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REVIEW

Cancer vaccines: Enhanced immunogenic modulation through therapeutic combinations

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ABSTRACT

Therapeutic cancer vaccines have gained significant popularity in recent years as new approaches for specific oncologic indications emerge. Three therapeutic cancer vaccines are FDA approved and one is currently approved by the EMA as monotherapy with modest treatment effects. Combining therapeutic cancer vaccines with other treatment modalities like radiotherapy (RT), hormone therapy, immunotherapy, and/or chemotherapy have been investigated as a means to enhance immune response and treatment efficacy. There is growing preclinical and clinical data that combination of checkpoint inhibitors and vaccines can induce immunogenic intensification with favorable outcomes. Additionally, novel methods for identifying targetable neoantigens hold promise for personalized vaccine development. In this article, we review the rationale for various therapeutic combinations, clinical trial experiences, and future directions. We also highlight the most promising developments that could lead to approval of novel therapeutic cancer vaccines.

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Introduction

While vaccines have historically been a preventative measure for infectious diseases and for prevention of virus-related cancers (i.e., hepatitis B virus [HBV] and human papilloma virus [HPV] vaccines), therapeutic cancer vaccines have generated significant interest within the medical and lay community as they offer the potential to direct a host's immune system against a tumor. There are three therapeutic cancer vaccines approved by the U.S. Food and Drug Administration (FDA): (1) Bacillus Calmette-Guerin (TheraCys®) – a live attenuated strain of *Mycobacterium bovis* for non-muscle invasive bladder carcinoma; (2) Sipuleucel-T (Provenge®) – a dendritic cell (DC) vaccine for metastatic castration resistant prostate cancer (mCRPC); and (3) talimogene laherparepvec (T-VEC or Imlygic®) – an oncolytic viral-based vaccine for advanced melanoma. Approval was based on modest improvements in overall survival (sipuleucel-T and T-VEC), disease free survival (TheraCys), and a durable response rate (T-VEC).[1-3]

In order to improve on these modest gains, tumor immune escape caused by natural selection of tumor cell clones lacking immunogenic antigens must be overcome.[4] Successful tumor clones can persist via acquired defects, epigenetic silencing of various components involved in antigen processing, or by upregulating inhibitory receptors leading to exhaustion of effector T-cells.[5]

There are currently 369 open "cancer vaccine" studies on clinicaltrials.gov with 232 studies in the United States alone (*as of 6/13/17*). Numerous cancer vaccines have been tested in multiple solid as monotherapy or in combination with chemotherapy, radiation, or other immunotherapy agents. In this manuscript, we will first review experiences with combination approaches and then discuss strategies that we believe have the most promise.

Vaccine platforms

There are multiple therapeutic cancer vaccine platforms including peptide-based, protein-based, viral-based, recombinant vector including yeast-based and bacterial-based, whole tumor cell and pulsed dendritic cells([6-12] Generally, most vaccines are well tolerated and have minimal side effects. Given the unique biology of different tumors types and the distinct variables that exist within an individual immune system, a discussion of optimal vaccine platform is beyond the scope of this review.

FDA approved cancer vaccines

The first FDA approved cancer vaccine was the intravesical BCG vaccine (TheraCys) in 1990 for the treatment and prophylaxis of primary or recurrent non-muscle invasive urothelial carcinoma following transuretheral resection.[3] TheraCys prolonged disease-free survival (DFS) to 30 months in patients with bladder carcinoma in situ (CIS) and to 22.5 months in patients with Ta/T1 urothelial carcinoma, compared to 4.9 months DFS in CIS and 10.5 months in Ta/T1 patients treated with topical doxorubicin.[3,13]

Sipuleucel-T (Provenge), an autologous DC vaccine containing a recombinant fusion protein, PA2024, that consists of prostate acid phosphatase (PAP) and granulocyte-macrophage

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colony-stimulating factor (GM-CSF) was FDA approved for patients with minimally symptomatic or asymptomatic mCRPC in 2010.[1] Sipuleucel-T has been shown to generate anti-tumor immune responses including PAP-specific T-cells and antigen cascade (immune response to antigens not contained in the vaccine).[14,15] A pivotal phase III study demonstrated a statistically significant 4.1 month improvement in median overall survival (OS); 25.8 months in the Sipuleucel-T group compared to 21.7 months in the placebo group.[1] Sipuleucel-T was initially approved by the European Medicines Association (EMA) in 2013 but was withdrawn in 2015 by the manufacturing company (Dendreon UK Ltd) who cited commercial reasons.

T-VEC (talimogene laherparepvec) is an oncolytic herpes virus in which two viral genes are deleted and that is modified to produce GM-CSF for enhancing immunogenicity. T-VEC was approved by the FDA and the EMA in 2015 for treatment of advanced melanoma based on data from the phase III OPTiM trial. The vaccine virus infects both cancer and normal cells but can only replicate within a cancer cell. Injected intralesionally, the vaccine is designed to produce a systemic anti-tumor effect.[16] The OPTiM trial showed a higher durable response rate with T-VEC compared to GM-CSF alone (DRR; 16.3% vs 2.1%; $p < 0.001$), as well as a higher overall response rate (ORR; 26.4% vs 5.7%, respectively) and a longer median OS (23.3 vs 18.9 months, $p = 0.051$) in patients with Stage IIIB, IIIC or IV M1a melanoma.[2]

The makings of an effective vaccine

Much has changed since 2009 when the NCI ranked 75 antigens thought to be important for an effective cancer vaccine antigen which included criteria such as good therapeutic function, ability to elicit T-cell and/or antibody responses and association with an oncogenic process.[17] Broadly speaking, tumor antigens can be divided into tumor associated (TAA) or tumor specific (TSA) antigens. Since TAAs are expressed on both cancer and normal cells, it was thought their use would be hampered by generation of tolerance to high avidity TAA-specific T-cells.[18] However, multiple studies have demonstrated that TAA-based vaccines can produce anti-tumor immune responses, albeit with only modest clinical benefit. Nonetheless, clinical experiences with PROSTVAC,[19,20] a PSA-targeted vaccine for prostate cancer, and Sipuleucel-T[14,15] which was discussed above, have shown that these TAA-based vaccines can generate an anti-tumor immune response and tumor-specific T-cells. A Phase III trial of PROSTVAC has completed accrual with results expected by the end of 2017 (NCT01322490).

Another promising TAA-based vaccine is NeuVax, which is desiged to prevent clinical recurrence in high risk, disease free patients with HER2+ breast cancer.[21,22] The NeuVax contains an immunogenic peptide called Nelipepimut-S (aka E75) from the HER2 protein and is combined with GM-CSF. Early phase trials have demonstrated clinical benefit in women with node-positive or high-risk node-negative HER2+ breast cancer.[22] A Phase III study with NeuVax is currently ongoing (NCT01479244).

Use of TSA-targeted vaccines is attractive because such vaccines target antigens found only on tumor cells, and should theoretically limit issues with tolerance and toxicity. Identifying immunogenic tumor specific neoantigens is challenging and increasingly complicated since neoantigens are dynamic and change in response to various stimuli (i.e., treatment effects, immune infiltration, tumor mutations).[23,24] Furthermore, not every protein product of tumor-exclusive mutations will yield an immunogenic peptide antigen i.e. an epitope that will be presented on MHC and recognized by T-cells, making screening for T-cell epitopes labor intensive.

Yadav et al. reported a novel method for identifying immunogenic neoantigens.[25] Briefly, whole exome sequencing was performed on MC-38 and TRAMP-C1 murine tumor cell lines, followed by selection for high-confidence mutations based on overlap with RNA-Seq transcriptome analysis. Out of over 1,300 candidate amino acid mutations, 7 (all in MC-38) were confirmed by mass spectroscopy to be expressed on MHC class I. Manual verification with synthetic peptides narrowed to 6 epitopes. After peptide vaccination with adjuvant, tumor infiltrating lymphocytes (TILs) specific for the 3 out of 6 of the peptide's associated neoantigens were detected in MC-38 tumor-bearing mice. Compared to other TILs, they displayed an activated phenotype (PD-1 and TIM-3 high). In vivo, most mice vaccinated with the more immunogenic mutant peptides had no tumor growth following challenge with MC-38 inoculation, compared to controls. This approach provides an innovative pathway for developing personalized cancers vaccines, by selecting only target antigens from one's own tumor that are predicted to be immunogenic[26] and then incorporating into any of the various available vaccine platforms.

In addition to choosing a viable vaccine platform, selection of the appropriate patient population is also vital to effective therapeutic cancer vaccine design. In 2007 the Cancer Vaccine Consortium outlined recommendations for therapeutic vaccine trial design based on review of prior trials.[27,28] This group recommended using early stage disease and/or low volume disease. While many trials have evaluated therapeutic cancer vaccines in the advanced disease setting, it is worth noting that the only approved FDA therapeutic cancer vaccines are approved in the limited disease setting.[1-3]

Vaccine combination strategies

Vaccination can induce antigen-specific T-cells;[19] however, vaccines alone are seldom sufficient to induce a strong enough immune response for tumor eradication. The pharmaceutical pipeline continues to release a variety of investigational agents that modulate the immune response. Checkpoint inhibitors, immunoagonists and immunocytokines can induce a spectrum of alterations upon cancer and/or immune cells that can enhance immune destruction of tumor cells.[29-31] Additionally, there is evidence that combining therapeutic cancer vaccines with traditional modalities such as radiation, immunotherapy, hormone therapy and/or chemotherapy may be synergistic. (Table 2).

Vaccine plus cytokines

Tumors often secrete their own immunosuppressive cytokines including TGF-ß, IL-4, IL-6 and IL-10. Co-administration of

Table 1. Vaccine platforms (Table adapted from multiple sources[6-12]).

Platform	Rationale	Immunogenicity	Toxicity	HLA Restriction	Pros	Cons
Peptide – Based	Elicit immunity to tumor associated self-antigens or tumor specific antigens	Low	Low	Yes	• Easy to produce with high purity in large amounts (e.g., cost effective) • High specificity through use of defined epitopes • No additional treatments/procedures for patients • Not pharmacologically active (little toxicity) • Repeated booster vaccines to help with sustained immune response	• Only target one or a few epitopes – needs multiple peptides to prevent immune escape • Immunological compatibility only in patients with a specific HLA subtype. • Peptides with low affinity for MHC may be poorly immunogenic • Immune responses may be transient or of low magnitude • Often requires that it is given with immunoadjuvant to enhance immunogenicity
Protein – Based	Elicit immunity to tumor associated self-antigens or tumor specific antigens	Moderate	Low	No	• Multiple epitopes	• Give with immunoadjuvant to enhance immunogenicity • More costly than peptide-based vaccines • Higher antigen load
Viral – Based	Use natural ability to trigger immune responses and carry genetic material into cells for production of antigens	High	High	No	• Introduce target antigen into immune cells • Easy to produce on large scale	• Immune response against priming virus requires different virus for a booster
Bacteria – Based	Use natural ability to trigger immune responses and carry genetic material into cells for production of antigens	High	High	No	• Effective antigen delivery – express foreign antigens • Stimulate mucosal and systemic responses • Easy to produce on large scale	• Risk of undesired infections (but may be treated with antibiotics)
Dendritic Cells/ Antigen Presenting Cells	DC's are the body's most effective APCs Can have protein, peptide or viral infected cells	High	Low	Yes	• More control over APC stimulation and antigen presentation • HLA restricted but autologous cells	• Logistically difficult (APC selection, ex vivo maturation) • Expensive – collection and manipulation • Need for a leukapheresis facility • Risk of leukapheresis (central line insertion, hypotension, electrolyte imbalances, vascular injury) • Difficult to ensure consistent vaccine production (varying leukapheresis yields, varying activated APCs, possible bacterial contamination)
Whole Tumor Cells	Deliver multiple relevant tumor antigens	Moderate	Low	No	• Broad array of antigens represented (minimizes immune escape) • Readily produce allogenic cell lines on larger scale • Not HLA restricted due to whole proteins present	• Cancer cells themselves are not that immunogenic • Autologous cell vaccines are labor-intensive and difficult to standardize

HLA Restriction – Different HLA subtypes present different antigens. Antigen is only immunological comparable in patients with a specific HLA subtype (i.e., HLA-A2+)

Table 2. Rationale for therapeutic cancer vaccine combinations.

