Next Generation Therapeutic Strateg-Es: Evolving cancer immunotherapy through agents that Engage, Expand and Enable the anti-tumor immune response

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Abstract
The development and FDA approval of immune checkpoint blocking antibodies have brought new light to cancer immunotherapy. While immune checkpoint blockade (ICB) has demonstrated clinical benefit in certain tumors as monotherapy, effective therapy of established tumors necessitates a combination of multiple immuno-oncology agents targeting diverse functions of the immune system. These combination strategies should be tactically designed to Engage the immune system by inducing a tumor-antigen specific T-cell population, Expand the number of antigen-specific cytotoxic T cells and increase their migration to the tumor microenvironment, and once there, Enable prolonged and persistent effector function. Although viral therapeutic cancer vaccines have demonstrated little efficacy as monotherapies, they have substantial potential to Engage the immune system as one branch of a multipronged treatment strategy. This review will summarize prior and ongoing Phase II and III clinical trials built upon the foundation of viral therapeutic cancer vaccines. We examine their efficacy as a monotherapy, and more importantly, when combined with additional agents that Expand and Enable the immune system. It is clear that the future of cancer immunotherapy will include evolving treatment strategies made up of multiple agents, and we are optimistic that in this context viral therapeutic cancer vaccines will emerge as an important part of next generation effective therapeutic strategies.

KEYWORDS
cancer, combination therapy, immunotherapy, oncology, vaccine, viral vaccine

1 | INTRODUCTION

Over the past century, cancer treatment evolved from monotherapy using basic chemotherapy drugs to strategies combining advanced chemotherapy with radiation, surgery, and targeted molecular therapies. It has already become clear that cancer treatment in the 21st century will be defined by immunotherapy.

The first drug targeting immune checkpoints, ipilimumab, was approved by the FDA in 2011, and six more checkpoint blockade drugs have since been approved across numerous indications. There are thousands of open clinical trials for immunotherapy strategies, and the 2018 Nobel Prize in Physiology or Medicine was awarded for the discovery of immune checkpoint molecules. Despite these successes, monotherapy with immune checkpoint
blockade (ICB) is not effective across all tumor types or even in all patients with similar tumors. Immune checkpoint blockade drugs function by blocking inhibitory receptors and rescuing exhausted intratumoral T cells, resulting in an increased ability of cytotoxic T cells to eliminate the cancer. Therefore, immune checkpoint inhibiting drugs are reliant on the presence of exhausted intratumoral tumor antigen-specific T cells.

Determining the amount and activity of antigen-specific T cells in the tumor and tumor microenvironment (TME) is a key determining factor for immunotherapy efficacy. The relationship between immune function and tumor immune context was first described in colorectal cancer, where the presence of T cells was shown to be highly important. Based on these and related findings, the immune context of various tumors is described as a spectrum ranging from "cold" tumors that have little to no immune effector infiltration and high levels of suppressive immune cells such as myeloid-derived suppressor cells (MDSCs) to "hot" tumors that are highly inflamed and have significant amounts of effector immune infiltrate, especially T cells and cytotoxic T cells, and low MDSC infiltration.

Because ICB is dependent in part on the presence of intratumoral antigen-specific T cells, it is most effective in "hot" tumors such as bladder, head and neck, melanoma, and non-small cell lung cancer. In immunologically "cold" tumors that lack T-cell infiltration there are no targets for immune checkpoint blockade drugs, and these treatment strategies are ineffective. In order to treat patients presenting with "cold" tumors, alternative strategies must be employed to engage the immune system by bolstering immune surveillance and elimination.

1.1 | Engage: Priming the immune system

One of the most deeply researched strategies for engaging the immune system is through vaccination. Since their invention in the late 18th century, prophylactic vaccines have been incredibly efficacious in preventing the spread of infectious disease. While certain cancers are caused by infectious diseases (human papillomavirus, hepatitis B virus) and effective preventative vaccines have been developed for them, the majority of cancers are the result of spontaneous mutation or environmental factors, and therefore cannot be prevented in this way. However, the mechanism of prophylactic vaccines is one that can be co-opted for the treatment of already existing tumors.

Therapeutic vaccines generate a tumor antigen-specific T-cell response by stimulating a patient's immune system with a tumor antigen in an immunogenic formulation that activates an immune response. Cancer vaccines can thus create a population of tumor antigen-specific cytotoxic T cells, resulting in an increased number of cancer-specific T cells, and turning "cold" tumors "hot." There have been several cancer vaccine success stories, but decades of research into therapeutic cancer vaccines have demonstrated that despite being well tolerated by patients, pursuing therapeutic cancer vaccines as a monotherapy or even in combination with cytokine to induce expansion is not a fruitful treatment strategy.

1.2 | Targeting diverse immune functions for effective immunotherapy

These results and others have shown that cancer immunotherapy in the 21st century is already beginning to follow the same trend as chemotherapy in the 20th century. The failures of monotherapy strategies for multiple classes of immuno-oncology (IO) agents have led researchers to the conclusion that a tactically designed combined approach, simultaneously targeting diverse immune-tumor interactions, is necessary for the optimal immunotherapeutic treatment strategy. Interactions between growing tumors and the immune system were concisely described by Dunn et al as the Three E’s of Immunoediting. Building off of this foundation,rationally selected combination immunotherapy should include agents capable of enabling at least three aspects of the immune system (Figure 1). It should: 1) utilize strategies such as vaccines, autologous cell transfer and immunogenic cell death to Engage the immune system through inducing antigen-specific T cells in the periphery, 2) use cytokines, costimulatory signals and endocrine deprivation to Expand the population of specific T cells and drive them to the tumor microenvironment, and 3) Enable the immune system by inhibiting mechanisms of self-regulation through the use of checkpoint blockade, metabolic support and agents that induce immunogenic modulation of the tumor.

In this review, we will focus on efforts to Engage the immune system through viral-based tumor vaccines. Although they have demonstrated little efficacy as monotherapy, these vaccines have enormous potential as one leg of a multipronged treatment strategy. We will discuss current clinical trials combining viral-based tumor vaccines with agents that Expand and Enable the immune system, as well as novel agents that will Evolve the current clinical strategies for cancer treatment.

