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Review

REVIEW

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

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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,

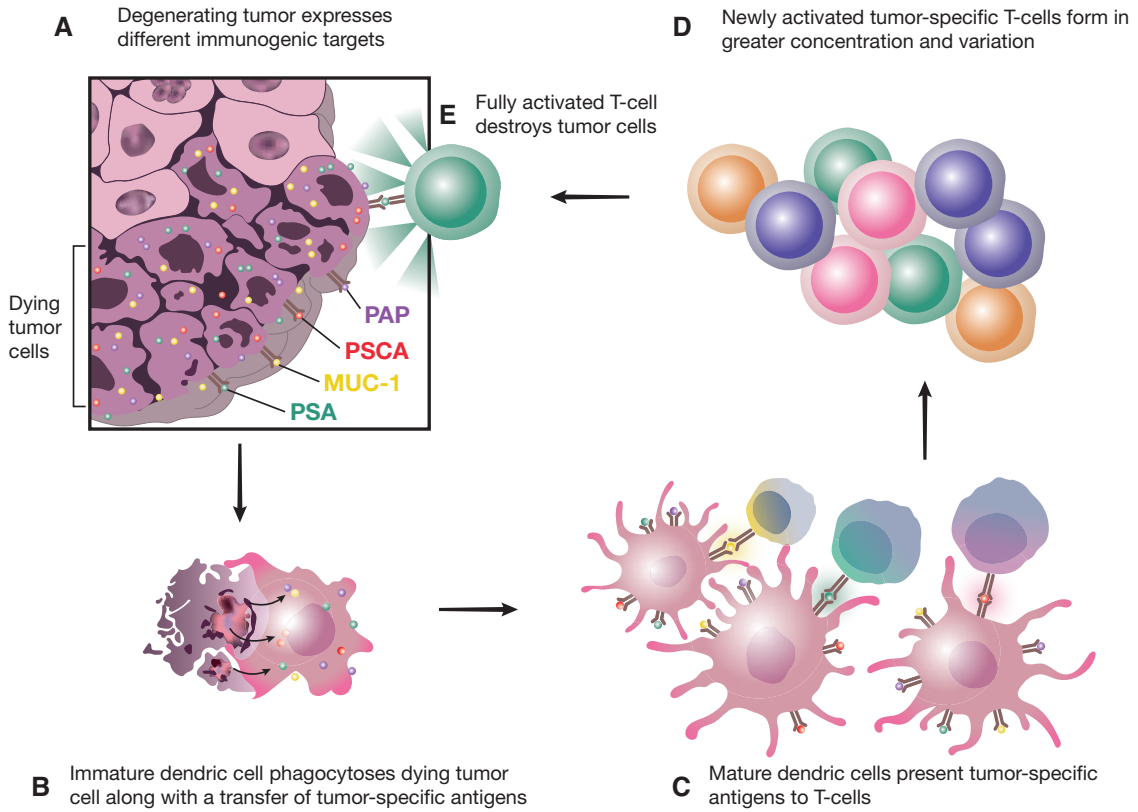

Figure 1. The process of antigen spread. **A)** An initial immune response is prompted following immunotherapy, releasing other antigens from dying tumor cells. **B)** Dendritic cells act as antigen-presenting cells (APCs), processing the "free" antigens, including neoantigens, and presenting these to 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®, 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)