	Rationale	References
Cytokines (IL, INF, TGF-β)	• Stimulate humoral and cellular immunity • Promote epithelial to mesenchymal transition • Promote differentiation of immature T-cells into Tregs and effectors T-cells • Promote dendritic cell maturation • Chemoattractant for neutrophils and MDSCs	32-37, 49, 50
Radiotherapy (External beam and radiopharmaceuticals)	• Enhance destruction of tumor cells via • upregulation of MHC, Fas, ICAM-1 and TAAs • Enhance vaccine-mediated tumor lysis • Increased inflammation and secretion of immunomodulatory cytokines • Sensitize tumor cells to immune-mediated killing	29, 55-57, 61
Checkpoint inhibitors (anti-CTLA-4/anti PD-1/PDL-1)	• Immunogenic intensification • Increased inflammation within the tumor • Reduction of Tumor burden • Activation of different T-cell population	77, 78, 93
Small molecules (TKIs/ HDACi)	• "Off-target" effects on immune cells (i.e., decreasing Tregs, decreasing MDSCs, increasing INF-g producing T-cells, and decreasing IL-4 producing T-cells • Sensitize cells to immune-mediated killing • Increase the protein expression of antigen presenting machinery	103-105, 108, 112
Endocrine Therapy	• Inducing thymic regeneration leading to increased production of naive T cells and CD4+ effector T cells. • Decrease Tregs within the tumor	115-118
Chemotherapy (including low dose CTX)	• Sensitize cells to immune-mediated killing • Decrease MDSCs and Tregs • Increase immune-supportive M1 macrophages, including CD4+ and CD8+ T-regs • Reduction of tumor burden • Induction of tumor immunsurveillance by NK cells • Enhance immunity by inhibiting Tregs, by enhancing DC maturation and by promoting of a durable T-cell memory response (low dose CTX)	121-123,127,128,131,132,138,141

immunostimulatory cytokines with vaccines offers a potential means to augment the effect of vaccine derived effector T-cells. While data from large trials are still lacking, smaller clinical trials have investigated multiple cytokines in combination with vaccines, at varying schedules, in several malignancies.

Vaccines plus GM-CSF

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a pro-inflammatory cytokine that stimulates humoral and cellular immunity.[32,33] Interestingly, two of the FDA-approved therapeutic cancer vaccines (Sipuleucel-T and T-VEC) are engineered to secrete GM-CSF. Hypothetically GM-CSF augments DC activity and thus enhances the immune response in each of these different platforms. However, its role, or lack thereof, in achieving the improved clinical outcomes seen with these agents is unclear. While GM-CSF is important for maturation of DC, activation and proliferation of T-cells, it also acts as a chemoattractant for myeloid-derived suppressor cells (MDSCs).[34,35] Since MDSCs contribute to the inhibitory milieu of the tumor micro environment (TME),[36,37] it is unclear how GM-CSF splays the balance between activation and inhibition in vivo.

Clinical studies have incorporated GM-CSF with the hope that it would be immune activating. Correlative studies in melanoma, breast and prostate cancer have not demonstrated a clear role for GM-CSF in antitumor immunity.[38-40] The E4697 phase III, double-blinded, placebo-controlled trial evaluated a peptide vaccine +/− GM-CSF (or placebo) in patients with completely resected stage IV or high-risk stage III melanoma did not show a survival benefit.[41] Furthermore, a systematic review of 26 studies which evaluated the addition of GM-CSF to various treatment modalities including chemotherapy, surgery and peptide vaccines in patients with advanced melanoma did not show a significant improvement in outcomes with the addition of GM-CSF to peptide vaccines.[42] The impact of GM-CSF's co-administration with the PROSTVAC vaccine in a completed Phase III study (NCT01322490) is under analysis. However, current evidence suggests GM-CSF is an inert companion for vaccines.

Vaccine plus IL-2

Interleukin-2 (IL-2) promotes differentiation of immature T-cells into both Tregs and effector T-cells.[43] Despite known activating effects on Tregs, high dose recombinant human IL-2 has produced durable remissions in select metastatic melanoma and metastatic renal cell carcinoma (mRCC) patients leading to FDA approval.[44-46] Studies of various vaccine platforms plus both low- and high-dose IL-2 have shown mixed results in recent years. The severe toxicity seen with higher doses has limited its use to select patients.[45]

A phase I/II trial of DC vaccine plus low-dose IL-2 in patients with mRCC or breast cancer showed that the combination was well tolerated but there were no observed clinical responses.[47] A phase III trial randomized 185 patients with locally advanced stage III and stage IV cutaneous melanoma to gp100 peptide vaccine plus high-dose IL-2 vs high-dose IL-2 alone.[48] The combination group had better overall clinical response (16% vs. 6%, $p = 0.03$) and modest extension of PFS (2.2 months vs. 1.6 months, $p = 0.008$). Given the negative studies of low-dose IL-2 and high toxicities seen with high-dose IL-2, a future role for IL-2 in vaccine formulations does not appear likely as agents targeting downstream targets with less toxicity become available.

Vaccine plus IL-7

Interleukin-7 (IL-7) is important for differentiation of hematopoietic stem cells into lymphoid progenitor cells and development of CTL responses. Preclinical data show that PBMCs subjected to two-step culturing involving neoantigens exposed to GM-CSF followed by IL-7 produced selective and sustained expansion of both CD4+ and CD8+ peptide-specific T-cells.[49] Other cytokine combinations were initially proliferogenic, but only IL-7 resulted in a sustained response.[49] An ongoing phase III trial is testing the combination of Sipuleucel-T plus subcutaneous IL-7 (CTY107), with the aim of augmenting proliferation of T-cell clones (NCT01881867).

Vaccine plus TGF-β modulation

Transforming growth factor β (TGF-β) is an important regulator of the cell cycle and is known to promote epithelial to mesenchymal transition (EMT).[50] Parts of the TGF-β signaling pathway are mutated in many malignancies, allowing invasion and metastasis, while TGF-β stimulation increases recruitment of MDSCs and Tregs.[50,51]

A neoadjuvant clinical trial of an allogeneic pancreatic adenocarcinoma vaccine containing GM-CSF (GVAX) produced tertiary lymphoid aggregates (TLAs) within TME. Microarray studies of TLAs from patients who survived greater than 3 years showed suppression of multiple portions of the TGF- β pathway.[52] Several small molecule TGF-β inhibitors are now under investigation and offer a means to dampen the immunosuppressive milieu within the TME. Galunisertinib has a safe toxicity profile based on two phase II trials in HCC and pancreatic cancer with no cardiac toxicity, which was a concern with first-generation TGF- β inhibitors.[53,54] To the best of our knowledge, no TGF-β inhibitors are currently being studied in combination with cancer vaccines. However, a novel bifunctional fusion protein called M7824 which consists of and anti-PD-L1 antibody and the extracellular domain of TGF-β receptor type two is currently being evaluated in a phase I trial in solid tumors. The receptor portion of the molecule essentially traps TGF-β and holds great promise as a companion to vaccine therapy (NCT02517398).

Vaccines plus radiotherapy

Rationale for combining radiotherapy (RT) with vaccines is multifold. Preclinical data have demonstrated that the combination of vaccines and RT is additive, with enhanced destruction of tumor cells via upregulation of MHC, Fas, ICAM-1 and TAAs, as well as by enhancing vaccine-mediated tumor lysis in mouse models.[55-57] Other studies have produced dramatic reduction in tumor burden in mice with combination RT plus vaccine, but not with either therapy alone, suggesting synergy.[58] T-cells specific for antigens not included in vaccine were also generated with combination, a phenomenon known as antigen cascade or antigen spreading. Moreover, non-lethal doses of radiation administered to tumor cell lines have also been shown to induce such phenotypes.[59,60] Lower doses of radiation can induce changes in tumor cells that make them more susceptible to T-cell killing through increased type I IFN secretion and increased expression of surface calreticulin.[29,61] The radiopharmaceutical, samarium-223, has been shown to have similar effects in vitro.[62] Additionally, the abscopal effect, wherein non-irradiated lesions regress following radiation to a distant area, has also been reported in multiple malignancies,[63-66] including melanoma[67] and non-small cell lung cancer (NSCLC).[68]

Since RT is a part of standard of care in many malignancies, many ongoing trials combining radiation and vaccine are designed to examine a vaccine's role in enhancing responses with RT as opposed to examining how RT may positively affect response to vaccines. Below is a brief summary of clinical experiences and ongoing trials.

Vaccines plus external beam radiotherapy

A phase I trial published in 2005 demonstrated increased PSA-specific T-cells in patients with locally invasive prostate cancer treated with RT plus a first generation fowlpox vaccine.[30] Patients were randomized to recombinant vaccinia (rV) -PSA, rV-B7.1 vaccine followed by monthly booster vaccines with recombinant fowlpox (rF)-PSA plus standard of care RT (19 patients) or RT alone (11 patients). Of the 17 patients in the combination arm who received all scheduled vaccinations, 13 had a $\geq$ 3-fold increase in PSA-specific T-cells. There was no such signal in the RT alone arm ($p < 0.0005$).

Several ongoing phase I studies are also evaluating the safety and efficacy of this approach. Self-adjuvanting mRNA cancer vaccine (RNActive®), called CV9202, targeting NY-ESO-1, MAGEC1, MAGEC2, 5 T4, survivin, and MUC1 is being tested in combination with RT for stage IV NSCLC (NCT01915524).[69] Preliminary data presented at the ASCO 2016 meeting demonstrated safety of CV9202 vaccine in combination with RT.[70] A personalized neoantigen vaccine for O^6-methylguanin-DNA-methyltransferase (MGMT) unmethylated glioblastoma (GBM) in combination with RT is currently ongoing (NCT02287428). G207 is an oncolytic herpes simplex virus-1 engineered to contain mutations that enable it to selectively replicate within and kill cancer cells, but not normal cells.[71] A phase I study indicated that G207 injected into recurrent high grade gliomas alone, or in combination with a single dose RT is well tolerated and active.[72,73] Another phase I study in pediatric patients with recurrent or progressive supratentorial tumors is currently recruiting subjects and will test G207 as monotherapy or combined with single dose RT (NCT02457845).

Another prostate cancer vaccine, aglatimagene besadenovec (ProstAtak®), is being evaluated in combination with RT in a phase III trial in patients with intermediate-high risk localized prostate cancer (NCT01436968). ProstAtak® is a cytotoxic immunotherapy derived from an adenovirus thymidine kinase (AdV-tk) vector that delivers herpes simplex virus into tumor cells when injected locally, creating a vaccine-like effect.[74] It utilizes valacyclovir as a prodrug. Patients receive three intraprostate ProstAtak® treatments administered via transrectal ultrasound starting between 15 days and 8 weeks after beginning standard of care RT. Results are pending.

Vaccines plus radiopharmaceuticals

Samarium-153 EDTMP (^{153}Sm) is a radiopharmaceutical targeted to osteoblastic lesions. A phase II trial randomized 44 mCRPC patients previously treated with docetaxel to ^{153}Sm with or without PSA-TRICOM. The median PFS was

3.7 months for the combination vs 1.7 months for ^{153}Sm alone (HR 0.51; p = 0.041). Although the results were not statistically significant, there was a trend of decreased RDP and increased PFS.[75,76] A phase II study in mCRPC with bone metastases is currently recruiting patients who are randomized to Sipuleucel-T with or without radium-223 (the FDA approved radiopharmaceutical which demonstrated improvements in OS in mCRPC) (NCT02463799).

Vaccine plus checkpoint inhibitors

Vaccines plus anti-CTLA-4

CTLA-4 is expressed on T-cells and mediates inhibitory effects on CD4 helper T-cells during interactions with antigen presenting cells, representing an important mechanism of autoregulation.[77] CTLA-4 signaling can also have activating effects on Tregs.[78] CTLA-4 blockade with monoclonal antibodies is a potential strategy for converting vaccine-generated immune responses into clinically significant ones.