2 | VIRAL VECTOR BASED CANCER VACCINES

There are two initial choices in therapeutic vaccine design, the vaccine target and the platform. Ideal targets should be present on cancer cells but not normal cells, highly immunogenic and necessary for tumor survival. Vaccine targets are grouped into two major categories, tumor-associated antigens (TAA), which are self-antigens upregulated in tumor cells compared to normal tissue, and tumor-specific antigens, which are only expressed in tumor tissue. Tumor-associated antigens include stem cell/EMT markers, oncocalen antigens, cancer-testis antigens, and tissue lineage markers. Tumor-associated antigens have the advantage of being common to multiple patients and tumor types. However, because they are self-antigens, cancer vaccines must combat immune tolerance against them to stimulate a rare population of T cells. Additionally, some tumor-associated antigens are still expressed on normal tissues, which
can lead to off-target effects and toxicity. Tumor-specific antigens such as oncoviral antigens, oncogenes, and neoantigens are specific to tumor tissue. While oncoviruses associated with cancer etiology have led to the development of prophylactic cancer vaccines, neoantigen vaccines remain a work in progress.

Numerous vaccine-delivery platforms have been examined in the decades of tumor vaccine research, including whole-tumor-cell, peptides, yeast, bacteria, plasmid DNA, dendritic cells, viruses, and platform combinations. The breadth of the differences between platforms is beyond the scope of this review, and is well discussed in others.

Viral vectors currently in use include mammalian poxviruses (vaccinia virus, modified virus Ankara (MVA)), avian poxviruses (fowlpox (FPV), canarypox), adenoviruses (human and nonhuman primate), alphaviruses, measles virus, herpes simplex virus, vesicular stomatitis virus, retroviruses, lentiviruses, cytomegalovirus, and Sendai virus. Each viral vector has its own advantages and disadvantages, which have previously been well reviewed.

### 2.1 Oncolytic viruses

In addition to their use as vaccine platforms, viruses have found great success as vectors for gene delivery or as oncolytic agents. While no viral-based gene therapies have been approved for use in the United States, an adenovirus that induces overexpression of tumor suppressor p53 was approved for use in head and neck cancer in China in 2003, and more than a dozen additional gene therapy-based drugs are on the market worldwide. Oncolytic viruses are viruses that selectively infect tumor cells. These viruses then undergo a lytic life cycle, resulting in tumor cell death, spreading the virus and releasing tumor-associated antigens. This immunogenic cell death enhances the immune response and boosts immune-mediated cell killing.

Oncolytic virus-based therapies have been developed across multiple viral platforms, including adenoviruses, coxsackie virus, herpes simplex virus, measles virus, vaccinia and others. Currently, one oncolytic viral therapy, talimogene laherparepvec (T-VEC), has been approved for use in melanoma, with clinical trials underway in most solid tumors. T-VEC is a herpesvirus that was genetically modified to increase its immunogenicity by removing the genes ICP34.5 and ICP47 and replacing them with the gene for GM-CSF, an immunostimulatory cytokine.

Treatment with T-VEC plus GM-CSF resulted in an overall survival (OS) of 23.3 months compared to 18.9 in the GM-CSF alone arm, with 88.5% of patients estimated to survive at 5 years post-treatment. There are currently approximately 100 ongoing clinical trials utilizing oncolytic viruses, and it is clear that this will be an ongoing direction of research and treatment for years to come.

### 3 ENGAGING THE IMMUNE SYSTEM

We have identified three broad clinical modalities that are effective at engaging the immune system, resulting in increased numbers of antigen-specific T cells: 1. Chemotherapy- and radiation-induced immunogenic cell death, 2. Adoptive immune therapy, 3.
Therapeutic vaccines (Figure 1). Substantial preclinical and clinical data have demonstrated that certain doses of standard-of-care chemotherapy and radiation cause immunogenic cell death.\(^26\) In immunogenic cell death, tumor cell death results in changes to cell surface markers and the release of soluble factors that stimulate the presentation of tumor antigens to T cells, increasing the proportion of antigen-specific T cells.\(^27\) Adoptive immune therapy utilizes exogenous expansion of a patient’s own immune cells, often including genetic engineering to make the cells specific for tumor antigen (CAR-T).\(^28\) Three CAR-T therapies are currently FDA approved, targeting the CD19 antigen in acute lymphoblastic leukemia, diffuse large B-cell lymphoma,\(^29\) and mantle cell lymphoma.\(^30,31\) Despite these successes, CAR-T and adoptive immune therapy face challenges such as antigen selection, the cost and labor associated with personalized therapy, and antigen escape. It is likely that, similar to therapeutic vaccines, successful application of CAR-T will involve combination with additional agents that Enable and Expand the immune system.\(^32\)

The earliest research into therapeutic vaccines focused solely on the vaccine itself, targeting TAAs to promote immune destruction of the tumor. Phase I studies consistently demonstrated the safety of cancer vaccines,\(^9\) and several viral vaccine platforms have moved from Phase I to Phase II studies in the monotherapy form (Table 1).

### 3.1 Adenoviral vaccines

Two different adenovirus-based vaccines have entered Phase II studies. Patients with hormone-refractory and recurrent prostate cancers are being treated with an adenoviral vaccine utilizing the TAA prostate-specific antigen (PSA), a highly prevalent antigen in prostate cancer that is also utilized as a serum biomarker for cancer progression.\(^30\) High proportions of both populations exhibited anti-PSA T-cell immune responses, with the majority of patients experiencing a decrease in serum PSA or an increase in PSA doubling time.\(^33\) A separate study treated patients with colon, lung, or breast cancer with ETBX-011, an adenovirus-5 vaccine targeting carcinoembryonic antigen (CEA), a common TAA across many solid tumors.\(^35,41,42\) The 19 patients in the Phase II cohort experienced a 12-month survival probability of 48%, and 10/19 (53%) had a positive CEA-directed cell-mediated immune response by ELISPOT. Importantly, this trial demonstrated that pre-existing immunity to Adenovirus subtype-5 did not significantly impact survival outcomes.\(^31\)

