Published OnlineFirst May 20, 2019; DOI: 10.1158/1078-0432.CCR-19-0183

Clinical Trials: Immunotherapy

Clinical Cancer Research

A Phase I Dose-Escalation Trial of BN-CV301, a Recombinant Poxviral Vaccine Targeting MUC1 and CEA with Costimulatory Molecules

Margaret E. Gatti-Mays[1], Julius Strauss[1], Renee N. Donahue[1], Claudia Palena[1], Jaydira Del Rivero[2], Jason M. Redman[3], Ravi A. Madan[4], Jennifer L. Marté[4], Lisa M. Cordes[5], Elizabeth Lamping[1], Alanvin Orpia[6], Andrea Burmeister[6], Eva Wagner[7], Cesar Pico Navarro[8], Christopher R. Heery[8], Jeffrey Schlom[1], and James L. Gulley[4]

Abstract

Purpose: BN-CV301 is a poxviral-based vaccine comprised of recombinant (rec.) modified vaccinia Ankara (MVA-BN-CV301; prime) and rec. fowlpox (FPV-CV301; boost). Like its predecessor PANVAC, BN-CV301 contains transgenes encoding tumor-associated antigens MUC1 and CEA as well as costimulatory molecules (B7.1, ICAM-1, and LFA-3). PANVAC was reengineered to make it safer and more antigenic.

Patients and Methods: This open-label, 3+3 design, dose-escalation trial evaluated three dose levels (DL) of MVA-BN-CV301: one, two, or four subcutaneous injections of 4×10^8 infectious units (Inf.U)/0.5 mL on weeks 0 and 4. All patients received FPV-CV301 subcutaneously at 1×10^9 Inf.U/0.5 mL every 2 weeks for 4 doses, then every 4 weeks. Clinical and immune responses were evaluated.

Results: There were no dose-limiting toxicities. Twelve patients enrolled on trial [dose level (DL) 1 = 3, DL2 = 3, DL3 = 6). Most side effects were seen with the prime doses and lessened with subsequent boosters. All treatment-related adverse events were temporary, self-limiting, grade 1/2, and included injection-site reactions and flu-like symptoms. Antigen-specific T cells to MUC1 and CEA, as well as to a cascade antigen, brachyury, were generated in most patients. Single-agent BN-CV301 produced a confirmed partial response (PR) in 1 patient and prolonged stable disease (SD) in multiple patients, most notably in *KRAS*-mutant gastrointestinal tumors. Furthermore, 2 patients with *KRAS*-mutant colorectal cancer had prolonged SD when treated with an anti-PD-L1 antibody following BN-CV301.

Conclusions: The BN-CV301 vaccine can be safely administered to patients with advanced cancer. Further studies of the vaccine in combination with other agents are planned.

See related commentary by Repáraz et al., p. 4871

Introduction

BN-CV301 is a poxviral-based vaccine comprised of recombinant modified vaccinia Ankara (MVA-BN-CV301; prime) and recombinant fowlpox (FPV-CV301; boost). BN-CV301 contains transgenes encoding two tumor-associated antigens (TAA), mucin 1 (MUC1) and carcinoembryonic antigen (CEA), as well as three costimulatory molecules (B7.1, ICAM-1, and LFA-3, designated TRICOM). CEA and MUC1 have been identified on numerous adenocarcinomas including colorectal cancer, breast cancer, non–small cell lung cancer (NSCLC), bladder cancer, and pancreatic cancer.

The transmembrane MUC1 C-terminal (MUC1-C) has been shown to induce an epithelial–mesenchymal transition (EMT) leading to stemness, drug resistance, and immune evasion (1–3). MUC1-C also acts as an oncogene and overexpression is associated with a poor prognosis (4–7). Several studies have demonstrated that MUC1-C interacts with various receptor tyrosine kinases (i.e., EGFR and HER2) and promotes activation and downstream pathways (1, 2, 8). Furthermore, MUC1-C overexpression has been associated with immune evasion from anti-PD-L1 agents in triple-negative breast cancer and NSCLC (3, 9).

An earlier version of BN-CV301, PANVAC, has been evaluated in the preclinical and clinical settings. Preclinical studies have demonstrated that PANVAC activated antigen-specific human T cells *in vitro* (10), cellular immune responses *in vivo*, and antitumor efficacy against tumors expressing either human MUC1 or CEA in mice. Phase I and II clinical trials involving PANVAC as a single agent, or in combination with granulocyte macrophage colony-stimulating factor (GM-CSF) ± chemotherapy, demonstrated an

[1]Laboratory of Tumor Immunology and Biology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland. [2]Pediatric Oncology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland. [3]Medical Oncology Service, National Cancer Institute, National Institutes of Health, Bethesda, Maryland. [4]Genitourinary Malignancies Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland. [5]Oncology Clinical Pharmacy, National Cancer Institute, National Institutes of Health, Bethesda, Maryland. [6]Leidos Biomedical Research, Inc., Frederick, Maryland. [7]Bavarian Nordic GmbH, Martinsried, Germany. [8]Bavarian Nordic, Inc., Morrisville, North Carolina.

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

M.E. Gatti-Mays and J. Strauss contributed equally to this article. J. Schlom and J.L. Gulley contributed equally to this article.