Ipilimumab and tremelimumab are anti-CTLA-4 antagonist monoclonal antibodies. Single agent use of ipilimumab has produced dramatic improvement in OS in advanced melanoma and is now FDA approved. However, ipilimumab has failed to achieve comparable clinical results in other solid tumors. For example, two phase III trials using ipilimumab in mCRPC failed to improve OS.[79,80] Both ipilimumab and tremelimumab are at various stages of clinical investigation alone or in combination with cancer vaccines.

In a phase I dose-escalation trial, a fixed dose of PROSTVAC was tested with escalating doses (1, 3, 5, and 10 mg/kg) of ipilimumab in mCRPC patients.[81] There were no increases in immune-mediated AEs with combination. Fourteen of the 24 chemotherapy-naïve patients had a PSA decline with 6 patients having a PSA decrease > 50%. The median OS chemotherapy-naïve patients was 31.3 months, which was longer than historical controls of PROSTVAC alone.[38] There was a trend toward improved OS and the presence of certain immune cell subsets in peripheral blood.[82] A trial testing tremelimumab in combination with several other agents, including a vaccine, is currently recruiting patients with mCRPC (NCT02616185).

Ipilimumab may also prove effective in a host of other malignancies and/or with other vaccine platforms, as illustrated by a study in 30 previously treated pancreatic adenocarcinoma randomized patients 1:1 to ipilimumab or ipilimumab plus GVAX.[83] No patients in the ipilimumab alone arm had a biochemical CA19–9 response, and two patients had stable disease (7 and 22 weeks). However, in the combination arm, 3 patients had prolonged stable disease (31, 71, and 81 weeks) and seven patients had a decline in CA-19–9. One year OS also favored combination (7 vs. 27%).[84] A phase III study showed that patients with advanced melanoma who received ipilimumab with or without the gp100 peptide vaccine (HLA-A*0201–restricted peptides derived from the melanosomal protein, glyco- protein 100) had OS of 10 months compared to patients who only received the gp100 vaccine (6.4 months).[85]

As mentioned above, CTLA-4 mediates inhibitory effects on CD4 helper T-cells during interactions with antigen presenting cells.[77] For this reason, combining CTLA-4 blockade with antigen presenting cell administration i.e. DC vaccines is an exciting strategy. In a phase II trial, 39 pretreated advanced melanoma patients were given TriMixDC-MEL (autologous DC melanoma vaccine) intravenously and subcutaneously plus ipilimumab 10mg/kg every 3 weeks for four treatments. Patients who remained progression-free received maintenance therapy every 12 weeks.[86] The primary endpoint was met with a disease control rate of 51% at 6 months. Seven complete responses (CR) and 1 partial response (PR) were observed. The TriMixDC-MEL plus Ipilimumab ORR of 38% was better than monotherapy with ipilimumab in this population (10–15%) and was comparable to the ORR seen with anti-PD-1 monotherapy (ORR 25–43%) but not as high as seen in patients with dual anti-CTLA-4 plus anti-PD-1 therapy (ORR 57–61%).[87-90]

Vaccines plus PD-1/PD-L1 inhibitors

Programmed cell death protein 1 (PD-1) is expressed on T-cells, as well as some B cells and NK cells, and binds to PD-L1 and PD-L2.[77] Antagonist antibodies that target the PD-1/PD-L1 axis have also achieved impressive and durable results in many solid tumors. Since 2015, multiple PD-1/PD-L1 inhibitors have received FDA approval for use in metastatic squamous NSCLC (nivolumab, pembrolizumab and atezolizumab), mRCC (nivolumab) unresectable/metastatic melanoma (pembrolizumab, nivolumab), locally advanced/metastatic urothelial carcinoma (atezolizumab, nivolumab, pembrolizuman, avelumab and durvalumab), recurrent/metastatic head and neck squamous cell carcinoma (pembrolizumab, nivolumab) metastatic merkel cell carcinoma (avelumab).[91]

Preclinical data shows that combination of an anti-PD-1 antibody and a multi-peptide vaccine (immunogenic peptides derived from breast cancer antigens, neu, legumain, and β-catenin) prolonged PFS in mice with breast tumors.[92] There are several immune cell interactions that can be affected by PD-1/PD-L1 axis inhibition. Blockade of either of these targets can prevent PD-1/PD-L1 interaction-mediated inhibition of cytotoxicity at the effector cell:tumor cell synapse. Exerting a negative effect on each of these interactions with PD-1/PD-L1 blockade is promising as a means to enhance clinical activity of anti-tumor vaccines.[93]

The gp100 peptide vaccine failed to enhance the clinical benefit produced by nivolumab in advanced melanoma patients.[94] However, nivolumab plus a multipeptide vaccine produced promising DFS data in the adjuvant setting for patients with high-risk resected melanoma. Median relapse-free survival (RFS) was 47.1 months with this combination which was highly favorable compared to historical median RFS (5 to 7.2 months) with other approaches.[95] Correlative studies were consistent with antigen-specific immune responses, and a trend towards lower levels of MDSC and Tregs was seen in non-relapsing patients. Tumor PD-L1 expression did not correlate with outcomes.

Preliminary clinical data combining a DNA vaccine encoding PAP in combination or in sequence with pembrolizumab were presented in November 2016 and showed promising results (NCT02499835).[96] Four out of six mCRPC patients treated with combination showed a decline in PSA, and imaging in 3 of 6 patients showed decreased tumor volume at 12 weeks.[96]

Combination of vaccines and anti-PD-1 treatment are also underway in NSCLC. Viagenpumatucel-L is a cell based vaccine derived from a gp96-Ig secreting NSCLC tumor cell line

selected for its ability to induce antigen specific T-cells.[97] A phase II study stratifying patients by high or low volume of TILs is currently enrolling patients and will test the vaccine in combination with nivolumab (NCT02439450). As seen with anti-CTLA-4 monoclonal antibodies, anti-PD-1 and anti-PD-L1 agents are also being tested in combination with different vaccine platforms for many malignancies (clinicaltrials.gov). Results are pending.

Vaccines plus small molecules

Tyrosine kinase inhibitors

Tyrosine kinase inhibitors (TKIs) are utilized in the treatment of several malignancies. VEGF-targeting TKIs (sunitinib, pazopanib, axitinib), multi-targeted TKI cabozantinib, and lenvatinib plus everolimus are FDA approved for treatment of mRCC. While these agents can improve outcomes, and produce objective responses, TKI's have not produced the durable CRs that have been observed in some mRCC patients treated with high-dose IL-2 therapy.[98-102]

Growing preclinical and clinical data suggest that these agents often have "off target" effects on immune cells that enhance and/or damper the antitumor response.[103,104] For example sunitinib (inhibits KIT, PDGFR, KDR kinases, FLT3 kinase) decreases Tregs, decreases MDSCs, increases interferon-γ producing T-cells, and decreases IL-4 producing T-cells – all of which are important for an effective anti-tumor immune response.[105] Complicating the picture, sunitinib has also been found to inhibit activation of peripheral T-cells.[106] In a mouse model, sunitinib plus CEA-TRICOM vaccine decreased the angiogenesis in central areas of tumor whereas sunitinib alone only decreases angiogenesis in the periphery.[107] Furthermore, the combination of sunitinib or sorafenib plus CEA-TRICOM decreased tumor volume and intratumoral pressure. These effects on tumor vasculature appear to enhance migration of effector T-cells into the TME.[104]

Another TKI, cabozantinib (inhibits c-MET, VEGFR2) also appears to have anti-tumor immune effects based on preclinical studies. It sensitizes murine tumor cells to immune-mediated killing, and when combined with CEA-TRICOM vaccine, reduces Treg and MDSC infiltration of the TME. As with sorafenib and sunitinib, cabozantinib plus CEA-TRICOM appears to normalize tumor vascularity favoring immune migration.[108] For these reasons, and their effectiveness as monotherapy, combining vaccines with standard of care TKIs is an attractive potential means to enhance their activity.

AGS-003 is a dendritic cell vaccine derived by co-electroporation of DCs with the patient's amplified tumor RNA and synthetic CD40L RNA. A phase II study treated 22 mRCC patients with AGS-003 plus sunitinib.[109] The vaccine was well tolerated. Out of 21 evaluable patients, 9/21 (43%) had a PR and 4/21 (19%) had SD. Median OS was 30.2 months, comparing favorably to historical data for mRCC patients treated with bevacizumab (22 months).[110] A randomized phase III trial of AGS-003 plus sunitinib vs sunitinib alone is in progress (NCT01582672).

Vaccines with HDACi

Epigenetic silencing of genes involved in the immune-response, is one mode of immune escape utilized by tumor.[111] Histone deacetylase 1 inhibitors (HDACi) such as vorinostat and entinostat have been shown to sensitize tumor cells to antigen-specific T-cell mediated lysis and to increase the protein expression of antigen presenting machinery in breast cancer and prostate cancer cell lines.[112] While HDACi are promising, researchers have demonstrated enhanced cell migration and metastasis with use of HDACi in some human cancer cell lines and in mice.[113] HDACi have little activity as single agents but have demonstrated efficacy when combined with cytotoxic and non-cytotoxic chemotherapy agents.[114] Due to the immunomodulatory properties of HDACi there is growing interest using HDACi in combination with various drugs including checkpoint inhibitors and vaccines.

Vaccines plus endocrine therapy

In hormonally-driven tumors such as prostate cancer and breast cancer, the hormonal milieu is important in cancer development and progression. Breast cancer patients who received the aromatase inhibitor letrozole were found to have fewer Tregs within the TME.[115] Furthermore, androgen deprivation in prostate cancer creates an immunostimulatory atmosphere, induces thymic regeneration and increases the number of effector T-cells.[116-118]

The E9802 phase II trial tested the PROSTVAC vaccine followed by anti-androgen therapy in non-metastatic prostate cancer patients with biochemical recurrence (BCR). The combination was well tolerated. An increase in PSA doubling time between pre- and post-vaccine administration was observed (5.3 to 7.3 months).[119] Notably, timing of vaccine treatment appears to be important. The survival data from a phase II trial suggested clinical benefit if vaccine was given prior to ADT. Survival analyses revealed a median OS advantage for the patients initially randomized to the vaccine arm who later received nilutamide compared to patients who received nilutamide first followed by vaccine (6.2 versus 3.7 years; $p = 0.045$).[120]

Two phase II trials studying the androgen receptor antagonist, enzalutamide, with and without PROSTVAC in early and metastatic prostate cancer are ongoing. (NCT01867333, NCT01875250). The combination of vaccine with hormonal therapy in breast and prostate cancer is attractive since vaccines are minimally toxic and can easily be incorporated in standard of care regimens.

Vaccines plus chemotherapy

Many cytotoxic agents cause DNA damage or alter tumor phenotype, making the tumor more susceptible to CTL killing.[121,122] Chemotherapy agents such as docetaxel may have indirect effects on the immune system that improve their efficacy.[123] Chemotherapy dosing at maximum tolerated dosing (MTD) results in depletion of T-cells with both CD4+ and CD8+ cells effected but CD8+ cells recover more quickly.[124] In addition, NK cells are also impaired. Standard of care dosing for most chemotherapies is often much lower than the MTD and allows for a residual immune response.[123] Several chemotherapeutic agents, including gemcitabine, taxanes, topoisomerase inhibitors, platinum compounds, and 5-FU have been

shown to produce immunomodulatory effects.[121,123,125,126] These effects are discussed in more detail below.