### 3.2 Poxviral vaccines

The majority of monotherapy therapeutic vaccine trials have been performed using poxviruses. Several trials have utilized the vaccine TroVax, a modified vaccinia Ankara–based vaccine that targets the oncofetal antigen ST4. ST4 expression is associated with a tumor-initiating phenotype and highly expressed in tumor cells compared to normal tissue in multiple solid tumors.\(^37\) Three monotherapy TroVax trials have been conducted. In colorectal cancer, 14/16\(^38\) and 18/19\(^43\) patients had ST4-specific antigen responses. Dangoor et al found that 7/16 patients eventually recurred,\(^38\) and Elkord et al demonstrated that patients with a greater than average ST4-specific T-cell proliferative response had a significant survival advantage compared to those who did not, indicating that immune response to the vaccine and density of CD3 cells was likely driving increased survival.\(^34\) Following these data, a double-blind Phase II study examining TroVax in ovarian, fallopian tube, and peritoneal cancer was initiated and is currently ongoing with a primary endpoint of RECIST-defined progression at 25 weeks.\(^43\)

### 3.3 Prime/boost

Two monotherapy Phase II trials have been conducted that utilized a “diversified prime/boost” vaccination method. In this strategy patients are “primed” with a recombinant vaccinia vaccine, followed by subsequent vaccinations (boosts) with avipox vaccines. This approach is commonly utilized when treating with vaccinia-based vaccines, as vaccinia-immune patients are able to mount an immune response to the vaccine antigen after the first vaccination but not to the second.\(^18\) This strategy was tested in a Phase II trial utilizing rF-PSA (recombinant vaccinia) followed by rF-PSA (recombinant fowlpox) in prostate cancer. 45.3% of patients were free of PSA progression at 19.1 months, and 46% of patients had an increase in PSA-reactive T cells.\(^39\) This trial also reported a trend favoring the treatment group receiving vaccinia prime, which was verified in a later trial that also demonstrated the improved efficacy of prime-boost as opposed to vaccination with fowlpox alone.\(^18,39,44\)

Monotherapy prime-boost has also been investigated targeting the cancer-testis antigen NY-ESO-1 in fallopian tube cancer, ovarian cancer, peritoneal cancer, and melanoma. This recent Phase II trial found that melanoma patients had an objective response rate of 14% with a median progression-free survival (PFS) of 9 months and a median OS of 48 months. Epithelial ovarian cancer patients had a PFS of 21 months and an OS of 48 months. The trial found that 40% of melanoma patients exhibited CD8+ T-cell immunity to NY-ESO-1, compared to 14% of ovarian cancer patients.\(^45\) While there are currently no registered follow-up clinical trials utilizing rV/rF-NY-ESO-1, there are currently >50 clinical trials utilizing various NY-ESO-1 vaccine platforms, adoptive immune therapy, and combination therapy.\(^46\)

These monotherapy vaccine studies have demonstrated efficacy in inducing anti-tumor T-cell responses and positive biomarker responses; however, the clinical benefit of vaccine alone has been negligible. This holds true across all therapeutic vaccine delivery platforms and has informed the combination of therapeutic vaccines with other agents since the early days of clinical vaccine development.\(^47\)
| Clinical Trial #   | Indication                  | Status                        | Vaccine        | Trial Phase | n  | Outcomes/Key Points                                                                                           | Ref. |
|-------------------|------------------------------|-------------------------------|----------------|-------------|----|-------------------------------------------------------------------------------------------------------------|------|
| NCT00583024       | Hormone Refractory Prostate Cancer | Active, not recruiting             | Ad-PSA         | II          | 32 | 100% pts exhibited anti-PSA T cell immune responses 75% pts had decrease in serum PSA or increase in PSA doubling time | 33   |
| NCT00583752       | Recurrent Prostate Cancer    | Active, not recruiting             | Ad-PSA         | II          | 40 | 71% pts had anti-PSA T cell immune response 58% pts demonstrated decrease in serum PSA or increase in PSA doubling time | 33   |
| NCT00003871       | Prostate Cancer              | Completed                       | rF-PSA, rV-PSA | II          | 66 | At 19.1 months 45.3% of pts remained free of PSA progression, 78.1% PFS 46% of pts demonstrated increase in PSA-reactive T cells | 34   |
| NCT01147965       | Colon Cancer | Lung Cancer | Breast Cancer | Completed                               | ETBX-011 | I/II | 37 | 19 pts in Phase II had 12-month survival probability of 52.6% Pre-existing Ad5 immunity did not impact outcomes | 35   |
| NCT00259844       | Colorectal Cancer            | Completed                       | TroVax         | II          | 20 | 12/16 pts had T-cell response 14 pts developed ST4-specific Ab response At 8.4 months 7/16 had disease recurrence | 36   |
| NCT00259844       | Colorectal Cancer            | Completed                       | TroVax         | II          | 20 | 18/19 pts had increased ST4-specific Ab response Pts with >median ST4-specific Ab response had insignificant survival advantage Pts with >median ST4-specific proliferative response had significant survival advantage | 37   |
| NCT01556841       | Ovarian Cancer | Fallopian Tube Cancer | Peritoneal Cancer | Completed | TroVax | II      | 69 | No data                                                                                                      | 38   |
| NCT00112957       | Fallopian Tube Cancer | Ovarian Cancer | Peritoneal Cavity Cancer | Melanoma | Completed | TroVax | II      | 49 | Melanoma: Objective response rate 14%, median PFS 9 months, median OS 48 months Epithelial ovarian cancer: median PFS 21 months, median OS 48 months | 39   |