Corresponding Author: James L. Gulley, Center for Cancer Research, National Cancer Institute, National Institutes of Health, 10 Center Drive, 13N240, Bethesda, MD 20892. Phone: 301-408-8870; Fax: 240-541-4575; E-mail: gulleyj@mail.nih.gov

Clin Cancer Res 2019;25:4933–44

doi: 10.1158/1078-0432.CCR-19-0183

Translational Relevance

BN-CV301 is a poxviral-based vaccine containing transgenes encoding for MUC1 and CEA. The predecessor of BN-CV301, PANVAC, was modified to improve safety and immune responses. The amino acid sequences in the CEA and MUC1 tumor-associated antigens, including those in the MUC1-C region, were modified to produce a stronger immune response. The C-terminal transmembrane unit of MUC1 (MUC1-C) is important for cell growth and cell survival, and functions as an oncoprotein. In this trial, BN-CV301 activated $CD8^+$ and $CD4^+$ T cells against MUC1 and CEA and to a cascade antigen, brachyury. Furthermore, we saw preliminary evidence of clinical efficacy in colorectal cancer, a traditionally "cold" tumor, both as a single agent and followed by an anti-PD-L1 antibody. This phase I trial demonstrated that the BN-CV301 vaccine can be safely administered to patients with advanced cancer. Further trials of the vaccine in combination with other agents are planned.

acceptable safety profile with some hints of clinical benefit (11–14). Treatment with PANVAC was well tolerated with only low-grade injection-site reactions and transient flu-like symptoms (15). The pilot trial of PANVAC had four prolonged responders (1 patient with colorectal cancer, 1 patient with appendiceal cancer, and 2 patients with ovarian cancer), but multiple patients had prolonged survival after coming off trial and several patients had unexpected clinical responses to subsequent therapies (15). On the basis of this pilot trial there was an expansion cohort for patients with breast or ovarian cancer, again resulting in a few prolonged responders. One patient with breast cancer continues to have a complete response ongoing for 9.5 years and received a total of 55 PANVAC vaccines with concurrent trastuzumab prior to being taken off trial due to lack of available vaccine (13, 16). Encouraging responses in patients with breast cancer led to a small randomized, controlled phase II trial showing that docetaxel + PANVAC resulted in longer progression-free survival than docetaxel alone (7.9 months vs. 3.9 months, respectively; ref. 12). Furthermore, in a phase II trial, patients with complete resection of oligometastatic colorectal cancer who received PANVAC had improved survival compared with unvaccinated historical and contemporary controls (14). Collectively, these trials demonstrated preliminary evidence of clinical efficacy of PANVAC as an adjunct to other therapies.

To improve safety and immune response, PANVAC was modified in several ways, resulting in the new version of the vaccine, called BN-CV301. Like its predecessor PANVAC, BN-CV301 utilizes a prime-boost dosing regimen. Because of the replication competency of the vaccinia vector employed in the priming dose of PANVAC, its administration offered particular risks to specific segments of the general population, including immunocompromised individuals and persons diagnosed with atopic dermatitis (17–19). In addition, traditional replication-competent smallpox vaccines are known to carry the risk of rare but serious cardiac complications, including myo/pericarditis. Considering these potential safety issues, a second-generation cancer immunotherapy strategy using an attenuated vaccinia virus, MVA, was developed. MVA-BN can infect mammalian cells and express transgenes, but it cannot produce infective viral particles, alleviating most of the safety concerns of PANVAC. More than 13,000 patients have been vaccinated with various MVA-BN–based infectious disease vaccines and no inflammatory cardiac adverse reactions have been reported. The expected improved safety profile has allowed the dosing regimen to be changed for BN-CV301 compared with PANVAC. Specifically, priming doses of MVA-BN-CV301 were given in four separate injection sites, resulting in a higher total virus dose and potential for exposure to a greater number of dendritic cells (DC) in four draining lymph node regions. In addition, two priming doses of MVA-BN-CV301 were given prior to switching to heterologous boosting with FPV-CV301, which is then given for a longer duration than in some previous trials with PANVAC.

The transgenes in the original PANVAC vectors were modified to contain one $CD8^+$ enhancer epitope for CEA (10, 20) and one $CD8^+$ enhancer for MUC1 in the VNTR region of the molecule (21). The BN-CV301 vectors contain additional MUC1 enhancer epitopes, especially in the MUC1-C region. These enhancer epitopes have been described in detail previously (22) and span HLA-A2, HLA-A3 and HLA-A24 MHC Class I alleles, which encompass the majority of the population. All of the CEA and MUC1 agonist epitopes in BN-CV301 were also shown to enhance T-cell responses and subsequent tumor lysis versus the use of the corresponding native epitopes (20–22). Consequently, BN-CV301 is expected to promote a stronger antigen-specific targeted immune response in comparison with PANVAC.

The primary objective of this phase I trial was to assess the safety of BN-CV301 and to identify the recommended dose of BN-CV301 for use in future clinical trials.

Patients and Methods

Preclinical evaluation of antigenicity

Culture and infection of DCs. Peripheral blood mononuclear cells (PBMC) were separated from heparinized blood of HLA-A2 healthy donors obtained from the NIH Blood Bank by centrifugation on a Ficoll density gradient (Lymphocyte Separation Medium, LSM, MP Biomedicals). DCs were prepared using a modification of a previously published procedure (23); briefly, PBMCs (1.5×10^8) were resuspended in 50 mL AIM-V medium (Thermo Fisher Scientific) and allowed to adhere to T-150 flasks (Corning Costar Corp.). After 2 hours at 37°C, the nonadherent cells were removed with a gentle rinse. Adherent cells were cultured for 6 to 7 days in AIM-V medium containing 100 ng/mL of recombinant human (rh) GM-CSF and 20 ng/mL of recombinant human IL4 (rhIL4). The culture medium was replenished every 3 days. For infection, DCs (1×10^6) were incubated in 1 mL of Opti-MEM medium (Thermo Fisher Scientific) at 37°C with MVA-BN-CV301, FPV-CV301, or the control empty vectors, MVA-wild type (WT) and FPV-WT. DCs were infected for 2 hours with FPV-based constructs at a multiplicity of infection (MOI) of 20 or 40, and for 1 hour with MVA-based constructs at an MOI of 5 or 10, followed by the addition of 10 mL of fresh, warmed RPMI1640 complete medium containing 100 ng/mL of rhGM-CSF and 20 ng/mL of rhIL4. After 24 hours, DCs were analyzed by flow cytometry or used as antigen-presenting cells (APC) for *in vitro* stimulation of human antigen-specific T cells.