Vaccine plus low dose cyclophosphamide (CTX)

Metronomic (low) dosing of CTX has been shown to inhibit Tregs,[127] enhance DC maturation, and promote memory T-cell responses, making it a candidate for enhancing cancer immunotherapies.[122,128] A single-arm feasibility study gave HER2+ metastatic breast cancer patients allogeneic HER2+ GM-CSF-secreting whole-cell breast cancer vaccine one day after receiving CTX 300 mg/m^2 and trastuzumab 2mg/kg (NCT00399529). All patients were HER2+ and 13 of 20 patients were hormone receptor positive. The clinical benefit rate (CBR = CR+ PR+SD) at 6 months was 55% (p = 0.013) and at 12 months was 40%.[129]

Results from an international phase II/III study in metastatic breast cancer patients testing CTX plus OPT-822, a vaccine targeted to a glycolipid overexpressed in breast cancer, or placebo were presented at ASCO 2016 (NCT01516307).[130] Similar to the above study, the vaccination plus CTX arm did not improve PFS or OS. However, PFS and OS were higher in patients who developed an immune response to the vaccination.[130] There is currently an ongoing phase II study is comparing viagenpumatucel-L plus CTX to CTX alone in patients with advanced NSCLC who have failed multiple prior therapies (NCT02117024).

Vaccines plus gemcitabine

Preclinical studies have shown that gemcitabine can increase antigen cross-presentation, decrease MDSC and Tregs, and increase immune-supportive M1 macrophages, circulating CD4+ and CD8+T cells[131,132] These effects were observed in human in a phase I/II trial in ovarian cancer patients treated with gemcitabine, Pegintron and p53 synthetic long peptide (SLP) vaccine.[133] However, effects on outcome in patients treated with vaccine plus gemcitabine have been mixed. Results from a phase II study of algenpantucel-L plus gemcitabine and 5-fluorouracil-based standard adjuvant chemoradiotherapy for resected pancreatic cancer demonstrated 12-month PFS of 62% and 12-month OS of 86%.[134] While these compared favorably to historical data,[135] a phase III study randomized patients 1:1:1 to receive chemotherapy alone, chemotherapy followed by a telomerase vaccine (GV1001), or concurrent chemotherapy and vaccine in metastatic pancreatic cancer did not meet its primary OS endpoint.[136,137]

Vaccines plus docetaxel

Combination of vaccine plus docetaxel has been shown to antigen specific CD8 T-cells and decrease tumor burden in murine models.[138] Additionally, other preclinical data suggest docetaxel can make tumor cells more susceptible to CD8 T-cell-mediated cytotoxicity via enhanced calreticulin expression.[31]

A phase II trial evaluated docetaxel alone vs modified vaccinia Ankara vaccine (TroVax; targeted tumor antigen 5T4) followed by docetaxel in patients with mCRPC. Although the study was closed prematurely due to accrual issues with only 25 patients enrolled, a superior median PFS of 9.67 months was observed in the TroVax + docetaxel compared to 5.1 months in the docetaxel alone arm ($p = 0.097$).[139]

A phase II trial evaluated docetaxel alone verse docetaxel combined with PANVAC (contains MUC-1, CEA and co-stimulatory molecules B7.1, ICAM-1, LFA-3) in metastatic breast cancer patients.[40] Forty-eight patients were enrolled; 23 were randomized to docetaxel alone and 25 were randomized to combination arm. A significant increase in median PFS was observed in the PANVAC plus docetaxel vs. the docetaxel only arm (7.9 months vs 3.9 months; $p = 0.09$).[40] Secondary analyses demonstrated no correlation between generation of the T-cell specific immune response and time to progression.[40] Patients who received only docetaxel also developed T-cell responses to the TAA supporting the hypothesized immunomodulatory properties of docetaxel.

Several ongoing studies are currently evaluating combination of docetaxel and different vaccines in prostate cancer: PROSTVAC plus docetaxel in castration sensitive prostate cancer (phase II; NCT02649855) and docetaxel plus DCVAC/PCa in metastatic castration resistant disease (phase III; NCT02111577).

Vaccine plus irinotecan

Irinotecan blocks DNA repair and stimulates a complex immune response including activation of tumor-suppressor proteins and induction of tumor immunosurveillance by NK cells and activated CD8+ T-cells.[121]

A phase II multicenter study tested G17DT (a vaccine consisting of the N-terminus of gastrin 17, a growth factor, conjugated to diptheroid toxin that elicits anti-gastrin 17 antibodies) plus irinotecan in metastatic CRC patients who were progressing on irinotecan. Of the 161 patients, PR was observed in 3%, SD in 32% and PD in 65% of treated patients. Aside from increased injection site reactions seen in 52% of patients, the side effect profile was similar to irinotecan alone. Sixty-two percent of patients had measurable anti-gastrin 17 antibodies, which was associated with a survival benefit (9.0 vs. 5.6 months; p < 0.001).[140]

Vaccine plus platinum-based chemotherapy

Carboplatin-paclitaxel combination also has immunomodulatory anti-tumor effects in preclinical studies.[121] Recently published work demonstrated that carboplatin-paclitaxel plus HPV16 peptide vaccine increased survival in murine tumor models, correlating with decreased number of myeloid cells in tumor and peripheral blood.[141] Additionally, carboplatin-paclitaxel increased ex vivo T-cell activity to recall antigens.

A phase 2b/3 trial in the first line setting for stage IV NSCLC showed some benefit with TG4010 (modified Ankara vaccine expressing MUC-1 and IL-2) added to platinum-based chemotherapy.[142] Patients were randomized to TG4010 plus chemotherapy (n = 111) or placebo plus chemotherapy (n = 111). The combination group had a longer median PFS (5.9 vs 5.1 months; p = 0.019) and more confirmed responses (40% vs 29%, respectively). Interestingly, there were delayed responses and more durable responses observed in the TG4010 group (median duration was 30.1 weeks in responders who received TG4010 and 18.7 weeks in placebo group responders).[143]

Expert opinion

As detailed above, there are many vaccine platforms and target antigens to be tested in different malignancies and different clinical settings. Despite intensive study during the past 20 years, it was not vaccines, but immune checkpoint inhibitors that first revolutionized cancer therapy by altering the treatment landscape in many cancers including melanoma, non-small cell lung cancer, and urothelial carcinoma.[85,144,145] The three approved cancer vaccines have produced only modest improvements in OS (sipuleucel-T, T-VEC), DFS (TheraCys) and DRR (T-VEC) in patients with early stage or a limited disease burden. If there is to be an therapeutic role for vaccines especially in more diverse clinical settings and in patients with advanced or metastatic cancers, it will likely be as part of combination therapy.[26] While marveling at the successes of checkpoint inhibitors, it is important to bear in mind that these therapies to date have not worked in most malignancies or even provide benefit for the majority of patients with malignancies in which these agents are known to be active. The biology of resistance to these therapies is far from being understood, but one possible explanation is a lack of tumor-specific T-cells, leaving the checkpoint inhibitors without any effector cells to 'unleash.' For the following reasons, vaccines may be able to correct this deficit.

We know that various vaccine platforms are capable of generating tumor-specific T-cells, and in some cases, have been shown to increase tumor infiltrating T-cells.[146] Findings from early phase studies support the hypothesis that vaccines plus checkpoint inhibitors can create a situation for success where there may have been none with monotherapy. For example, in mCRPC, ipilimumab plus PROSTVAC appears to have a survival benefit compared to historical controls of ipilimumab alone.[38,81] Ipilimumab plus GVAX produced periods of SD for 3 pancreatic adenocarcinoma patients (31, 71, and 81 weeks), compared to SD for 2 patients (7 and 22 weeks) with ipilimumab alone.[83] The phase II TriMixDC-MEL plus ipilimumab in melanoma also produced comparable or superior response rates compared to phase II data of some checkpoint inhibitors.[86-90] Early phase data testing nivolumab plus vaccine in the adjuvant setting for high risk melanoma compare favorably to historical relapse free survival data.[95] In making the argument for the promise of vaccine plus checkpoint inhibitors, we have intentionally included examples from different malignancies. This highlights the need to consider available preclinical data, relevant to an individual tumor type's biology, when selecting agents for combination with vaccine.

Furthermore, vaccines will be enhanced as antigen selection is refined. The potential influence of neoantigen expression on response to checkpoint inhibitors[147] has been noted, however the exact role(s), mechanism(s), and utility as target antigens are yet unclear. Utilizing high throughput whole exome and transcriptome sequencing techniques, as described by Yadav et al.[25] offers a means to identify highly immunogenic antigens for incorporation into any number of the vaccine platforms described above. As suggested by others, personalized tumor vaccines, based on synthesized neoantigens derived from whole tumor exome sequencing or use of autologous tumor for antigen sourcing, should be a prioritized strategy.[26] The potential power of combining such 'smart target' vaccines with checkpoint inhibitors remains untested but holds great promise.

Conclusion

Experience suggests that the therapeutic cancer vaccines currently in clinical development are unlikely to dramatically impact cancer outcomes as single agents. Several combination approaches including vaccine plus cytokines, checkpoint inhibitors, small molecule inhibitors, radiotherapy and chemotherapy have been tested. Based on these findings, it appears that combining therapeutic vaccines with immune checkpoint inhibitors holds the greatest potential for improving clinical outcomes. Addition of vaccines to various cancer treatment modalities can augment immune responses with minimal additional toxicity. Additionally, optimizing target antigen selection via novel high throughput sequencing is a developing technique that has generated excitement. Traditional study endpoints and assessment criteria may not be appropriate given the different mechanisms of action and response patterns seen with immunotherapy agents. As we learn more about the mechanisms of immune evasion, various therapies will be combined and perhaps specific sequencing of this multi-modality approach will prove to be superior for achieving durable responses. A one-size-fits-all approach is not effective and our goal should be to better understand the biology and to develop predictive biomarkers. Cancer vaccines are here to stay, as evidenced by the many ongoing clinical trials evaluating immunotherapy combinations.

Disclosure of potential conflicts of interest

No potential conflict of interest was reported by the authors.

References

[1] Kantoff PW, Higano CS, Shore ND, Berger ER, Small EJ, Penson DF, Redfern CH, Ferrari AC, Dreicer R, Sims RB, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. N Engl J Med. 2010;363(5):411-422. doi:10.1056/NEJMoa1001294. PMID:20818862

[2] Andtbacka RH, Kaufman HL, Collichio F, Amatruda T, Senzer N, Chesney J, Delman KA, Spitler LE, Puzanov I, Agarwala SS, et al. Talimogene laherparepvec improves durable response rate in patients with advanced melanoma. J Clin Oncol. 2015;33(25):2780-88. doi:10.1200/JCO.2014.58.3377. PMID:26014293

[3] Sylvester RJ, van der MEIJDEN AP, Lamm DL. Intravesical bacillus Calmette-Guerin reduces the risk of progression in patients with superficial bladder cancer: A meta-analysis of the published results of randomized clinical trials. J Urol. 2002;168(5):1964-70. doi:10.1016/S0022-5347(05)64273-5. PMID:12394686

[4] Bui JD, Schreiber RD. Cancer immunosurveillance, immunoediting and inflammation: Independent or interdependent processes? Curr Opin Immunol. 2007;19(2):203-208. doi:10.1016/j.coi.2007.02.001. PMID:17292599

[5] Matsushita H, Vesely MD, Koboldt DC, Rickert CG, Uppaluri R, Magrini VJ, Arthur CD, White JM, Chen YS, Shea LK, et al. Cancer exome analysis reveals a T-cell-dependent mechanism of cancer immunoediting. Nature. 2012;482(7385):400-404. doi:10.1038/nature10755. PMID:22318521

[6] Schlom J. Therapeutic cancer vaccines: Current status and moving forward. J Natl Cancer Inst. 2012;104(8):599-613. doi:10.1093/jnci/djs033. PMID:22395641

[7] Schlom J, Hodge JW, Palena C, Tsang KY, Jochems C, Greiner JW, Farsaci B, Madan RA, Heery CR, Gulley JL. Therapeutic cancer vaccines. Adv Cancer Res. 2014;121:67-124. doi:10.1016/B978-0-12-800249-0.00002-0. PMID:24889529

[8] Peres Le P, da Luz FA, Pultz Bo A, Brígido PC, de Araújo RA, Goulart LR, Silva MJ. Peptide vaccines in breast cancer: The immunological basis for clinical response. Biotechnol Adv. 2015;33 (8):1868-77. doi:10.1016/j.biotechadv.2015.10.013. PMID:26523780