Ad, Adenovirus; NY-ESO-1, New York esophageal squamous cell carcinoma 1; OS, overall survival; PFS, progression-free survival; PSA, prostate-specific antigen; rF, recombinant-fowlpox, rV, recombinant-vaccinia; TroVax, modified vaccinia Ankara-based vaccine that targets the oncofetal antigen 5T4.
| Clinical Trial # | Indication | Status | Vaccine   | IO Agent | Additional Treatment | Trial Phase | n   | Outcomes/Key Points                                                                                                                                       | Ref. |
|-----------------|------------|--------|-----------|----------|----------------------|-------------|-----|------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| NCT02772562    | Prostate Cancer After Radical Prostatectomy | Active, not recruiting | PROSTVAC-V/F |          |          | II | 44 | No data                                                                                                                                                   | 52   |
| NCT01875250    | Biochemically Recurrent Prostate Cancer | Active, not recruiting | PROSTVAC-V/F | Enzalutamide |          | II | 40 | Enzalutamide alone median PSA decline of 99% Enzalutamide alone increased naïve T cells and NK cells, decreased MDSCs Vaccine data not yet available | 53   |
| NCT02649439    | Biochemically Recurrent Prostate Cancer | Active, not recruiting | PROSTVAC-V/F |          |          | II | 100 | 8/22 pts had delayed PSA decline after PROSTVAC-V/F compared to 1/13 pts not receiving PROSTVAC-V/F                                                                 | 54   |
| NCT02649855    | Metastatic Castration-resistant Prostate Cancer | Active, not recruiting | PROSTVAC-V/F | Docetaxel | Abiraterone Enzalutamide | II | 76 | Regardless of immunotherapy sequence, 33% of pts progressed 12 pts started abiraterone/enzalutamide after progression Median time to PSA progression was 5.54 months, 5/12 did not have PSA response | 53   |
| NCT01867333    | Metastatic Castration-resistant Prostate Cancer | Active, not recruiting | PROSTVAC-V/F | Enzalutamide |          | II | 59 | No significant difference in PSA progression between enzalutamide alone and enzalutamide + PROSTVAC-V/F                                                                 | 55   |
| NCT00078585    | Metastatic Castration-resistant Prostate Cancer | Completed | PROSTVAC-V/F |          |          | II | 56 | No difference in PFS 3 years post-study PROSTVAC-V/F arm had OS of 25/82 (30%) versus 7/40 (17%) control Median survival of 25.1 months for PROSTVAC-V/F vs 16.6 for control | 56   |
| NCT00003125    | Breast Cancer | Completed | ALVAC-CEArV-CEA | IL-2 GM-CSF |          | II | 24 | No data                                                                                                                                                   | 49   |
| NCT02015104    | High Grade Non-muscle Invasive Bladder Cancer | Completed | PANVAC-V/F | BCG |          | II | 32 | No data                                                                                                                                                   | 57   |
| NCT00108732    | Recurrent Prostate Carcinoma | Completed | PROSTVAC-V/F | GM-CSF | GM-CSF Bicalutamide Goserelin Acetate | II | 52 | In PROSTVAC-V/F arm 25/40 (63%) of pts were progression free at 6 months When androgen ablation was added 20/27 (74%) pts had CR at 7 months                                                                 | 58   |

(Continues)
An early identified strategy for improving therapeutic vaccine efficacy was to combine vaccine treatment with therapies that were known to increase the number of tumor-infiltrating lymphocytes (TIL), both through promoting immune cell proliferation and by driving immune cell trafficking to the tumor microenvironment. This is done through several strategies: treatment with proinflammatory cytokines, delivery of costimulatory molecules and endocrine deprivation.

4.1 | Proinflammatory cytokines

The cytokines most frequently combined with immunotherapy in the clinic are Interleukin-2 (IL-2) and granulocyte-macrophage colony-stimulating factor (GM-CSF). IL-2 directly stimulates T-cell growth and differentiation into effector and memory T cells after antigen stimulation. It was first approved for treatment of metastatic renal cell carcinoma in 1992, and has served as a backbone of immunotherapy strategies ever since. Similarly, GM-CSF is a proinflammatory cytokine that stimulates differentiation of granulocytes, macrophages and dendritic cells, promoting antigen processing and presentation. A recombinant GM-CSF was approved by the FDA in 1991 to promote white blood cell growth following bone marrow transplantation, and is now also used in cancer therapy. GM-CSF can also be directly delivered to the tumor site as part of a therapeutic vaccine, as in the case of T-VEC.

We have identified one Phase II clinical trial conducted solely with a therapeutic viral vaccine and concomitant cytokine treatment. This trial evaluated the efficacy of recombinant vaccinia and avipox vaccines delivering CEA in combination with IL-2 and GM-CSF to patients with numerous types of solid tumor; however, no results are yet reported (Table 2). While few trials examine the efficacy of vaccine and cytokine alone, cytokines are a common agent in the multi-combination trials found in Tables 3 and 4.

4.2 | Costimulatory molecules

T-cell activation requires two signals from molecules on antigen-presenting cells: stimulation by the peptide (antigen)/major histocompatibility complex and a second signal from a costimulatory molecule. While there are several costimulatory drugs currently in development (agonist antibodies for OX40 and glucocorticoid-induced tumor necrosis factor receptor (GITR)), the only costimulatory strategy currently in combination with viral vaccines in Phase II trials is the viral vaccine platform TRICOM of Costimulatory Molecules (TRICOM). TRICOM vectors express TAAs as well as three costimulatory molecules: B7-1, ICAM-1, and LFA-3. Preclinical and clinical data have demonstrated that TRICOM enhances T-cell response to TAAs greater than other strategies with only one or two costimulatory molecules. TRICOM is a key component of the PROSTVAC
and PANVAC vaccines, and is currently in use in a multitude of Phase I and II clinical trials. The PROSTVAC therapeutic vaccine consists of a prime/boost with recombinant vaccinia-PSA-TRICOM followed by fowlpox-CEA-mucin 1 (MUC1)-TRICOM and is utilized in patients with prostate cancer. For other solid tumors the vaccine platform PANVAC is used, which is the same vaccinia/fowlpox TRICOM prime boost but using the TAA s CEA and MUC1 instead of PSA. A Phase II trial of PANVAC-V(vaccinia virus)/F(fowlpox virus) in combination with Bacillus Calmette-Guerin (standard-of-care) for patients with high-grade nonmuscle invasive bladder cancer has been completed, however, results have yet to be reported.

Initial PROSTVAC-V/F studies demonstrated safety and clinical efficacy in Phase I and II trials. One Phase II study in patients at high risk of relapse following radical prostatectomy is currently ongoing. In a previous Phase II study of metastatic castration-resistant prostate cancer patients, those who received PROSTVAC-V/F and those who received control vectors exhibited similar progression-free survival. However, 3 years post-study, the PROSTVAC-V/F arm reported 25/82 (30%) patients still alive compared to 7/40 (17%) in the control arm. The median survival of the PROSTVAC-V/F arm was 8.5 months longer than control, and there was a 44% decrease in the death rate. A later Phase II trial for patients with biochemically recurrent prostate cancer found that 8/22 (36%) patients had delayed PSA declines, whereas only 1/13 (8%) patients not receiving vaccine exhibited a similar decline. In a third Phase II trial for patients with local prostate cancer undergoing radical prostatectomy, PROSTVAC-V/F treatment resulted in a 2x increase in CD8 T-cell infiltrate in 17/24 patients, a 2x increase in CD4 T-cell infiltrate in 12/24 patients with high-grade nonmuscle invasive peripheral blood in 12/24 patients, and a TAA response in peripheral blood in 12/24 patients.