FACS analysis of infected DCs. Flow cytometry was performed on infected DCs using phycoerythrin (PE)-labeled antibodies against human B7.1 (CD80), ICAM-1 (CD54), and LFA-3 (CD58) or a control isotype IgG (BD Biosciences). The anti-CEA mAb COL-1 (24) and anti-MUC1 antibodies DF3 and DF3-P (25, 26) were also used.

Activation of human CEA- and MUC1-specific T cells. For T-cell stimulation, DCs (2×10^4) were coincubated with HLA-A2–restricted T-cell lines specific for CEA or two different epitopes of MUC1, at a ratio of T-cell-to-DCs of 1:10. Culture supernatants were collected at 24 hours and evaluated for secretion of IFNγ by ELISA (BioSource International).

Clinical

Patients. Patients were eligible for trial if they had evaluable (not necessarily measurable) metastatic or unresectable locally advanced solid tumors with no known curative therapy. When available, *KRAS* status was determined using historical genomic profile reports (i.e., FoundationOne, Caris). Patients with surgically resected or ablated metastatic disease at high risk of relapse were also eligible. Patients must have completed at least one prior line of standard therapy at least 4 weeks prior to enrollment on trial, with resolution of any grade $\geq$2 adverse events (AE). Patients could continue maintenance therapy where appropriate (i.e., endocrine therapy for estrogen receptor–positive/progesterone receptor–positive breast cancer; HER2-targeted therapy for $HER2^+$ breast cancer, capecitabine $\pm$ bevacizumab for colorectal cancer; erlotinib for EGFR-mutated NSCLC; androgen deprivation therapy for prostate cancer) as long as the patient had been receiving this treatment $\geq$2 months prior to the start of trial treatment. Patients were required to be $\geq$18 years old with a good performance status (Eastern Cooperative Oncology Group 0–1) and normal organ function. Exclusion criteria included chronic infection, including hepatitis B or C and HIV, active brain metastases, leptomeningeal disease, autoimmune disorders of clinical significance, concurrent systemic corticosteroids (physiologic doses defined as $\leq$ prednisone 5 mg per day or equivalent allowed), history of allergic reaction to components of vaccines, serious uncontrolled medical issues, and pregnancy.

Trial design and oversight. This phase I dose-escalation trial followed a standard 3+3 design to demonstrate the safety and immunogenicity of the BN-CV301 vaccine in patients with advanced solid malignancies (NCT02840994). Three vaccine dose levels (DL) of the priming dose of MVA-BN-CV301 were evaluated with patients receiving one (DL1), two (DL2), or four (DL3) injections. Each of these injections contained 4×10^8 infectious units per 0.5 mL (Inf.U/0.5 mL) and was injected in a different arm or leg. After two priming doses at week 0 and week 4, a booster dose of the FPV-CV301 (1×10^9 Inf.U/0.5 mL) was given subcutaneously at one site every 2 weeks from week 8 to week 14, every 4 weeks from week 18 to week 50 and every 13 weeks in all three dose levels (Fig. 1A).

The phase Ib component of this trial is ongoing and is evaluating the safety and tolerability of BN-CV301 + anti-PD-1 therapy (nivolumab or pembrolizumab) in patients with NSCLC who relapsed after or who are refractory to first-line chemotherapy. This phase I trial was conducted in accordance with the Declaration of Helsinki after approval by the Scientific Review Committee and Institutional Review Board (IRB) of the Intramural NCI and the Center for Cancer Research, NCI (Bethesda, MD). All patients provided informed written consent. This trial was sponsored by Bavarian Nordic and the NCI. Ongoing safety oversight was conducted by the IRB and an appointed Safety Monitoring Team. Any serious AEs were reported to the U.S. Food and Drug Administration for review, per guidelines. Informed consent was obtained from each participant, including consent for treatment, primary and secondary endpoints, and correlative studies.

Safety assessment. All patients were monitored for dose-limiting toxicities (DLT) during the 7 days after the first dose of BN-CV301. On day 7, patients were called by trained research staff to elicit symptoms of interest. A 12-lead EKG was performed at baseline, as well as during the week 2 clinic visit. Safety was assessed on the basis of reported AEs through time on trial. Reported AEs were graded according to the NCI Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 4.03. Treatment-emergent AEs occurring from the first dose of BN-CV301 through 30 days after the last dose of BN-CV301 were recorded. All BN-CV301–related serious AEs or AEs of special interest were collected until 100 days following administration of the last dose of BN-CV301.

Clinical assessment. All patients were assessed in the clinic every 2 weeks $\pm$ 3 days for the first 14 weeks on trial, every 4 weeks $\pm$ 7 days through week 50, then every 13 weeks $\pm$ 7 days for the duration of treatment. Routine laboratory tests and targeted physical exams were performed at each time point. Tumor markers were also monitored as appropriate. Restaging with CT of the chest, abdomen, and pelvis was performed at baseline, week 6, week 12, week 18, every 8 weeks until week 50 and then every 13 weeks using RECIST 1.1 criteria to assess the response.

Exploratory analyses. PBMCs collected from patients before and during treatment were separated by Ficoll-Hypaque density gradient centrifugation and cryopreserved in human AB serum and 10% DMSO (1×10^7 cells/mL) and stored in liquid nitrogen until assayed. Antigen-specific responses were assessed by intracellular cytokine staining following a period of *in vitro* stimulation (IVS) with overlapping 15-mer peptide pools encoding MUC1 and CEA, as well as the cascade antigen brachyury, as described previously (27). The TAA peptide pools contain agonist epitopes that had previously been identified (20, 22). Antigen-specific responses to *KRAS* were assessed in patients where the specific mutation was known and compared with WT *KRAS*. Peptide pools encoding for HLA and CEFT (a mixture of peptides of cytomegalovirus, Epstein–Barr virus, influenza, and tetanus toxin) served as negative and positive controls, respectively. Peptide mixes were purchased from JPT and GenScript, reconstituted in DMSO, and used immediately.

