapy-induced immunogenic modulation of tumor cells enhances killing by cytotoxic T lymphocytes and is distinct from immunogenic cell death. *Int J Cancer*. 2013;133(3):624-636.
75. Yuan J, Adamow M, Ginsberg BA, et al. Integrated NY-ESO-1 antibody and CD8+ T-cell responses correlate with clinical benefit in advanced melanoma patients treated with ipilimumab. *Proc Natl Acad Sci U S A*. 2011;108(40):16723-16728.
76. Madan RA, Mohebtash M, Arlen PM, et al. Ipilimumab and a poxviral vaccine targeting prostate-specific antigen in metastatic castration-resistant prostate cancer: A phase 1 dose-escalation trial. *Lancet Oncol*. 2012;13(5):501-508.
77. O'Sullivan Coyne G, Gulley JL. Adding fuel to the fire: Immunogenic intensification. *Hum Vaccin Immunother*. 2014;10(11):3306-3312.
78. van den Eertwegh AJ, Versluis J, van den Berg HP, et al. Combined immunotherapy with granulocyte-macrophage colony-stimulating factor-transduced allogeneic prostate cancer cells and ipilimumab in patients with metastatic castration-resistant prostate cancer: A phase 1 dose-escalation trial. *Lancet Oncol*. 2012;13(5):509-517.
79. McNeel DG, Gardner TA, Higano CS, et al. A transient increase in eosinophils is associated with prolonged survival in men with metastatic castration-resistant prostate cancer who receive sipuleucel-T. *Cancer Immunol Res*. 2014;2(10):988-999.
80. Campbell CT, Gulley JL, Oyeleran O, Hodge JW, Schlom J, Gildersleeve JC. Humoral response to a viral glycan correlates with survival on PROSTVAC-VF. *Proc Natl Acad Sci U S A*. 2014;111(17):E1749-1758.