4.3 Endocrine deprivation

Preclinical evidence has demonstrated that endocrine deprivation therapy promotes T-cell recruitment to the tumor microenvironment. An early Phase II multi-institution study in men with hormone-sensitive nonmetastatic prostate cancer examined PROSTVAC-V/F in combination with endocrine deprivation in two stages. First, they were treated with PROSTVAC-V/F with progression at 6 months as the primary endpoint, and if they progressed they were treated with the endocrine deprivation drugs bicalutamide and goserelin acetate. At 6 months, 25/40 (63%) remained progression free. Of the 27 patients eligible for endocrine deprivation, 20/27 achieved a complete response at 7 months. A second Phase II delivered PROSTVAC-V/F in combination with the antiandrogen flutamide to prostate cancer patients. With 64 patients accrued, this trial found that the median time for progression for patients who received PROSTVAC-V/F + flutamide was 6.9 months compared to 4.5 months for flutamide alone. These data demonstrated both that PROSTVAC-V/F is safe and that further studies of PROSTVAC-V/F in combination with endocrine deprivation should be conducted in the hormone-sensitive nonmetastatic prostate cancer population.

There are two ongoing Phase II studies combining vaccine and endocrine deprivation in metastatic castration-resistant prostate cancer (mCRPC). The first examined PROSTVAC-V/F + enzalutamide, and found that there was no difference in PSA progression between patients receiving PROSTVAC-V/F and those receiving enzalutamide alone; however, further analyses are ongoing. A second Phase II study is investigating the efficacy of the endocrine deprivation agents abiraterone and enzalutamide in mCRPC after patients have progressed on a combination of standard-of-care chemotherapy and PROSTVAC-V/F. Preliminary analysis has found 15/46 (33%) of patients progressed within 1 year of docetaxel and PROSTVAC-V/F, and that subsequent treatment with enzalutamide or abiraterone had limited benefit with no difference between the two.

In biochemically recurrent prostate cancer, there is one ongoing Phase II clinical trial investigating enzalutamide + PROSTVAC-V/F. Early reports found that patients who received enzalutamide alone had a median PSA decline of 99%, and immune analysis of 12 patients indicated that enzalutamide alone increased naive T cells while also decreasing the number of MDSCs. While the vaccine + enzalutamide arm of this trial is not yet evaluable, these preliminary data support further combinations of vaccine and endocrine deprivation.

Based on the strength of these Phase II trials, a Phase III trial was conducted in patients with mCRPC. 432 patients received PROSTVAC-V/F + GM-CSF vs 433 patients receiving placebo, with a primary endpoint of OS. While PROSTVAC-V/F was well tolerated, no difference in OS was reported between the PROSTVAC-V/F arm and the placebo arm.

The data from these trials identify two primary points of contention with the Engage + Expand strategy. First, it is clear that, as with all cancer treatment strategies, the stage of the cancer and point at which treatment is given are irreconcilably important for vaccine efficacy, and likely account for most variability between the different trials reported. Second, these data implicate the paradigm of simple single or double-agent immunotherapeutic strategies and strongly support the need for tactically designed multipronged therapy that targets multiple aspects of immune function. While Engage + Expand ensures that TAA-specific immune cells are present, it does not guarantee that they are active. The presence of inactive or exhausted immune cells is a prevalent issue in immunotherapy, and the ability to Enable inactive cytotoxic immune cells has made immunotherapy the cancer therapy of the new century.

5 ENABLING THE IMMUNE SYSTEM

The development of immune checkpoint blockade drugs was a sea-change moment in immunotherapy, identifying a new therapeutic strategy that has demonstrated efficacy as a monotherapy but also brings enormous potential to multipronged therapeutic strategies. ICB
### TABLE 3 Enabling the immune anti-tumor response

| Clinical Trial #   | Indication                                                                 | Status          | Vaccine            | IO Agent        | Additional treatments | Trial Phase | n  | Outcomes/Key Points                                                                 | Ref. |
|--------------------|-----------------------------------------------------------------------------|-----------------|--------------------|------------------|-----------------------|-------------|----|-------------------------------------------------------------------------------------|------|
| NCT03815942        | Intermediate Risk Prostate Cancer | Recruiting      | ChAdOx1-MVA 5T4    | Nivolumab        |                       | I/II        | 38 | Preliminary results show 5/23 (22%) receiving vaccine + nivolumab had >50% decrease in PSA | 62   |
| NCT03632941        | HER2+ Breast Cancer                                                          | Recruiting      | VRP-HER2           | Pembrolizumab    |                       | II          | 41 | No data                                                                              | 62   |
| NCT03113487        | PD-L1 Positive | Recurrent Fallopian Tube Carcinoma | Recruiting      | MVA-p53          | Pembrolizumab        | II          | 28 | No data                                                                              | 63   |
| NCT03547999        | Metastatic Colorectal Cancer                                                | Recruiting      | CV301              | Nivolumab        | mFOLFOX6             | II          | 80 | No data                                                                              | 64   |
| NCT02933255        | Metastatic Castration-resistant Prostate Cancer                            | Recruiting      | PROSTVAC-V/F       | Nivolumab        |                       | I/II        | 29 | No data                                                                              | 65   |
| NCT04020094        | Prostate Cancer                                                             | Recruiting      | MVA-BN-Brachyury   | Atezolizumab     |                       | II          | 22 | No data                                                                              | 66   |
| NCT03628716        | Bladder Cancer                                                              | Active, not recruiting | CV301              | Atezolizumab     |                       | II          | 70 | No data                                                                              | 65   |
| NCT02506114        | Prostate Cancer                                                             | Active, not recruiting | PROSTVAC-V/F      | Ipilimumab       |                       | II          | 15 | No data                                                                              | 67   |
| NCT03050814        | Metastatic Colorectal Cancer                                                | Active, not recruiting | Ad-CEA            | Avelumab         | Bevacizumab FOLFOX   | II          | 27 | No data                                                                              | 68   |
| NCT00450619        | Metastatic Castration-resistant Prostate Cancer                            | Completed       | PROSTVAC-V/F       | GM-CSF           | Samarium−153 Lexidronam Pentasodium | II          | 46 | Median PFS 3.7 months for Sm−153-EDTMP + PROSTVAC-V/F compared to 1.7 months for Sm−153-EDTMP alone 4/21 pts in combination arm had PSA decline >30% compared to 0/18 in Sm−153-EDTMP alone | 69   |
| NCT00179309        | Breast Cancer                                                               | Completed       | PANVAC-V/F         | GM-CSF           | Docetaxel            | II          | 50 | PANVAC-V/F + docetaxel arm had PFS of 7.9 months vs 3.9 months in docetaxel alone arm | 70   |
| NCT00052351        | Breast Cancer                                                               | Completed       | rF-CEA/TRICOM      | GM-CSF           | Radiation Paclitaxel Doxorubicin Cyclophosphamide | II          | 88 | No data                                                                              | 71   |