[9] Slingluff CL. The present and future of peptide vaccines for cancer: Single or multiple, long or short, alone or in combination? Cancer J. 2011;17(5):343-350. doi:10.1097/PPO.0b013e318233e5b2. PMID:21952285

[10] Hatem S. Developing an effective breast cancer vaccine. Cancer Control. 2010;17(3):183-190. PMID:20664516

[11] Emens LA, Jaffee EM. Toward a breast cancer vaccine: Work in progress. Oncology (Williston Park). 2003;17(9):1200-11; discussion 1214, 1217-1208. PMID:14569849

[12] Bolhassani A, Zahedifard F. Therapeutic live vaccines as a potential anticancer strategy. Int J Cancer. 2012;131(8):1733-43. doi:10.1002/ijc.27640. PMID:22610886

[13] Lamm DL, Blumenstein BA, Crawford ED, Montie JE, Scardino P, Grossman HB, Stanisic TH, Smith JA Jr, Sullivan J, Sarosdy MF, et al. A randomized trial of intravesical doxorubicin and immunotherapy with bacille Calmette-Guérin for transitional-cell carcinoma of the bladder. N Engl J Med. 1991;325(17):1205-09. doi:10.1056/NEJM199110243251703. PMID:1922207

[14] GuhaThakurta D, Sheikh NA, Fan LQ, Kandadi H, Meagher TC, Hall SJ, Kantoff PW, Higano CS, Small EJ, Gardner TA, et al. Humoral immune response against nontargeted tumor antigens after treatment with sipuleucel-T and its association with improved clinical outcome. Clin Cancer Res. 2015;21(16):3619-30. doi:10.1158/1078-0432.CCR-14-2334. PMID:25649018

[15] Sheikh NA, Petrylak D, Kantoff PW, Dela Rosa C, Stewart FP, Kuan LY, Whitmore JB, Trager JB, Poehlein CH, Frohlich MW, et al. Sipuleucel-T immune parameters correlate with survival: An analysis of the randomized phase 3 clinical trials in men with castration-resistant prostate cancer. Cancer Immunol Immunother. 2013;62(1):137-147. doi:10.1007/s00262-012-1317-2. PMID:22865266

[16] Kohlhapp FJ, Kaufman HL. Molecular pathways: Mechanism of action for talimogene laherparepvec, a new oncolytic virus immunotherapy. Clin Cancer Res. 2016;22(5):1048-54. doi:10.1158/1078-0432.CCR-15-2667. PMID:26719429

[17] Cheever MA, Allison JP, Ferris AS, Finn OJ, Hastings BM, Hecht TT, Mellman I, Prindiville SA, Viner JL, Weiner LM, et al. The prioritization of cancer antigens: A national cancer institute pilot project for the acceleration of translational research. Clin Cancer Res. 2009;15(17):5323-37. doi:10.1158/1078-0432.CCR-09-0737. PMID:19723653

[18] Cloosen S, Arnold J, Thio M, Bos GM, Kyewski B, Germeraad WT. Expression of tumor-associated differentiation antigens, MUC1 glycoforms and CEA, in human thymic epithelial cells: Implications for self-tolerance and tumor therapy. Cancer Res. 2007;67(8):3919-26. doi:10.1158/0008-5472.CAN-06-2112. PMID:17440107

[19] Gulley JL, Madan RA, Tsang KY, Jochems C, Marté JL, Farsaci B, Tucker JA, Hodge JW, Liewehr DJ, Steinberg SM, et al. Immune impact induced by PROSTVAC (PSA-TRICOM), a therapeutic vaccine for prostate cancer. Cancer Immunol Res. 2014;2(2):133-141. doi:10.1158/2326-6066.CIR-13-0108. PMID:24778277

[20] Kantoff PW, Schuetz TJ, Blumenstein BA, Glode LM, Bilhartz DL, Wyand M, Manson K, Panicali DL, Laus R, Schlom J, 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. 2010;28(7):1099-1105. doi:10.1200/JCO.2009.25.0597. PMID:20100959

[21] Schneble EJ, Berry JS, Trappey FA, Clifton GT, Ponniah S, Mittendorf E, Peoples GE. The HER2 peptide nelipepimut-S (E75) vaccine (NeuVax^{TM}) in breast cancer patients at risk for recurrence: Correlation of immunologic data with clinical response. Immunotherapy. 2014;6(5):519-531. doi:10.2217/imt.14.22. PMID:24896623

[22] Mittendorf EA, Clifton GT, Holmes JP, Schneble E, van Echo D, Ponniah S, Peoples GE. Final report of the phase I/II clinical trial of the E75 (nelipepimut-S) vaccine with booster inoculations to prevent disease recurrence in high-risk breast cancer patients. Ann Oncol. 2014;25(9):1735-42. doi:10.1093/annonc/mdu211. PMID:24907636

[23] Anagnostou V, Smith KN, Forde PM, Niknafs N, Bhattacharya R, White J, Zhang T, Adleff V, Phallen J, Wali N, et al. Evolution of neoantigen landscape during immune checkpoint blockade in non-small cell lung cancer. Cancer Discov. 2017;7(3):264-276. doi:10.1158/2159-8290.CD-16-0828. PMID:28031159

[24] Verdegaal EM, de Miranda NF, Visser M, Harryvan T, van Buuren MM, Andersen RS, Hadrup SR, van der Minne CE, Schotte R, Spits H, et al. Neoantigen landscape dynamics during human melanoma-T cell interactions. Nature. 2016;536(7614):91-95. doi:10.1038/nature18945. PMID:27350335

[25] Yadav M, Jhunjhunwala S, Phung QT, Lupardus P, Tanguay J, Bumbaca S, Franci C, Cheung TK, Fritsche J, Weinschenk T, et al. Predicting immunogenic tumour mutations by combining mass spectrometry and exome sequencing. Nature. 2014;515(7528):572-576. doi:10.1038/nature14001. PMID:25428506

[26] Dillman RO. Is there a role for therapeutic cancer vaccines in the age of checkpoint inhibitors? Hum Vaccin Immunother. 2017;13 (3):528-532. doi:10.1080/21645515.2016.1244149. PMID:27808593

[27] Finke LH, Wentworth K, Blumenstein B, Rudolph NS, Levitsky H, Hoos A. Lessons from randomized phase III studies with active cancer immunotherapies–outcomes from the 2006 meeting of the Cancer Vaccine Consortium (CVC). Vaccine. 2007;25(Suppl 2): B97-B109. doi:10.1016/j.vaccine.2007.06.067. PMID:17916465

[28] Hanna MG. Cancer vaccines: Are we there yet? Hum Vaccin Immunother. 2012;8(8):1161-65. doi:10.4161/hv.21660. PMID:22854657

[29] Gameiro SR, Jammeh ML, Wattenberg MM, Tsang KY, Ferrone S, Hodge JW. Radiation-induced immunogenic modulation of tumor enhances antigen processing and calreticulin exposure, resulting in enhanced T-cell killing. Oncotarget. 2014;5(2):403-416. doi:10.18632/oncotarget.1719. PMID:24480782

[30] Gulley JL, Arlen PM, Bastian A, Morin S, Marte J, Beetham P, Tsang KY, Yokokawa J, Hodge JW, Ménard C, et al. Combining a recombinant cancer vaccine with standard definitive radiotherapy in patients with localized prostate cancer. Clin Cancer Res. 2005;11 (9):3353-62. doi:10.1158/1078-0432.CCR-04-2062. PMID:15867235

[31] Hodge JW, Garnett CT, Farsaci B, Palena C, Tsang KY, Ferrone S, Gameiro SR. 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. doi:10.1002/ijc.28070. PMID:23364915

[32] Yu TW, Chueh HY, Tsai CC, Lin CT, Qiu JT. Novel GM-CSF-based vaccines: One small step in GM-CSF gene optimization, one giant leap for human vaccines. Hum Vaccin Immunother. 2016;12(12):3020-28. doi:10.1080/21645515.2016.1221551. PMID:27560197

[33] Dranoff G. GM-CSF-based cancer vaccines. Immunol Rev. 2002;188:147-154. doi:10.1034/j.1600-065X.2002.18813.x. PMID:12445288

[34] Shi Y, Liu CH, Roberts AI, Das J, Xu G, Ren G, Zhang Y, Zhang L, Yuan ZR, Tan HS, et al. Granulocyte-macrophage colony-stimulating factor (GM-CSF) and T-cell responses: What we do and don't know. Cell Res. 2006;16(2):126-133. doi:10.1038/sj.cr.7310017. PMID:16474424

[35] Gomez-Cambronero J, Horn J, Paul CC, Baumann MA. Granulocyte-macrophage colony-stimulating factor is a chemoattractant cytokine for human neutrophils: Involvement of the ribosomal p70 S6 kinase signaling pathway. J Immunol. 2003;171(12):6846-55. doi:10.4049/jimmunol.171.12.6846. PMID:14662891

[36] Dolcetti L, Peranzoni E, Ugel S, Marigo I, Fernandez Gomez A, Mesa C, Geilich M, Winkels G, Traggiai E, Casati A, et al. Hierarchy of immunosuppressive strength among myeloid-derived suppressor cell subsets is determined by GM-CSF. Eur J Immunol. 2010;40 (1):22-35. doi:10.1002/eji.200939903. PMID:19941314

[37] Talmadge JE. Pathways mediating the expansion and immunosuppressive activity of myeloid-derived suppressor cells and their relevance to cancer therapy. Clin Cancer Res. 2007;13(18 Pt 1):5243-48. PMID:17875751

[38] Gulley JL, Arlen PM, Madan RA, Tsang KY, Pazdur MP, Skarupa L, Jones JL, Poole DJ, Higgins JP, Hodge JW, 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. PMID:19890632

[39] Slingluff CL, Petroni GR, Olson WC, Smolkin ME, Ross MI, Haas NB, Grosh WW, Boisvert ME, Kirkwood JM, Chianese-Bullock KA. Effect of granulocyte/macrophage colony-stimulating factor on circulating CD8+ and CD4+ T-cell responses to a multipeptide melanoma vaccine: Outcome of a multicenter randomized trial. Clin Cancer Res. 2009;15(22):7036-44. PMID:19903780

[40] Heery CR, Ibrahim NK, Arlen PM, Mohebtash M, Murray JL, Koenig K, Madan RA, McMahon S, Marté JL, Steinberg SM, et al. Docetaxel alone or in combination with a therapeutic cancer vaccine (PANVAC) in patients with metastatic breast cancer: A randomized clinical trial. JAMA Oncol. 2015;1(8):1087-95. PMID:26291768

[41] Lawson DH, Lee S, Zhao F, Tarhini AA, Margolin KA, Ernstoff MS, Atkins MB, Cohen GI, Whiteside TL, Butterfield LH, et al. Randomized, placebo-controlled, phase III trial of yeast-derived granulocyte-macrophage colony-stimulating factor (GM-CSF) versus peptide vaccination versus GM-CSF plus peptide vaccination versus placebo in patients with no evidence of disease after complete surgical resection of locally advanced and/or stage IV melanoma: A trial of the eastern cooperative oncology group-American college of radiology imaging network cancer research group (E4697). J Clin Oncol. 2015;33(34):4066-76. PMID:26351350

[42] Hoeller C, Michielin O, Ascierto PA, Szabo Z, Blank CU. Systematic review of the use of granulocyte-macrophage colony-stimulating factor in patients with advanced melanoma. Cancer Immunol Immunother. 2016;65(9):1015-34. PMID:27372293

[43] Liao W, Lin JX, Leonard WJ. IL-2 family cytokines: New insights into the complex roles of IL-2 as a broad regulator of T helper cell differentiation. Curr Opin Immunol. 2011;23(5):598-604. PMID:21889323

[44] Atkins MB, Lotze MT, Dutcher JP, Fisher RI, Weiss G, Margolin K, Abrams J, Sznol M, Parkinson D, Hawkins M, et al. High-dose recombinant interleukin 2 therapy for patients with metastatic melanoma: Analysis of 270 patients treated between 1985 and 1993. J Clin Oncol. 1999;17(7):2105-16. PMID:10561265

[45] McDermott DF, Regan MM, Clark JI, Flaherty LE, Weiss GR, Logan TF, Kirkwood JM, Gordon MS, Sosman JA, Ernstoff MS, et al. Randomized phase III trial of high-dose interleukin-2 versus subcutaneous interleukin-2 and interferon in patients with metastatic renal cell carcinoma. J Clin Oncol. 2005;23(1):133-141. PMID:15625368

[46] FDA. Proleukin (Aldesleukin) drug label. 2012; https://www.accessdata.fda.gov/drugsatfda_docs/label/2012/103293s5130lbl.pdf. Accessed April 24, 2017.