Cryopreserved PBMCs from patients before therapy and on weeks 6 (2 weeks after second MVA-BN-CV301 prime), 10 (2 weeks after first FPV-CV301 boost) and 18 or 22 (4 weeks after fourth or fifth FPV-CV301 boost, where available) were assayed as described previously (27). Using a BD Fortessa flow cytometer equipped with a UV, violet, blue, red, and yellow/green laser, 3×10^5 events in the live gate were acquired. FCS files were analyzed with FlowJo v9.7 for Macintosh (TreeStar). Nonviable cells were excluded, and fluorescence minus one controls were used for gating. The absolute number of $CD4^+$ or $CD8^+$ T lymphocytes producing cytokine or positive for the degranulation marker CD107a was calculated per 1×10^6 cells plated at the start of the IVS. The background signal (obtained with the HLA peptide pool) and any value obtained

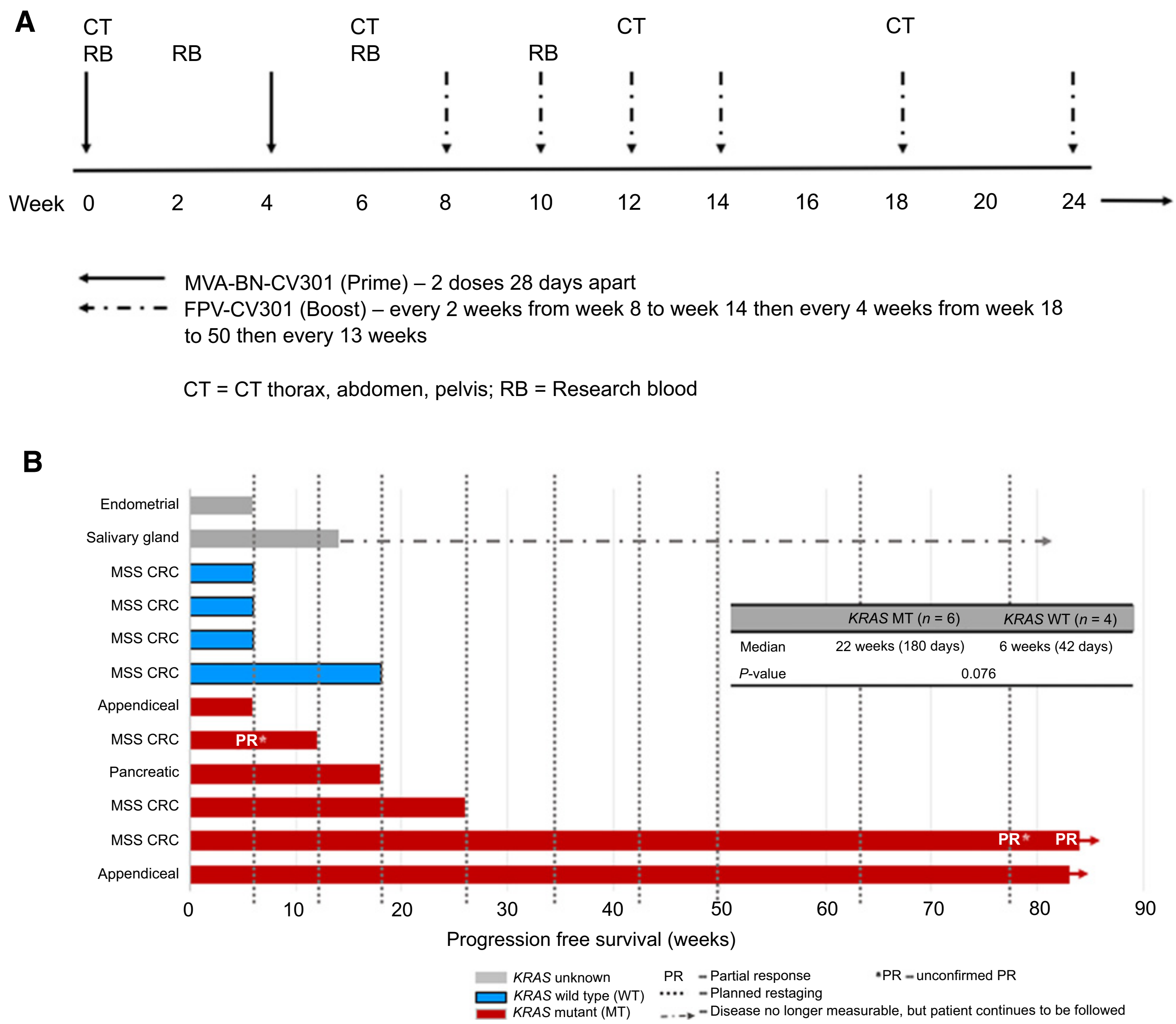


Figure 1.

BN-phase I schema and clinical outcomes. **A,** BN-CV301 phase I study schema. **B,** Progression-free survival on BN-CV301 for individual patients by *KRAS* status. *Post hoc KRAS* analysis performed with an exact two-tailed log-rank test suggests there is a trend toward a difference by *KRAS* status but is limited by sample size. MSS CRC, microsatellite-stable colorectal cancer.

prior to vaccination were subtracted from those obtained after vaccination ([post-TAA – post-HLA] – [pre-TAA – pre-HLA]). A response to each TAA was scored as positive if a patient had more than 250 $CD4^+$ or $CD8^+$ T cells that produced IFNγ, TNF, IL2, or were positive for CD107a at the end of the stimulation assay per 1×10^6 cells that were plated at the start of the assay.

Statistical analysis

This was a dose-escalation phase I clinical trial. Patients with presumed CEA/MUC1-expressing tumors were enrolled using a 3+3 design. Descriptive statistics are reported throughout the article. Because of differing clinical outcomes noted among patients with *KRAS*-mutant and WT cancers, an exploratory, *post hoc* analysis of the impact of *KRAS* mutation status on clinical outcome was evaluated using an exact two-tailed log-rank test with an *a priori P* value of significance < 0.05.