(Continues)
agents are the primary mechanism for enabling the immune system, reversing the mechanisms of T-cell exhaustion and allowing infiltrating T cells to have greater and longer efficacy. There are two additional modes of enabling the immune system, metabolic support and radiation/chemotherapy induced immunogenic modulation (Figure 1).

### 5.1 Checkpoint inhibition

The combination of viral vaccines with ICB agents represents an exciting new clinical strategy, and we have identified eight ongoing Phase II trials combining solely viral vaccine and ICB antibodies. There are three ongoing trials combining PROSTVAC-V/F with checkpoint blockade. One combines PROSTVAC-V/F with the anti-CTLA4 antibody ipilimumab, with a primary endpoint examining the proportion of patients with increased CD3+ T-cell infiltration.

Two additional PROSTVAC-V/F trials are currently recruiting, one combining PROSTVAC-V/F with the viral vaccine MVA-BN-Brachyury and anti-PD-L1 antibody atezolizumab, with the primary endpoint of increased CD8+ T-cell infiltration, and the other combining PROSTVAC-V/F with the anti-PD-1 antibody nivolumab in patients with mCRPC to evaluate T-cell infiltration.

There are two Phase II clinical trials that deliver the therapeutic vaccine CV301. Similar to PROSTVAC and PANCVAC, CV301 is a combination of vaccinia and fowlpox-based vaccines delivered on a prime/boost strategy utilizing TRICOM and expressing the TAAs CEA and MUC1. One trial combines CV301 with atezolizumab in patients with bladder cancer with a primary outcome of objective response rate, while the other combines nivolumab with the combinatorial chemotherapy regimen mFOLFOX6 in patients with metastatic colorectal cancer for a primary outcome of OS.

Two additional currently recruiting Phase II trials are also applying vaccines built on the modified vaccinia Ankara virus. The first is treating patients with PD-L1 positive fallopian tube, ovarian or peritoneal cancers, and is treating with an MVA-based vaccine expressing the TAA p53 in combination with pembrolizumab with a primary outcome of response rate. The second utilizes an MVA vaccine encoding the TAA 5T4 in conjunction with Chimpanzee adenovirus OX1 (ChAdOx1-5T4). The combination of ChAdOx1 with MVA is an alternative prime/boost strategy to the vaccinia/fowlpox method. This trial combines ChAdOx1/MVA-5T4 with nivolumab in patients with prostate cancer. While the study is ongoing, of the 23 mCRPC patients evaluable, 5/23 (22%) had a >50% reduction in PSA compared to baseline.

One final Phase II trial is examining the efficacy of VRP-HER2, a vaccine made of alphavirus-like replicon particles expressing HER2, in combination with pembrolizumab in HER2+ breast cancer. As this trial is currently recruiting and ongoing, no data have yet been reported.

### 5.2 Immunogenic modulation

Instead of directly enabling the immune system, it is also possible to promote increased immune efficacy by sensitizing tumor cells directly.
| Clinical Trial # | Indication | Status | IO Agent | Vaccine | Additional Treatment | Ref. | Outcomes/Key Points | Ref. |
|------------------|------------|--------|----------|---------|---------------------|------|---------------------|------|
| NCT03493945     | Metastatic Colorectal Cancer | Recruiting | N-803 | MVA-BN-Baculovirus | FPV-Baculovirus | N-803 | | |
| NCT03185791     | Prostate Cancer | Recruiting | N-803 | MVA-BN-Baculovirus | FPV-Baculovirus | N-803 | | |
| NCT03207048     | Triple Negative Breast Cancer | Recruiting | N-803 | MVA-BN-Baculovirus | FPV-Baculovirus | N-803 | | |
| NCT03207111     | Squamous Cell Carcinoma | Recruiting | N-803 | MVA-BN-Baculovirus | FPV-Baculovirus | N-803 | | |

**TABLE 4**

**Integrated strategies for immunotherapy and Evolution of immunotherapy**

| Clinical Trial # | Indication | Status | IO Agent | Vaccine | Additional Treatment | Ref. | Outcomes/Key Points | Ref. |
|------------------|------------|--------|----------|---------|---------------------|------|---------------------|------|
| NCT03493945     | Metastatic Colorectal Cancer | Recruiting | N-803 | MVA-BN-Baculovirus | FPV-Baculovirus | N-803 | | |
| NCT03185791     | Prostate Cancer | Recruiting | N-803 | MVA-BN-Baculovirus | FPV-Baculovirus | N-803 | | |
| NCT03207048     | Triple Negative Breast Cancer | Recruiting | N-803 | MVA-BN-Baculovirus | FPV-Baculovirus | N-803 | | |
| NCT03207111     | Squamous Cell Carcinoma | Recruiting | N-803 | MVA-BN-Baculovirus | FPV-Baculovirus | N-803 | | |

**Next Gen IO**

| Function | Description | Ref. |
|----------|-------------|------|
| HDACi    | HDACi Epigenetic modulator Enables immune response through immunogenic modulation | 83   |
| NHS-I12  | NHS-I12 IL-2 fused to anti-histone antibody Enables immune response through immunogenic modulation | 84   |
| anti-IL-8 receptor | anti-IL-8 receptor binding antibody Enables tumor progression/immune escape | 85   |
| PDL1-N803 fusion | PDL1-N803 fusion Enables immune response through checkpoint blockade, Expands TIL | 86   |
| DDRi    | DDRi Inhibits DNA damage response Enables immune response through immunogenic modulation | 87,88 |
| OX40 Agonist | OX40 Agonist Costimulatory molecule Expands immune response | 89   |
| 41BB Agonist | 41BB Agonist Costimulatory molecule Expands immune response | 89   |
| anti-TIGIT | anti-TIGIT Checkpoint inhibitor Blocks suppression of T cells | 91   |
| anti-LAG-3 | anti-LAG-3 Checkpoint inhibitor Blocks suppression of T cells | 91   |
| anti-Siglec-15 | anti-Siglec-15 Checkpoint inhibitor Blocks suppression of T cells | 92   |
| anti-LAIR-1 | anti-LAIR-1 Blocks suppression of T cells | 93   |