[47] Baek S, Kim CS, Kim SB, Kim YM, Kwon SW, Kim Y, Kim H, Lee H. Combination therapy of renal cell carcinoma or breast cancer patients with dendritic cell vaccine and IL-2: Results from a phase I/II trial. J Transl Med. 2011;9:178. doi:10.1186/1479-5876-9-178. PMID:22013914

[48] Schwartzentruber DJ, Lawson DH, Richards JM, Conry RM, Miller DM, Treisman J, Gailani F, Riley L, Conlon K, Pockaj B, et al. gp100 peptide vaccine and interleukin-2 in patients with advanced melanoma. N Engl J Med. 2011;364(22):2119-27. doi:10.1056/NEJMoa1012863. PMID:21631324

[49] Pathangey LB, McCurry DB, Gendler SJ, Dominguez AL, Gorman JE, Pathangey G, Mihalik LA, Dang Y, Disis ML, Cohen PA. Surrogate in vitro activation of innate immunity synergizes with interleukin-7 to unleash rapid antigen-driven outgrowth of CD4+ and CD8+ human peripheral blood T-cells naturally recognizing MUC1, HER2/neu and other tumor-associated antigens. Oncotarget. 2017;8(7):10785-808. PMID:27974697

[50] Li MO, Flavell RA. TGF-beta: A master of all T cell trades. Cell. 2008;134(3):392-404. doi:10.1016/j.cell.2008.07.025. PMID:18692464

[51] Bierie B, Moses HL. Transforming growth factor beta (TGF-beta) and inflammation in cancer. Cytokine Growth Factor Rev. 2010;21(1):49-59. doi:10.1016/j.cytogfr.2009.11.008. PMID:20018551

[52] Lutz ER, Wu AA, Bigelow E, Sharma R, Mo G, Soares K, Solt S, Dorman A, Wamwea A, Yager A, et al. Immunotherapy converts nonimmunogenic pancreatic tumors into immunogenic foci of immune regulation. Cancer Immunol Res. 2014;2(7):616-631. doi:10.1158/2326-6066.CIR-14-0027. PMID:24942756

[53] de Gramont A, Faivre S, Raymond E. Novel TGF-β inhibitors ready for prime time in onco-immunology. Oncoimmunology. 2017;6(1):e1257453. doi:10.1080/2162402X.2016.1257453. PMID:28197376

[54] Faivre SJ, Santoro A, Gane E, et al. A phase 2 study of galunisertib, a novel transforming growth factor-beta (TGF-β) receptor I kinase inhibitor, in patients with advanced hepatocellular carcinoma (HCC) and low serum alpha fetoprotein (AFP). Paper presented at: ASCO2016; Chicago, IL.

[55] Chakraborty M, Abrams SI, Camphausen K, Liu K, Scott T, Coleman CN, Hodge JW. Irradiation of tumor cells up-regulates Fas and enhances CTL lytic activity and CTL adoptive immunotherapy. J Immunol. 2003;170(12):6338-47. doi:10.4049/jimmunol.170.12.6338. PMID:12794167

[56] Ferrara TA, Hodge JW, Gulley JL. Combining radiation and immunotherapy for synergistic antitumor therapy. Curr Opin Mol Ther. 2009;11(1):37-42. PMID:19169958

[57] Chakraborty M, Abrams SI, Coleman CN, Camphausen K, Schlom J, Hodge JW. External beam radiation of tumors alters phenotype of tumor cells to render them susceptible to vaccine-mediated T-cell killing. Cancer Res. 2004;64(12):4328-37. doi:10.1158/0008-5472.CAN-04-0073. PMID:15205348

[58] Kudo-Saito C, Schlom J, Camphausen K, Coleman CN, Hodge JW. The requirement of multimodal therapy (vaccine, local tumor radiation, and reduction of suppressor cells) to eliminate established tumors. Clin Cancer Res. 2005;11(12):4533-44. doi:10.1158/1078-0432.CCR-04-2237. PMID:15958639

[59] Garnett CT, Palena C, Chakraborty M, Tsang KY, Schlom J, Hodge JW. Sublethal irradiation of human tumor cells modulates phenotype resulting in enhanced killing by cytotoxic T lymphocytes. Cancer Res. 2004;64(21):7985-94. doi:10.1158/0008-5472.CAN-04-1525. PMID:15520206

[60] Chakraborty M, Wansley EK, Carrasquillo JA, Yu S, Paik CH, Camphausen K, Becker MD, Goeckeler WF, Schlom J, Hodge JW. The use of chelated radionuclide (samarium-153-ethylenediaminetetramethylenephosphonate) to modulate phenotype of tumor cells and enhance T cell-mediated killing. Clin Cancer Res. 2008;14(13):4241-49. doi:10.1158/1078-0432.CCR-08-0335. PMID:18594006

[61] Gajewski TF. The next hurdle in cancer immunotherapy: Overcoming the non-T-cell-inflamed tumor microenvironment. Semin Oncol. 2015;42(4):663-671. doi:10.1053/j.seminoncol.2015.05.011. PMID:26320069

[62] Malamas AS, Gameiro SR, Knudson KM, Hodge JW. Sublethal exposure to alpha radiation (223Ra dichloride) enhances various carcinomas' sensitivity to lysis by antigen-specific cytotoxic T lymphocytes through calreticulin-mediated immunogenic modulation. Oncotarget. 2016;7(52):86937-947. PMID:27893426

[63] Stamell EF, Wolchok JD, Gnjatic S, Lee NY, Brownell I. The abscopal effect associated with a systemic anti-melanoma immune response. Int J Radiat Oncol Biol Phys. 2013;85(2):293-295. doi:10.1016/j.ijrobp.2012.03.017. PMID:22560555

[64] Okuma K, Yamashita H, Niibe Y, Hayakawa K, Nakagawa K. Abscopal effect of radiation on lung metastases of hepatocellular carcinoma: A case report. J Med Case Rep. 2011;5:111. doi:10.1186/1752-1947-5-111. PMID:21418591

[65] Nakanishi M, Chuma M, Hige S, Asaka M. Abscopal effect on hepatocellular carcinoma. Am J Gastroenterol. 2008;103(5):1320-21. doi:10.1111/j.1572-0241.2007.01782_13.x. PMID:18477367

[66] Wersäll PJ, Blomgren H, Pisa P, Lax I, Kälkner KM, Svedman C. Regression of non-irradiated metastases after extracranial

stereotactic radiotherapy in metastatic renal cell carcinoma. Acta Oncol. 2006;45(4):493-497. doi:10.1080/02841860600604611. PMID:16760190

[67] Grimaldi AM, Simeone E, Giannarelli D, Muto P, Falivene S, Borzillo V, Giugliano FM, Sandomenico F, Petrillo A, Curvietto M, et al. Abscopal effects of radiotherapy on advanced melanoma patients who progressed after ipilimumab immunotherapy. Oncoimmunology. 2014;3:e28780. doi:10.4161/onci.28780. PMID:25083318

[68] Golden EB, Demaria S, Schiff PB, Chachoua A, Formenti SC. An abscopal response to radiation and ipilimumab in a patient with metastatic non-small cell lung cancer. Cancer Immunol Res. 2013;1(6):365-372. doi:10.1158/2326-6066.CIR-13-0115. PMID:24563870

[69] Sebastian M, Papachristofilou A, Weiss C, Früh M, Cathomas R, Hilbe W, Wehler T, Rippin G, Koch SD, Scheel B, et al. Phase Ib study evaluating a self-adjuvanted mRNA cancer vaccine (RNActive(R)) combined with local radiation as consolidation and maintenance treatment for patients with stage IV non-small cell lung cancer. BMC Cancer. 2014;14:748. doi:10.1186/1471-2407-14-748. PMID:25288198

[70] Sebastian M, Klinkhardt U. Phase Ib trial of the RNActive cancer vaccine BI 1361849 (CV9202) and local radiotherapy (RT) in patients (pts) with stage IV NSCLC with disease control after 1st-line chemotherapy or during therapy with an EGFR-TKI: results of an interim analysis. J Clin Oncol. 2016;34(Suppl):Abstract e20627.

[71] Mineta T, Rabkin SD, Yazaki T, Hunter WD, Martuza RL. Attenuated multi-mutated herpes simplex virus-1 for the treatment of malignant gliomas. Nat Med. 1995;1(9):938-943. doi:10.1038/nm0995-938. PMID:7585221

[72] Markert JM, Razdan SN, Kuo HC, Cantor A, Knoll A, Karrasch M, Nabors LB, Markiewicz M, Agee BS, Coleman JM, et al. A phase 1 trial of oncolytic HSV-1, G207, given in combination with radiation for recurrent GBM demonstrates safety and radiographic responses. Mol Ther. 2014;22(5):1048-55. doi:10.1038/mt.2014.22. PMID:24572293

[73] Whisenhunt TR, Rajneesh KF, Hackney JR, Markert JM. Extended disease-free interval of 6 years in a recurrent glioblastoma multiforme patient treated with G207 oncolytic viral therapy. Oncolytic Virother. 2015;4:33-38. PMID:27512668

[74] Mesnil M, Yamasaki H. Bystander effect in herpes simplex virus-thymidine kinase/ganciclovir cancer gene therapy: Role of gap-junctional intercellular communication. Cancer Res. 2000;60(15):3989-99. PMID:10945596

[75] Heery CR, Madan RA, Bilusic M, Singh NK, Rauckhorst M, Steinberg SM, Dahut WL, Chen C, DiPaola RS, Stein MN, et al. A phase II randomized clinical trial of samarium-153 EDTMP (Sm-153) with or without PSA-tricom vaccine in metastatic castration-resistant prostate cancer (mCRPC) after docetaxel. J Clin Oncol. 2013 (Suppl 6):Abstract 102. doi:10.1200/jco.2013.31.6_suppl.102

[76] Heery CR, Madan RA, Stein MN, Stadler WM, Di Paola RS, Rauckhorst M, Steinberg SM, Marté JL, Chen CC, Grenga I, et al. Samarium-153-EDTMP (Quadramet®) with or without vaccine in metastatic castration-resistant prostate cancer: A randomized Phase 2 trial. Oncotarget. 2016;7(42):69014-023. doi:10.18632/oncotarget.10883. PMID:27486817

[77] Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer. 2012;12(4):252-264. doi:10.1038/nrc3239. PMID:22437870

[78] Peggs KS, Quezada SA, Chambers CA, Korman AJ, Allison JP. Blockade of CTLA-4 on both effector and regulatory T cell compartments contributes to the antitumor activity of anti-CTLA-4 antibodies. J Exp Med. 2009;206(8):1717-25. doi:10.1084/jem.20082492. PMID:19581407

[79] Kwon ED, Drake CG, Scher HI, Fizazi K, Bossi A, van den Eertwegh AJ, Krainer M, Houede N, Santos R, Mahammedi H, 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. doi:10.1016/S1470-2045(14)70189-5. PMID:24831977

[80] Beer TM, Kwon ED, Drake CG, Fizazi K, Logothetis C, Gravis G, Ganju V, Polikoff J, Saad F, Humanski P, et al. Randomized, double-blind, phase III trial of ipilimumab versus placebo in asymptomatic or minimally symptomatic patients with metastatic chemotherapy-naive castration-resistant prostate cancer. J Clin Oncol. 2017;35(1):40-47. doi:10.1200/JCO.2016.69.1584. PMID:28034081