Results

Preclinical antigenicity

MVA-BN-CV301– and FPV-CV301–infected DCs stimulate CEA- and MUC1-specific $CD8^+$ T cells. The ability of the recombinant BN-CV301 vectors to infect human DCs *in vitro* was first evaluated. As shown in Supplementary Table S1A, expression of the encoded costimulatory molecules B7.1, ICAM-1, and LFA-3 was markedly enhanced in MVA-BN-CV301 and FPV-CV301 versus the corresponding MVA-WT– and FPV-WT–infected cells, respectively. Expression of the encoded CEA and MUC1 tumor antigens was observed with both MVA-BN-CV301 and FPV-C301 vectors above the low levels observed in control-infected or uninfected DCs.

MVA-BN-CV301– and FPV-CV301–infected human DCs were subsequently used *in vitro* as APCs to stimulate HLA-A2–restricted human $CD8^+$ T cells specific for an epitope of CEA (T-CEA; ref. 28)

Published OnlineFirst May 20, 2019; DOI: 10.1158/1078-0432.CCR-19-0183

Table 1. Patient demographics ($n = 12$)

	***n* (%)**
Female	6 (50.0%)
Age in years, mean (range)	56.0 (39–77)
Race	
White	6 (50.0%)
Black	3 (25.0%)
Other	3 (25.0%)
Ethnicity: Hispanic	1 (8.3%)
Tumor type	
MSS colorectal cancer	7 (58.3%)
Appendiceal cancer	2 (16.7%)
Pancreatic cancer	1 (8.3%)
Endometrial cancer	1 (8.3%)
Salivary gland cancer	1 (8.3%)
Metastatic at diagnosis	6 (50.0%)
Cancer treatment	
Prior number of regimens, mean (range)	4.2 (1–8)
Patients with prior immunotherapy	6 (50.0%)
Patients on maintenance chemotherapy	1 (8.3%)

NOTE: *n* (%) unless otherwise stated.

or two distinct T-cell lines directed against an epitope of MUC1 (T-MUC1) or an epitope located on the C-terminal region of MUC1 (T-MUC1-C; ref. 22). As shown in Supplementary Table S1B, MVA-BN-CV301 and FPV-CV301 were equally efficient at stimulating T-cell lines directed against CEA or MUC1 epitopes, as denoted by the secretion of high levels of IFNγ, compared with the levels observed with control MVA-WT– or FPV-WT–infected DCs.

Altogether these results demonstrated that MVA-BN-CV301 and FPV-CV301 are able to efficiently infect and direct the expression of the encoded transgenes CEA, MUC1, B7.1, ICAM-1, and LFA-3 in human DCs. Moreover, the antigens CEA and MUC1 encoded by the vectors are being processed and presented in the context of MHC class I molecules, leading to the effective stimulation of antigen-specific $CD8^+$ T cells.

Patient demographics

In total, 12 patients were enrolled on trial between December 2016 to May 2017 (DL1, $n = 3$; DL2, $n = 3$; DL3, $n = 6$). The data cutoff date for analysis was December 11, 2018. At this time, 2 patients remain on trial and receive FPV-CV301 every 13 weeks.

Half of patients enrolled were female, and median age was 56 years (Table 1). Because of referral patterns, most patients had a gastrointestinal tumor, including 7 patients with microsatellite-stable (MSS) colorectal cancer, 3 of whom had *KRAS* mutations and 4 were *KRAS* WT. Two patients had appendiceal cancer (>50% mucinous; both with *KRAS* mutations), and 1 patient had pancreatic cancer (presumed *KRAS* mutation because >90% of pancreatic cancer patients have *KRAS* mutations; ref. 29). Other patients enrolled had endometrial cancer ($n = 1$) and salivary gland cancer ($n = 1$); *KRAS* status was unknown. Half of patients were initially diagnosed with cancer in the metastatic setting (*de novo*). Patients had a mean of 4.2 prior regimens in the advanced cancer setting. Fifty percent of patients had at least one prior therapy qualifying as immunotherapy (i.e., prior therapeutic cancer vaccine, cytokine or anti-PD-1/L1 agent). One appendiceal cancer patient remained on maintenance standard therapy with capecitabine + bevacizumab while receiving BN-CV301. All patients enrolled were evaluable for clinical, safety, and immune responses.

Toxicity

BN-CV301 was well tolerated with no DLTs (Supplementary Table S2). The maximum tolerated dose was not reached. The recommended phase II dose (R2PD) is DL3 of MVA-BN-CV301 (four injections of 4×10^8 Inf.U/0.5 mL, each one administered in a different arm and leg) followed by the FPV-CV301 dose of 1×10^9 Inf.U/0.5 mL. No deaths occurred on trial. There were no grade ≥3 AEs reported that were attributed to the BN-CV301 vaccine. The majority of AEs were reported during the priming doses of the vaccine (MVA-BN-CV301) and lessened over time. All reported AEs that were attributed to BN-CV301 were temporary, self-limiting and grade 1 or 2 in severity. Grade 1 or 2 injection-site reactions were reported in all 12 patients (more common and severe with MVA- than FPV-BN-CV301–priming doses). These reactions generally occurred within 24 hours of administration, resolved within 7 days and were managed with supportive care. The majority of patients also reported systemic symptoms including fatigue ($n = 11$; 91.7%), myalgias ($n = 9$; 75.0%), chills ($n = 7$; 58.3%), and headache ($n = 6$; 50.0%). Nonneutropenic fevers were reported in slightly more than half of patients ($n = 7$; 58.3%). Arthralgia, nausea, vomiting, and headache were also reported.