**Next Gen IO Description Function Ref.**

| Function | Description | Ref. |
|----------|-------------|------|
| HDACi    | HDACi Epigenetic modulator Enables immune response through immunogenic modulation | 83   |
| NHS-I12  | NHS-I12 IL-2 fused to anti-histone antibody Enables immune response through immunogenic modulation | 84   |
| anti-IL-8 receptor | anti-IL-8 receptor binding antibody Enables tumor progression/immune escape | 85   |
| PDL1-N803 fusion | PDL1-N803 fusion Enables immune response through checkpoint blockade, Expands TIL | 86   |
| DDRi    | DDRi Inhibits DNA damage response Enables immune response through immunogenic modulation | 87,88 |
| OX40 Agonist | OX40 Agonist Costimulatory molecule Expands immune response | 89   |
| 41BB Agonist | 41BB Agonist Costimulatory molecule Expands immune response | 89   |
| anti-TIGIT | anti-TIGIT Checkpoint inhibitor Blocks suppression of T cells | 91   |
| anti-LAG-3 | anti-LAG-3 Checkpoint inhibitor Blocks suppression of T cells | 91   |
| anti-Siglec-15 | anti-Siglec-15 Checkpoint inhibitor Blocks suppression of T cells | 92   |
| anti-LAIR-1 | anti-LAIR-1 Blocks suppression of T cells | 93   |

**Next Gen IO Description Function Ref.**

- 5-FU, 5-fluorouracil; aNK, activated natural killer cells; DDRi, DNA damage response inhibitor; FPV, fowlpox virus; GITR, glucocorticoid-induced tumor necrosis factor receptor; hNk, high avidity natural killer cells; HDACi, histone deacetylase inhibitor; IL, interleukin; IO, immune-oncology; LAG-3, lymphocyte activation gene-3; LAIR-1, leukocyte-associated immunoglobulin-like receptor; MVA, modified virus Ankara; NB, nanoparticle; SBRT, stereotactic body radiation therapy; Siglec-15, sialic acid-binding immunoglobulin-like lectin-15; TIGIT, T cell immunoglobulin and mucin domain; TIL, tumor-infiltrating lymphocytes.
While cytolytic cancer therapies aim to completely remove the tumor, most are not capable of killing all cancer cells. However, when patients who recurred following chemotherapy and radiation therapy were put on immunotherapy, it was observed that they had a higher clinical benefit than those who had not been previously treated. These clinical observations and the subsequent preclinical investigations have demonstrated that certain chemotherapies, radiation doses and new anticancer agents are capable of changing the phenotype of tumor cells to make them more sensitive to immune cell killing. This process is called immunogenic modulation, and has been demonstrated with sublethal doses of multiple radiotherapies, chemotherapies, endocrine deprivation, as well as certain cytotoxic small molecules. Several Phase II clinical trials supporting immunogenic modulation have been completed. In mCRPC, a recent trial examined the combination of PROSTVAC-V/F and GM-CSF with Samarium-153-ethylene diamine tetramethylene phosphonate (SM-153-EDTMP), a beta-particle emitting radiopharmaceutical that is preferentially absorbed by osteoblastic bone lesions. It was shown that patients receiving PROSTVAC-V/F in combination with SM-153-EDTMP/GM-CSF had PFS of 3.7 months, compared to 1.7 months in the SM-153-EDTMP alone group. Additionally, 4/21 (19%) patients in the combination group had a PSA decline >30%, whereas 0/18 did when treated with SM-153-EDTMP alone.

Two Phase II studies have been completed in breast cancer. One utilizing PANVAC-V/F and one a prime/boost of rF-CEA/TRICOM and rV-CEA/TRICOM. In patients with metastatic breast cancer, PANVAC-V/F was combined with GM-CSF and docetaxel. It was shown that the combination with docetaxel resulted in PFS of 7.9 months, as opposed to 3.9 months in the docetaxel arm alone. 11/16 (69%) of evaluable patients in the combination arm developed T-cell responses to CEA or MUC1 compared to 8/15 (53%) in the docetaxel alone arm. The trial treating with rF-CEA/TRICOM +rV-CEA/TRICOM combined the vaccine with GM-CSF and cyclophosphamide, doxorubicin, paclitaxel, and radiation therapy. However, while it is complete, no data have been reported.

One Phase II clinical trial has looked at the efficacy of vaccine in malignant pleural mesothelioma. This study used the MVA-ST4 vaccine TroVax in combination with the chemotherapies pemetrexed and cisplatin. It achieved its clinical endpoint of generating an immune response, reporting that 22/23 (95.6%) of patients developed an immune response to TAA ST4 post-vaccination. 17/23 (74%) mounted humoral responses, and 20/23 (87%) patients developed ≥2x cellular immune response compared to baseline.

In addition to these completed trials, one currently ongoing study in colorectal cancer is combining checkpoint inhibition with other enabling agents. This trial is utilizing the adenoviral vaccine Ad-CEA and additionally treating with the anti-PD-L1 antibodies avelumab, FOLFOX, and bevacizumab.

One therapeutic viral vaccine has entered Phase III in combination with enabling agents. Building off of successful monotherapy Phase II trials (Table 1), the poxvirus vaccine expressing the TAA ST4 TroVax completed a Phase III trial in clear cell renal carcinoma combining vaccine with cytokines IL-2 and IFNα and the small molecule tyrosine kinase inhibitor sunitinib. Unfortunately, no significant difference in OS was detected between the treatment and placebo arms. However, patients who demonstrated a high ST4-specific antibody response showed favorable survival compared to placebo, indicating that the vaccine may be efficacious in certain patients.