[81] Madan RA, Mohebtash M, Arlen PM, Vergati M, Rauckhorst M, Steinberg SM, Tsang KY, Poole DJ, Parnes HL, Wright JJ, 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. doi:10.1016/S1470-2045(12)70006-2. PMID:22326924

[82] Jochems C, Tucker JA, Tsang KY, Madan RA, Dahut WL, Liewehr DJ, Steinberg SM, Gulley JL, Schlom J. A combination trial of vaccine plus ipilimumab in metastatic castration-resistant prostate cancer patients: Immune correlates. Cancer Immunol Immunother. 2014;63(4):407-418. doi:10.1007/s00262-014-1524-0. PMID:24514956

[83] Le DT, Lutz E, Uram JN, Sugar EA, Onners B, Solt S, Zheng L, Diaz LA Jr, Donehower RC, Jaffee EM, et al. Evaluation of ipilimumab in combination with allogeneic pancreatic tumor cells transfected with a GM-CSF gene in previously treated pancreatic cancer. J Immunother. 2013;36(7):382-389. doi:10.1097/CJI.0b013e31829fb7a2. PMID:23924790

[84] Santegoets SJ, Stam AG, Lougheed SM, Gall H, Scholten PE, Reijm M, Jooss K, Sacks N, Hege K, Lowy I, et al. T cell profiling reveals high CD4+CTLA-4 + T cell frequency as dominant predictor for survival after prostate GVAX/ipilimumab treatment. Cancer Immunol Immunother. 2013;62(2):245-256. doi:10.1007/s00262-012-1330-5. PMID:22878899

[85] Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, Gonzalez R, Robert C, Schadendorf D, Hassel JC, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. 2010;363(8):711-723. doi:10.1056/NEJMoa1003466. PMID:20525992

[86] Wilgenhof S, Corthals J, Heirman C, van Baren N, Lucas S, Kvistborg P, Thielemans K, Neyns B. Phase II study of autologous monocyte-derived mRNA electroporated dendritic cells (TriMixDC-MEL) plus ipilimumab in patients with pretreated advanced melanoma. J Clin Oncol. 2016;34(12):1330-38. doi:10.1200/JCO.2015.63.4121. PMID:26926680

[87] Robert C, Schachter J, Long GV, Arance A, Grob JJ, Mortier L, Daud A, Carlino MS, McNeil C, Lotem M, et al. Pembrolizumab versus ipilimumab in advanced melanoma. N Engl J Med. 2015;372(26):2521-32. doi:10.1056/NEJMoa1503093. PMID:25891173

[88] Ribas A, Puzanov I, Dummer R, Schadendorf D, Hamid O, Robert C, Hodi FS, Schachter J, Pavlick AC, Lewis KD, et al. Pembrolizumab versus investigator-choice chemotherapy for ipilimumab-refractory melanoma (KEYNOTE-002): A randomised, controlled, phase 2 trial. Lancet Oncol. 2015;16(8):908-918. doi:10.1016/S1470-2045(15)00083-2. PMID:26115796

[89] Postow MA, Chesney J, Pavlick AC, Robert C, Grossmann K, McDermott D, Linette GP, Meyer N, Giguere JK, Agarwala SS, et al. Nivolumab and ipilimumab versus ipilimumab in untreated melanoma. N Engl J Med. 2015;372(21):2006-17. doi:10.1056/NEJMoa1414428. PMID:25891304

[90] Daud A, Ribas A, Robert C, Stephen Hodi F, Wolchok JD, Joshua AM, Hwu WJ, Weber JS, Gangadhar TC, Joseph RW, et al. Long-term efficacy of pembrolizumab (pembro; MK-3475) in a pooled analysis of 655 patients (pts) with advanced melanoma (MEL) enrolled in KEYNOTE-001. ASCO; 2015;33(suppl 15):9005-05.

[91] Administration FaD. Hematology/Oncology (Cancer) approvals & Safety Notifications. 2017; https://www.fda.gov/drugs/informationdrugs/approveddrugs/ucm279174.htm. Accessed April 5, 2017.

[92] Karyampudi L, Lamichhane P, Scheid AD, Kalli KR, Shreeder B, Krempski JW, Behrens MD, Knutson KL. Accumulation of memory precursor CD8 T cells in regressing tumors following combination therapy with vaccine and anti-PD-1 antibody. Cancer Res. 2014;74(11):2974-85. doi:10.1158/0008-5472.CAN-13-2564. PMID:24728077

[93] Gabrilovich DI. Myeloid-derived suppressor cells. Cancer Immunol Res. 2017;5(1):3-8. doi:10.1158/2326-6066.CIR-16-0297. PMID:28052991

[94] Weber JS, Kudchadkar RR, Yu B, Gallenstein D, Horak CE, Inzunza HD, Zhao X, Martinez AJ, Wang W, Gibney G, et al. Safety, efficacy, and biomarkers of nivolumab with vaccine in ipilimumab-refractory or -naive melanoma. J Clin Oncol. 2013;31(34):4311-18. doi:10.1200/JCO.2013.51.4802. PMID:24145345

[95] Gibney GT, Kudchadkar RR, DeConti RC, Thebeau MS, Czupryn MP, Tetteh L, Eysmans C, Richards A, Schell MJ, Fisher KJ, et al. Safety, correlative markers, and clinical results of adjuvant nivolumab in combination with vaccine in resected high-risk metastatic melanoma. Clin Cancer Res. 2015;21(4):712-720. doi:10.1158/1078-0432.CCR-14-2468. PMID:25524312

[96] McNeel DG. DNA vaccine with pembrolizumab elicits anti-tumor responses in patients with metastatic, castration-resistant prostate cancer (mCRPC). SITC. 2016; National Harbor, MD.

[97] Strbo N, Garcia-Soto A, Schreiber TH, Podack ER. Secreted heat shock protein gp96-Ig: Next-generation vaccines for cancer and infectious diseases. Immunol Res. 2013;57(1-3):311-325. doi:10.1007/s12026-013-8468-x. PMID:24254084

[98] Klapper JA, Downey SG, Smith FO, Yang JC, Hughes MS, Kammula US, Sherry RM, Royal RE, Steinberg SM, Rosenberg S. High-dose interleukin-2 for the treatment of metastatic renal cell carcinoma: A retrospective analysis of response and survival in patients treated in the surgery branch at the National Cancer Institute between 1986 and 2006. Cancer. 2008;113(2):293-301. doi:10.1002/cncr.23552. PMID:18457330

[99] Gore ME, Larkin JM. Challenges and opportunities for converting renal cell carcinoma into a chronic disease with targeted therapies. Br J Cancer. 2011;104(3):399-406. doi:10.1038/sj.bjc.6606084. PMID:21285971

[100] Yang JC, Sherry RM, Steinberg SM, Topalian SL, Schwartzentruber DJ, Hwu P, Seipp CA, Rogers-Freezer L, Morton KE, White DE, et al. Randomized study of high-dose and low-dose interleukin-2 in patients with metastatic renal cancer. J Clin Oncol. 2003;21(16):3127-32. doi:10.1200/JCO.2003.02.122. PMID:12915604

[101] Choueiri TK, Escudier B, Powles T, Mainwaring PN, Rini BI, Donskov F, Hammers H, Hutson TE, Lee JL, Peltola K, et al. Cabozantinib versus everolimus in advanced renal-cell carcinoma. N Engl J Med. 2015;373(19):1814-23. doi:10.1056/NEJMoa1510016. PMID:26406150

[102] Motzer RJ, Hutson TE, Ren M, Dutcus C, Larkin J. Independent assessment of lenvatinib plus everolimus in patients with metastatic renal cell carcinoma. Lancet Oncol. 2016;17(1):e4-5. doi:10.1016/S1470-2045(15)00543-4. PMID:26758760

[103] Ott PA, Adams S. Small-molecule protein kinase inhibitors and their effects on the immune system: Implications for cancer treatment. Immunotherapy. 2011;3(2):213-227. doi:10.2217/imt.10.99. PMID:21322760

[104] Kwilas AR, Donahue RN, Tsang KY, Hodge JW. Immune consequences of tyrosine kinase inhibitors that synergize with cancer immunotherapy. Cancer Cell Microenviron. 2015;2(1):e677. PMID:26005708

[105] Finke JH, Rini B, Ireland J, Rayman P, Richmond A, Golshayan A, Wood L, Elson P, Garcia J, Dreicer R, et al. Sunitinib reverses type-1 immune suppression and decreases T-regulatory cells in renal cell carcinoma patients. Clin Cancer Res. 2008;14(20):6674-82. doi:10.1158/1078-0432.CCR-07-5212. PMID:18927310

[106] Zhao W, Gu YH, Song R, Qu BQ, Xu Q. Sorafenib inhibits activation of human peripheral blood T cells by targeting LCK phosphorylation. Leukemia. 2008;22(6):1226-33. doi:10.1038/leu.2008.58. PMID:18337760

[107] Farsaci B, Donahue RN, Coplin MA, Grenga I, Lepone LM, Molinolo AA, Hodge JW. Immune consequences of decreasing tumor vasculature with antiangiogenic tyrosine kinase inhibitors in combination with therapeutic vaccines. Cancer Immunol Res. 2014;2(11):1090-1102. doi:10.1158/2326-6066.CIR-14-0076. PMID:25092771

[108] Kwilas AR, Ardiani A, Donahue RN, Aftab DT, Hodge JW. Dual effects of a targeted small-molecule inhibitor (cabozantinib) on immune-mediated killing of tumor cells and immune tumor microenvironment permissiveness when combined with a cancer vaccine. J Transl Med. 2014;12:294. doi:10.1186/s12967-014-0294-y. PMID:25388653

[109] Amin A, Dudek AZ, Logan TF, Lance RS, Holzbeierlein JM, Knox JJ, Master VA, Pal SK, Miller WH Jr, Karsh LI, et al. Survival with AGS-003, an autologous dendritic cell-based immunotherapy, in combination with sunitinib in unfavorable risk patients with advanced renal cell carcinoma (RCC): Phase 2 study results. J Immunother Cancer. 2015;3:14. doi:10.1186/s40425-015-0055-3. PMID:25901286

[110] Heng DY, Xie W, Regan MM, Warren MA, Golshayan AR, Sahi C, Eigl BJ, Ruether JD, Cheng T, North S, et al. Prognostic factors for overall survival in patients with metastatic renal cell carcinoma treated with vascular endothelial growth factor-targeted agents: Results from a large, multicenter study. J Clin Oncol. 2009;27(34):5794-99. doi:10.1200/JCO.2008.21.4809. PMID:19826129

[111] Hull EE, Montgomery MR, Leyva KJ. HDAC inhibitors as epigenetic regulators of the immune system: Impacts on cancer therapy and inflammatory diseases. Biomed Res Int. 2016;2016:8797206. doi:10.1155/2016/8797206. PMID:27556043

[112] Gameiro SR, Malamas AS, Tsang KY, Ferrone S, Hodge JW. Inhibitors of histone deacetylase 1 reverse the immune evasion phenotype to enhance T-cell mediated lysis of prostate and breast carcinoma cells. Oncotarget. 2016;7(7):7390-7402. doi:10.18632/oncotarget.7180. PMID:26862729

[113] Lin KT, Wang YW, Chen CT, Ho CM, Su WH, Jou YS. HDAC inhibitors augmented cell migration and metastasis through induction of PKCs leading to identification of low toxicity modalities for combination cancer therapy. Clin Cancer Res. 2012;18(17):4691-4701. doi:10.1158/1078-0432.CCR-12-0633. PMID:22811583

[114] Ramalingam SS, Maitland ML, Frankel P, Argiris AE, Koczywas M, Gitlitz B, Thomas S, Espinoza-Delgado I, Vokes EE, Gandara DR, et al. Carboplatin and Paclitaxel in combination with either vorinostat or placebo for first-line therapy of advanced non-small-cell lung cancer. J Clin Oncol. 2010;28(1):56-62. doi:10.1200/JCO.2009.24.9094. PMID:19933908