Clinical activity and outcomes

All patients completed the first two priming doses of MVA-BN-CV301 (Fig. 1A and B). The median progression-free survival was 15 weeks (range: 6 to ongoing at 82 weeks). Because of disease progression (PD) at the first restaging point at 6 weeks, 5 patients (42%) received only the two priming doses. Seven patients received at least one dose of FPV-CV301 booster (range: 2 to ongoing at 15 FPV-CV301 doses). Of these 7 patients, 1 patient with MSS colorectal cancer had a partial response (PR) at 78 weeks and later confirmed at 82 weeks on trial after a period of prolonged stable disease (SD). Another patient with MSS colorectal cancer had an unconfirmed PR at 6 weeks but progressed with a new lesion at the next restaging visit (Fig. 1B; Table 2). The remaining 5 patients had SD as the best clinical response. Nine of the 12

Table 2. Individual patient responses

Patient information	DL	Time to progression (weeks)	Best clinical response (Δ target lesions from baseline)
KRAS mutation			
MSS colorectal cancer	1	12	PR* at 6 wk (−38%)
MSS colorectal cancer	1	26	SD at 12 wk (−4%)
Appendiceal cancer	3	6	PD at 6 wk (+6% + new)
Pancreatic cancer	3	18	SD at 6 wk (−20%)
Appendiceal cancer on capecitabine + bevacizumab	3	80+	SD at 12 wk (−8%)
MSS colorectal cancer	3	82+	PR at 82 wk (−30.6%)
KRAS WT			
MSS colorectal cancer	2	6	PD at 6 wk (+23%)
MSS colorectal cancer	2	6	PD at 6 wk (+7% + new)
MSS colorectal cancer	2	18	SD at 6 wk (+11%)
MSS colorectal cancer	3	6	PD at 6 wk (+27%)
KRAS unknown or not tested			
Endometrial cancer	1	6	PD at 6 wk (+13%)
Salivary gland cancer	3	12+	SD at 12 wk (−2%)**

Abbreviations: +, ongoing treatment on trial; DL, dose level (DL1 = 1 MVA-BN-CV301 injection, DL2 = 2 MVA-BN-CV301 injections, DL3 = 4 MVA-BN-CV301 injections); new, new lesion; PR*, unconfirmed partial response; **tumor resected at 13 weeks on trial; patient remained on trial with no measurable disease but at high risk of recurrence; wk, week.