5.3 | Metabolic support

Similar to checkpoint inhibition, metabolic support targets the immune system. Depending on the make-up of the tumor microenvironment, cancer cells can deprive the TME of glucose and increase levels of lactate in the TME, both of which have been shown to inhibit the functions of TILs. It is possible that these could be addressed in part through “metabolic tuning” of T cells ex-vivo prior to re-introduction during adoptive immune therapy. Alternatively, metformin, a drug that changes mitochondrial respiration, has been shown to promote TIL function, indicating its potential as an immunomodulation modulating therapeutic agent in the clinic.

Another metabolic target is the cytosolic enzyme indoleamine 2,3-dioxygenase-1 (IDO1), which has been shown to have immunosuppressive effects. Widely expressed in human tumors, IDO1 catalyzes tryptophan, a necessary metabolite for T-cell function. In addition to depriving T cells of tryptophan, the catabolites of tryptophan produced by IDO1 induce T-cell apoptosis, block T-cell activation and trigger differentiation of immunosuppressive T-regulatory cells. Several IDO1 inhibitors are in development and have demonstrated sustained preclinical efficacy. They have been generally well tolerated in Phase I trials, and multiple Phase II trials are ongoing either utilizing IDO1-inhibitor monotherapy or in combination with other agents such as checkpoint inhibitors. Additionally, one Phase II trial is ongoing combining the IDO1-inhibitor epacadostat with viral-based vaccines (Table 3).

With several Phase II clinical trials already completed using enabling agents, and more ongoing trials capitalizing on the newest iteration in the form of immune checkpoint antibodies, it is clear that combining agents that Engage with those that Enable will continue to be a fruitful clinical strategy moving forward. Moreover, many of the trials described above also include agents to Expand the immune system, either cytokines, costimulatory molecules or both. However, there are concurrent trials that are not hesitating to move past combining one or two IO agents toward true combination immunotherapy.

6 | INTEGRATED STRATEGIES FOR IMMUNOTHERAPY

Similar to the path followed by chemotherapy in the 20th century, the endgame of immunotherapy will be tactically designed multi-agent combinations that target all functions of the immune system to create an ideal anti-tumor immune microenvironment. These
novel studies are just beginning and combine multiple classes of IO agents and standard-of-care treatment strategies.

Two recruiting Phase I/II trials are combining vaccine with the new IO agents bintrafusp alfa and N-803. Bintrafusp alfa is a bifunctional TGF-β Trap-anti-PD1 fusion protein, combining the checkpoint inhibition capabilities of anti-PD-L1 antibodies with the immunomodulatory effects of TGF-β inhibition. It has been demonstrated to synergize well with vaccine in vivo; a recent Phase I trial demonstrated its safety and had encouraging early results. N-803 is an IL-15 superagonist that has been demonstrated to promote the development of natural killer (NK) and T cells. In vivo N-803 in combination with anti-PD-L1 significantly decreased tumor size while increasing immune cell cytotoxicity.

One Phase II trial is focusing on prostate cancer and treating patients with a combination of PROSTVAC-V/F, CV301, bintrafusp alfa and N-803, with a primary endpoint of 30% decline in PSA. An additional Phase I/II trial is utilizing the novel Quick efficacy seeking trial (QuEST1) design to verify the safety of 4 IO agents: Prime/boost of MVA-BN-Brachyury/FPV-Brachyury, bintrafusp alfa, N-803, and the IDO-1 inhibitor epacadostat. Multiple arms will determine safety of an expanding combination of these agents, beginning in mCRPC patients.

There are seven additional trials in colorectal cancer, pancreatic cancer, triple negative breast cancer, and squamous cell carcinoma. These trials all utilize different combinations of the same extensive group of standard-of-care and IO agents (Table 4) and are attempting high-number combinations in patients. Of note, patients will be vaccinated with combinations of adenoviral (ETBX, previously utilized in monotherapy trials listed in Table 1) and yeast (GI-) vaccines expressing the correct TAA for their tumor type, and treated with the anti-PD-L1 antibody avelumab, N-803 and/or allogenic cell transfer of the high-avidity NK cell line haNK.

7 | THE EVOLUTION OF IMMUNOTHERAPY

While these true multi-agent trials are the next step in immuno-therapeutic strategies, they are not possible without an expanding selection of IO agents targeting diverse immune system functions and immune tumor interactions. There are numerous novel agents in development, a subset of which are listed in Table 4. These agents include novel costimulatory molecules that expand the immune response, such as agonists 41BB and OX40, second-line checkpoint inhibitors that engage the immune response including anti-TIGIT, -LAG-3, and -GITR, therapeutic antibodies that block immunosuppressive mechanisms like anti-Siglec-15, anti-LAIR-1, and anti-IL-8-receptor, targeted cytokine molecules such as NHS-IL-12, new agents that induce immunogenic modulation through mechanisms such as histone deacetylase (HDAC) inhibition and DNA damage response inhibition and new fusion molecules including the anti-PD-L1/N-803 fusion molecule N-809 that combine multiples of these functions together in order to make high-level combinatorial therapy more manageable and improve patient welfare.

In addition to the continued evolution of IO agents, it is clear that immunotherapies need to induce an evolution of the anti-cancer immune response within the patient. It has been clear for decades that immunotherapy is in part effective due to the process of antigen cascade (also known as antigen spreading). This occurs when an Engaged and Enabled immune system results in tumor cell death and tumor antigen uptake and presentation, either mediated by T-cell cytolysis or through radiation/chemotherapy-induced immunogenic cell death. These innate tumor antigens may be more efficacious than those delivered through engaging agents and also allow for a broad, evolving immune response within a patient beyond the response induced by treatment with IO agents. Antigen cascade has been observed in both the preclinical and clinical setting, and further research into the most effective methods of inducing an evolving immune response within individual patients is clearly necessary.

The broad applicability of the most commonly used therapeutic viral vaccine platforms and the ability to design them with the appropriate TAAs to target diverse cancers make them a highly attractive therapeutic strategy for engaging the immune system. With decades of mono- and minimal-combination vaccine clinical trials completed and a rapidly evolving slate of IO agents in development, it is clear that the coming decades of immunotherapy will see tactically designed multicomination clinical trials utilizing agents that Engage, Expand and Enable the immune system (Figure 1). With therapeutic viral vaccines as a foundation, we are optimistic that these evolving clinical strategies will result in effective therapy of established tumors.

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