[115] Generali D, Bates G, Berruti A, Brizzi MP, Campo L, Bonardi S, Bersiga A, Allevi G, Milani M, Aguggini S, et al. Immunomodulation of FOXP3+ regulatory T cells by the aromatase inhibitor letrozole in breast cancer patients. Clin Cancer Res. 2009;15(3):1046-51. doi:10.1158/1078-0432.CCR-08-1507. PMID:19188178

[116] Mercader M, Bodner BK, Moser MT, Kwon PS, Park ES, Manecke RG, Ellis TM, Wojcik EM, Yang D, Flanigan RC, et al. T cell infiltration of the prostate induced by androgen withdrawal in patients with prostate cancer. Proc Natl Acad Sci U S A. 2001;98(25):14565-570. doi:10.1073/pnas.251140998. PMID:11734652

[117] Koh YT, Gray A, Higgins SA, Hubby B, Kast WM. Androgen ablation augments prostate cancer vaccine immunogenicity only when applied after immunization. Prostate. 2009;69(6):571-584. doi:10.1002/pros.20906. PMID:19143030

[118] Drake CG, Doody AD, Mihalyo MA, Huang CT, Kelleher E, Ravi S, Hipkiss EL, Flies DB, Kennedy EP, Long M, et al. Androgen ablation mitigates tolerance to a prostate/prostate cancer-restricted antigen. Cancer Cell. 2005;7(3):239-249. doi:10.1016/j.ccr.2005.01.027. PMID:15766662

[119] DiPaola RS, Chen YH, Bubley GJ, Stein MN, Hahn NM, Carducci MA, Lattime EC, Gulley JL, Arlen PM, Butterfield LH, 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: ECOG 9802. Eur Urol. 2015;68(3):365-371. doi:10.1016/j.eururo.2014.12.010. PMID:25533418

[120] Madan RA, Gulley JL, Schlom J, Steinberg SM, Liewehr DJ, Dahut WL, Arlen PM. Analysis of overall survival in patients with nonmetastatic castration-resistant prostate cancer treated with vaccine, nilutamide, and combination therapy. Clin Cancer Res. 2008;14(14):4526-31. doi:10.1158/1078-0432.CCR-07-5048. PMID:18628467

[121] Zitvogel L, Kroemer G. Anticancer immunochemotherapy using adjuvants with direct cytotoxic effects. J Clin Invest. 2009;119(8):2127-30. PMID:19620780

[122] Emens LA, Middleton G. The interplay of immunotherapy and chemotherapy: Harnessing potential synergies. Cancer Immunol Res.

2015;3(5):436-443. doi:10.1158/2326-6066.CIR-15-0064. PMID:25941355

[123] Galluzzi L, Buqué A, Kepp O, Zitvogel L, Kroemer G. Immunological effects of conventional chemotherapy and targeted anticancer agents. Cancer Cell. 2015;28(6):690-714. doi:10.1016/j.ccell.2015.10.012. PMID:26678337

[124] Shurin MR, Naiditch H, Gutkin DW, Umansky V, Shurin GV. Chemo immuno modulation: Immune regulation by the antineoplastic chemotherapeutic agents. Curr Med Chem. 2012;19(12):1792-1803. doi:10.2174/092986712800099785. PMID:22414087

[125] Galetto A, Buttiglieri S, Forno S, Moro F, Mussa A, Matera L. Drug- and cell-mediated antitumor cytotoxicities modulate cross-presentation of tumor antigens by myeloid dendritic cells. Anticancer Drugs. 2003;14(10):833-843. doi:10.1097/00001813-200311000-00010. PMID:14597879

[126] Nowak AK, Lake RA, Marzo AL, Scott B, Heath WR, Collins EJ, Frelinger JA, Robinson BW. Induction of tumor cell apoptosis in vivo increases tumor antigen cross-presentation, cross-priming rather than cross-tolerizing host tumor-specific CD8 T cells. J Immunol. 2003;170(10):4905-13. doi:10.4049/jimmunol.170.10.4905. PMID:12734333

[127] Lutsiak ME, Semnani RT, De Pascalis R, Kashmiri SV, Schlom J, Sabzevari H. Inhibition of CD4(+)25+ T regulatory cell function implicated in enhanced immune response by low-dose cyclophosphamide. Blood. 2005;105(7):2862-68. doi:10.1182/blood-2004-06-2410. PMID:15591121

[128] Sistigu A, Viaud S, Chaput N, Bracci L, Proietti E, Zitvogel L. Immunomodulatory effects of cyclophosphamide and implementations for vaccine design. Semin Immunopathol. 2011;33(4):369-383. doi:10.1007/s00281-011-0245-0. PMID:21611872

[129] Chen G, Gupta R, Petrik S, Laiko M, Leatherman JM, Asquith JM, Daphtary MM, Garrett-Mayer E, Davidson NE, Hirt K, et al. A feasibility study of cyclophosphamide, trastuzumab, and an allogeneic GM-CSF-secreting breast tumor vaccine for HER2+ metastatic breast cancer. Cancer Immunol Res. 2014;2(10):949-961. doi:10.1158/2326-6066.CIR-14-0058. PMID:25116755

[130] Huang CS, Yu AL, Tseng LM, et al. Randomized phase II/III trial of active immunotherapy with OPT-822/OPT-821 in patients with metastatic breast cancer. ASCO Annual Meeting. 2016;34(Suppl 15):Abstract 1003 (Presented June 4, 2016).

[131] Rettig L, Seidenberg S, Parvanova I, Samaras P, Curioni A, Knuth A, Pascolo S. Gemcitabine depletes regulatory T-cells in human and mice and enhances triggering of vaccine-specific cytotoxic T-cells. Int J Cancer. 2011;129(4):832-838. doi:10.1002/ijc.25756. PMID:21710545

[132] Suzuki E, Kapoor V, Jassar AS, Kaiser LR, Albelda SM. Gemcitabine selectively eliminates splenic Gr-1+/CD11b+ myeloid suppressor cells in tumor-bearing animals and enhances antitumor immune activity. Clin Cancer Res. 2005;11(18):6713-21. doi:10.1158/1078-0432.CCR-05-0883. PMID:16166452

[133] Dijkgraaf EM, Santegoets SJ, Reyners AK, Goedemans R, Nijman HW, van Poelgeest MI, van Erkel AR, Smit VT, Daemen TA, van der Hoeven JJ, et al. A phase 1/2 study combining gemcitabine, Pegintron and p53 SLP vaccine in patients with platinum-resistant ovarian cancer. Oncotarget. 2015;6(31):32228-243. doi:10.18632/oncotarget.4772. PMID:26334096

[134] Hardacre JM, Mulcahy M, Small W, Talamonti M, Obel J, Krishnamurthi S, Rocha-Lima CS, Safran H, Lenz HJ, Chiorean EG. Addition of algenpantucel-L immunotherapy to standard adjuvant therapy for pancreatic cancer: A phase 2 study. J Gastrointest Surg. 2013;17(1):94-100; discussion p 100–101. doi:10.1007/s11605-012-2064-6. PMID:23229886

[135] Regine WF, Winter KA, Abrams RA, Safran H, Hoffman JP, Konski A, Benson AB, Macdonald JS, Kudrimoti MR, Fromm ML, et al. Fluorouracil vs gemcitabine chemotherapy before and after fluorouracil-based chemoradiation following resection of pancreatic adenocarcinoma: A randomized controlled trial. JAMA. 2008;299(9):1019-26. doi:10.1001/jama.299.9.1019. PMID:18319412

[136] Middleton G, Silcocks P, Cox T, Valle J, Wadsley J, Propper D, Coxon F, Ross P, Madhusudan S, Roques T, et al. Gemcitabine and capecitabine with or without telomerase peptide vaccine GV1001 in patients with locally advanced or metastatic pancreatic cancer (TeloVac): An open-label, randomised, phase 3 trial. Lancet Oncol. 2014;15(8):829-840. doi:10.1016/S1470-2045(14)70236-0. PMID:24954781

[137] Middleton G, Greenhalf W, Costello E, Shaw V, Cox T, Ghaneh P, Palmer DH, Neoptolemos JP. Immunobiological effects of gemcitabine and capecitabine combination chemotherapy in advanced pancreatic ductal adenocarcinoma. Br J Cancer. 2016;114(5):510-518. doi:10.1038/bjc.2015.468. PMID:26931369

[138] Garnett CT, Schlom J, Hodge JW. Combination of docetaxel and recombinant vaccine enhances T-cell responses and antitumor activity: Effects of docetaxel on immune enhancement. Clin Cancer Res. 2008;14(11):3536-44. doi:10.1158/1078-0432.CCR-07-4025. PMID:18519787

[139] Harrop R, Chu F, Gabrail N, Srinivas S, Blount D, Ferrari A. Vaccination of castration-resistant prostate cancer patients with TroVax (MVA-5T4) in combination with docetaxel: A randomized phase II trial. Cancer Immunol Immunother. 2013;62(9):1511-20. doi:10.1007/s00262-013-1457-z. PMID:23877659

[140] Rocha-Lima CM, de Queiroz Marques Junior E, Bayraktar S, Broome P, Weissman C, Nowacki M, Leslie M, Susnerwala S. A multicenter phase II study of G17DT immunogen plus irinotecan in pretreated metastatic colorectal cancer progressing on irinotecan. Cancer Chemother Pharmacol. 2014;74(3):479-486. doi:10.1007/s00280-014-2520-y. PMID:25030089

[141] Welters MJ, van der Sluis TC, van Meir H, Loof NM, van Ham VJ, van Duikeren S, Santegoets SJ, Arens R, de Kam ML, Cohen AF, et al. Vaccination during myeloid cell depletion by cancer chemotherapy fosters robust T cell responses. Sci Transl Med. 2016;8(334):334ra352. doi:10.1126/scitranslmed.aad8307

[142] Quoix E, Lena H, Losonczy G, Forget F, Chouaid C, Papai Z, Gervais R, Ottensmeier C, Szczesna A, Kazarnowicz A, et al. TG4010 immunotherapy and first-line chemotherapy for advanced non-small-cell lung cancer (TIME): results from the phase 2b part of a randomised, double-blind, placebo-controlled, phase 2b/3 trial. Lancet Oncol. 2016;17(2):212-223. doi:10.1016/S1470-2045(15)00483-0. PMID:26727163

[143] Quoix E, Ramlau R, Westeel V, Papai Z, Madroszyk A, Riviere A, Koralewski P, Breton JL, Stoelben E, Braun D, et al. Therapeutic vaccination with TG4010 and first-line chemotherapy in advanced non-small-cell lung cancer: a controlled phase 2B trial. Lancet Oncol. 2011;12(12):1125-33. doi:10.1016/S1470-2045(11)70259-5. PMID:22019520

[144] Reck M, Rodríguez-Abreu D, Robinson AG, Hui R, Csőszi T, Fülöp A, Gottfried M, Peled N, Tafreshi A, Cuffe S, et al. Pembrolizumab versus chemotherapy for PD-L1-positive non-small-cell lung cancer. N Engl J Med. 2016;375(19):1823-33. doi:10.1056/NEJMoa1606774. PMID:27718847

[145] Rosenberg JE, Hoffman-Censits J, Powles T, van der Heijden MS, Balar AV, Necchi A, Dawson N, O'Donnell PH, Balmanoukian A, Loriot Y, 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):1909-20. doi:10.1016/S0140-6736(16)00561-4. PMID:26952546

[146] Fong L, Carroll P, Weinberg V, Chan S, Lewis J, Corman J, Amling CL, Stephenson RA, Simko J, Sheikh NA, et al. Activated lymphocyte recruitment into the tumor microenvironment following preoperative sipuleucel-T for localized prostate cancer. J Natl Cancer Inst. 2014;106(11):dju268. doi:10.1093/jnci/dju268. PMID:25255802

[147] McGranahan N, Furness AJ, Rosenthal R, Ramskov S, Lyngaa R, Saini SK, Jamal-Hanjani M, Wilson GA, Birkbak NJ, Hiley CT, et al. Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade. Science. 2016;351(6280):1463-69. doi:10.1126/science.aaf1490. PMID:26940869