Published OnlineFirst May 20, 2019; DOI: 10.1158/1078-0432.CCR-19-0183

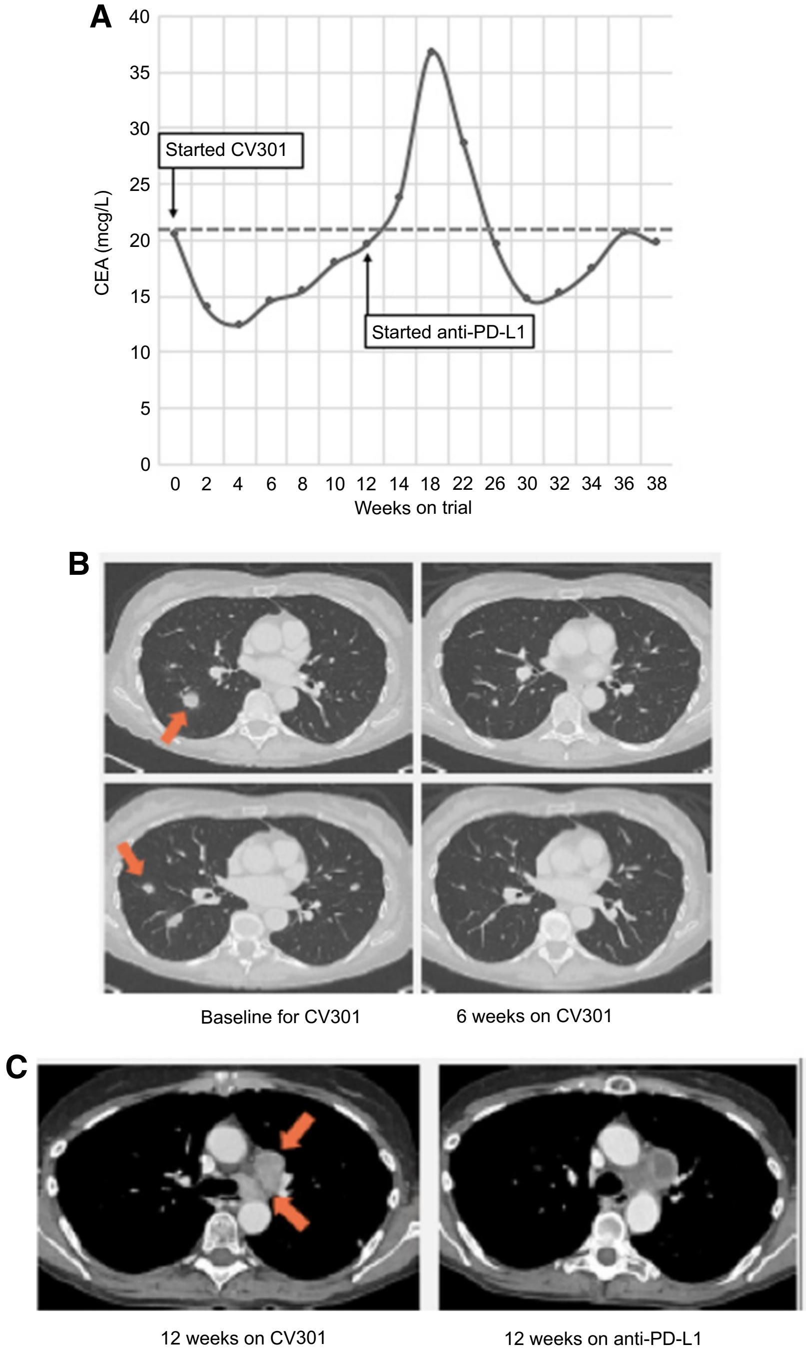


Figure 2.
Two patients with *KRAS* mutation, MSS metastatic colorectal cancer had a ≥35% decrease in tumor markers associated with prolonged SD after treatment with BN-CV301 followed by an anti-PD-L1 antibody. Patient #1: A 62-year-old female with *KRAS*-mutant, MSS colorectal cancer with PD despite 6 prior regimens (DL = 1). She had an initial decrease in CEA (**A**) and an unconfirmed PR at the first restaging (6 weeks; **B**), followed by growth of a nontarget lesion (new mediastinal adenopathy) at the 12-week restaging. The patient then enrolled on an anti-PD-L1 trial and experienced a subsequent decrease in CEA as well as a radiographic response (**C**) to treatment with necrosis of mediastinal adenopathy and decreasing tumor markers at week 12 of treatment with the anti-PD-L1 antibody. This patient had SD for 43 weeks while on an anti-PD-L1 antibody. Patient #2: A 54-year-old female with *KRAS*-mutant, MSS colorectal cancer with PD despite 8 prior regimens (DL = 1). While on BN-CV301 trial, CEA and tumor burden were stable, but the patient eventually developed PD at 26 weeks. (*Continued on the following page.*)

patients eventually had PD, including the patient with the unconfirmed PR at 6 weeks. One patient with salivary gland cancer underwent resection of the tumor and continued to receive the vaccine for 6 months in the adjuvant setting due to high risk of recurrence. After 6 months, the patient was taken off trial but continues to be monitored for recurrence.

Two patients remain on trial at this time. One patient with *KRAS*-mutant, MSS colorectal cancer on vaccine along with maintenance capecitabine + bevacizumab maintains SD at 81+ weeks. Another patient with *KRAS*-mutant, MSS colorectal cancer on single-agent vaccine achieved a PR. In this patient, the target lesion in the liver decreased by 30.6% from baseline at week 78 and was confirmed 4 weeks later (Fig. 1B; Table 2).

Preliminary signal in *KRAS*-mutated cancers

While this is a small sample of patients with known *KRAS* status ($n = 10$), it is notable that patients with *KRAS* mutations (three MSS colorectal cancer, two appendiceal cancer, and one pancreatic cancer) remained stable longer than patients with *KRAS* WT cancers. The six *KRAS* mutation carriers remained on

Published OnlineFirst May 20, 2019; DOI: 10.1158/1078-0432.CCR-19-0183

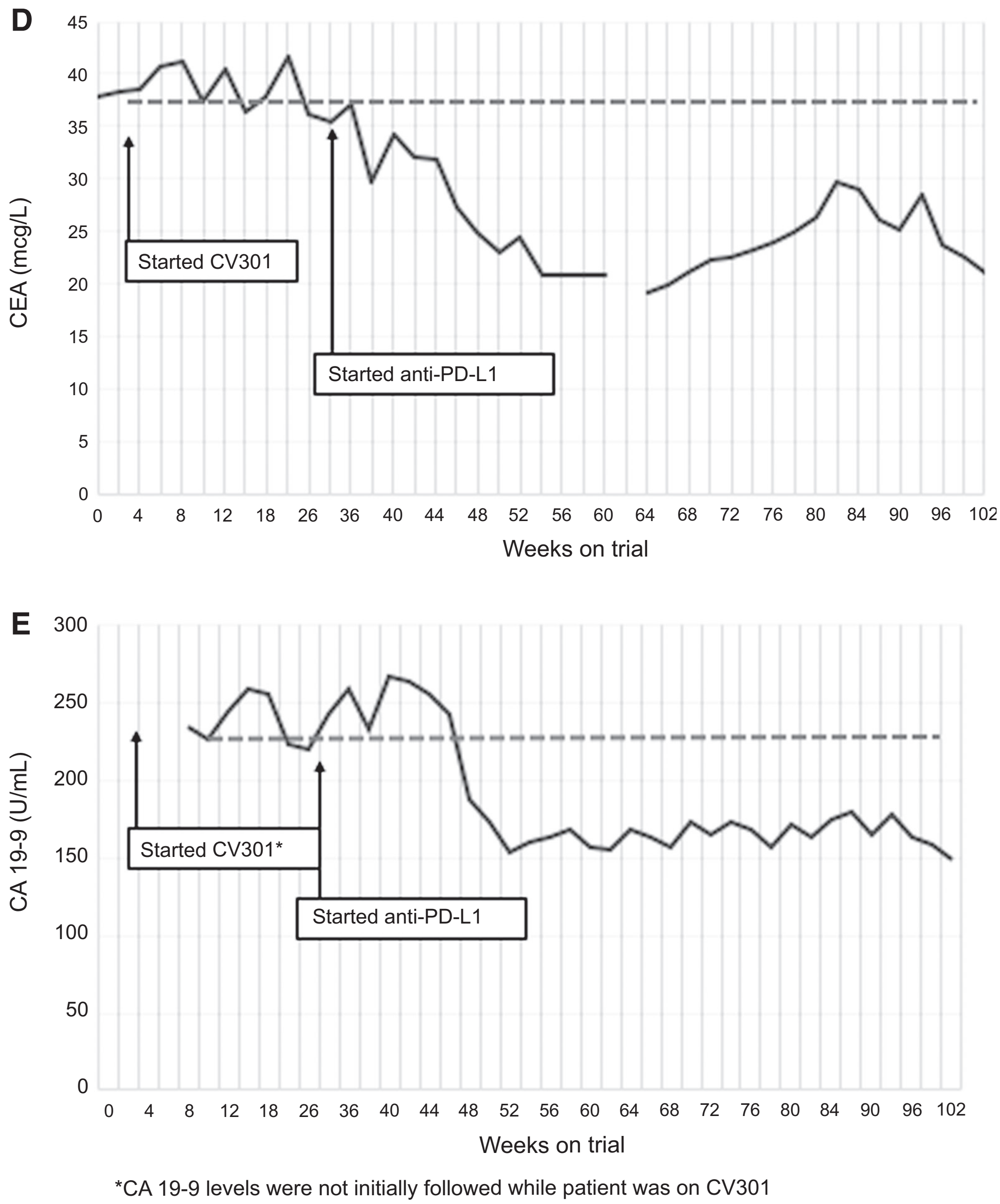


Figure 2.
(*Continued.*) The patient was then enrolled on an anti-PD-L1 trial and experienced a subsequent decrease in tumor markers (**D** and **E**). Radiographically, the patient has continued SD on the anti-PD-L1 antibody ongoing at 71 weeks. Prior trials have found a median progression-free survival of 10 weeks (2.2 months) in patients with MSS metastatic colorectal cancer who receive an anti-PD-L1 antibody. Dotted lines represent baseline tumor markers.