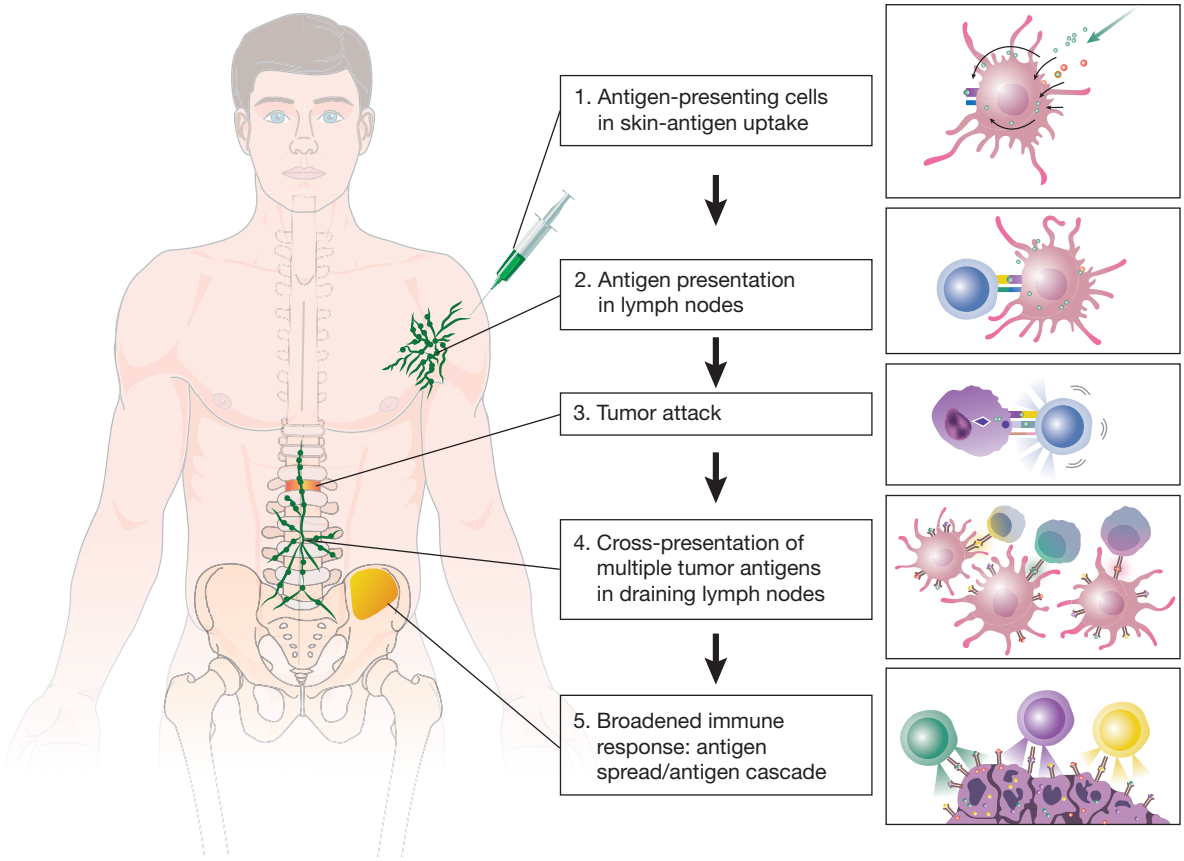

Figure 2. Antigen spread following treatment with immunotherapy (36). Adapted (with permission of Springer) from: Jochems C, Schlom J, Gulley JL. Novel technologies for vaccine development. TRICOM poxviral-based vaccines for the treatment of cancer. In: Lukashevich IS, Shirwan H, eds. Vienna: Springer; 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-α ($P = .0111$). 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 β (TCRβ) cDNA libraries, very few vaccine-specific CTLs were found in regressing metastases, but several TCRβ sequences were identified to correspond to nonvaccine tumor antigens. Further TCRβ 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

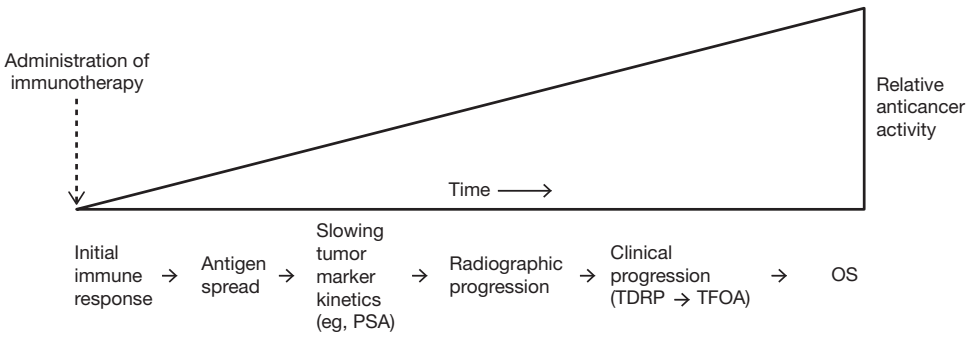

Figure 3. The anticancer activity of immunotherapy increases over time. Schematic representing progression and broadening of response over time following treatment with cancer-targeting immunotherapy. Antigen spread leads to more relevant targets (eg, neoantigens) for a given patient, and this highly 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; TOFA = time to first opioid analgesic.

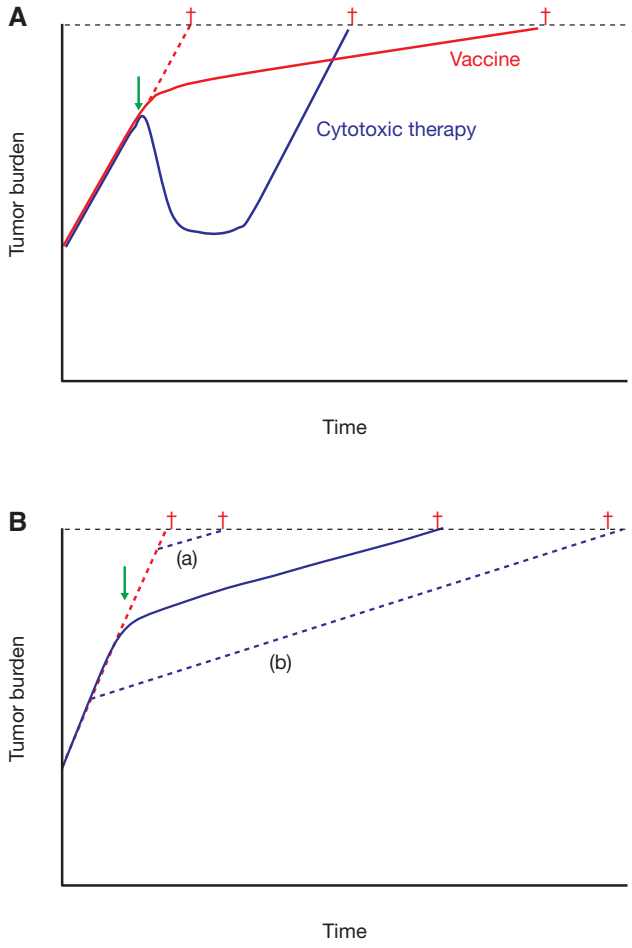

Figure 4. Tumor growth rates with immunotherapy (vaccine) and chemotherapy (cytotoxic therapy). **A)** Tumor growth rate with no therapy (**dotted black line**), with cytotoxic therapy (**blue line**), and with vaccine (**red line**), demonstrating the slow yet prolonged response with immunotherapy resulting 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 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 (≤22.1 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 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 <1.5x upper limit of normal, hemoglobin levels ≥11.0 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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