study for 6 weeks, 12 weeks, 18 weeks, and 26 weeks, and 2 patients remain on study at 80+ weeks while the 4 *KRAS* WT patients remained on study for 5 weeks, 6 weeks, 6 weeks and 18 weeks, respectively (*KRAS* mutation median progression-free time of 22 weeks vs. *KRAS* WT 6 weeks; $P = 0.076$). Furthermore, most patients with the *KRAS* mutation had SD with a decrease in the size of index lesions (although not more than a 30% reduction as required for a PR) for their best clinical response (Fig. 1B; Table 2). T-cell responses to *KRAS* mutations were compared to *KRAS* WT in 3 patients where the specific mutation was known, and peptides could be designed (2 patients with *KRAS* G12D mutation, 1 patient with *KRAS* G12C mutation). There was a slightly greater response to the specific *KRAS* mutations than the WT peptides in 2 of the 3 patients (Supplementary Fig. S1) with patient 10 achieving a PR at week 78.

The 2 patients remain on trial at the time of the data cutoff point and continue to receive FPV-CV301 every 13 weeks as long as they tolerate treatment and there is no evidence of disease progression. It is important to note that both of these prolonged responders received BN-CV301 at the highest DL tested (DL3). More *KRAS* mutants were enrolled in the highest DL compared with the lower DLs (DL3 had four *KRAS* mutants; DL2 had zero *KRAS* mutants; DL1 had two *KRAS* mutants). However, the two *KRAS* mutants in DL1 had prolonged SD on single-agent vaccine followed by prolonged SD when transitioned to an anti-PD-L1 agent as will be discussed next. Only 1 of the *KRAS* WT patients (DL2) experienced SD beyond the first restaging at 6 weeks. While it is possible the prolonged responses are due to dose-effect, it is also plausible that the clinical responses are due to immune responses to *KRAS* mutations or other cascade TAAs.

Preliminary signal of BN-CV301 followed by anti-PD-L1

Following BN-CV301 treatment, 3 patients were transitioned to another trial evaluating an anti-PD-L1 agent. While mostly driven by patient interest in clinical trial participation, the clinical decision to enroll these patients on the anti-PD-L1 trial was supported by preclinical data demonstrating synergy with the concurrent (or sequential) use of therapeutic cancer vaccines and anti-PD-1/L1 agents. One patient with pancreatic cancer quickly progressed. The patient with a *KRAS*-mutant, MSS colorectal cancer with an unconfirmed PR at 6 weeks on trial was transitioned to an anti-PD-L1 trial when the 12-week scan showed a new mediastinal lymph node lesion. Shortly after starting on the anti-PD-L1 trial, tumor markers started to decline. At the 12-week restaging visit on the anti-PD-L1 trial, this patient had central necrosis of the affected mediastinal lymph node (Fig. 2A–C). Another patient with *KRAS* mutation, MSS colorectal cancer has prolonged SD per RECIST after switching to anti-PD-L1 therapy (SD ongoing at 80 weeks). Both patients had a notable decrease ($\geq$35%) in tumor markers (CEA, CA19-9) shortly after starting on anti-PD-L1 (Fig. 2D and E) and a prolonged period of SD, both of which are uncommon in patients with *KRAS*-mutant, MSS colorectal cancer on checkpoint inhibitors.

Identification of MUC1-, CEA-, and brachyury-specific T cells

Sufficient PBMCs were available to analyze MUC1-, CEA-, and brachyury-specific T cells prior to vaccination ($n = 12$) and at weeks 6 ($n = 12$), 10 ($n = 7$), and 18 or 22 ($n = 4$) of the study. The FACS-based assay for T cells expressing the type I cytokines IFNγ, IL2, TNF, and/or the degranulation marker CD107a following stimulation with overlapping peptide pools is described in detail in the Materials and Methods section. All assays for a given patient's samples before and after vaccine were performed in the same controlled experiment.

Including all DLs and time points examined, 11 of 12 patients (92%) developed $CD4^+$ and/or $CD8^+$ T-cell responses after vaccination to the antigens MUC1 and CEA that were encoded in the vaccine, as well as to brachyury, a "cascade" antigen not encoded in the vaccine. The induction of antigen-specific T cells was rapid, with most patients having responses to MUC1 (75%), CEA (67%), or brachyury (58%) within 2 weeks after the second MVA-BN-CV301 priming vaccination (week 6). CEA-, MUC1-, and brachyury-specific T cells were observed at all DLs at this early timepoint; MUC1-specific T cells were generated in 1 of 3 patients at DL1, 2 of 3 at DL2, and 6 of 6 at DL3; CEA-specific T cells were induced in 1 of 3 patients at DL1, 3 of 3 at DL2, and 4 of 6 at DL3; and brachyury-specific T cells were developed in 1 of 3 patients at DL1, 2 of 3 at DL2, and 4 of 6 at DL3. TAA responses were evaluated in 7 patients with sufficient PBMCs after the first FPV-CV301 boost. In these patients, although not statistically significant, the magnitude of the MUC1-, CEA-, and brachyury-specific T cells producing cytokine or positive for CD107a was increased on average 2.8-, 2.3-, and 1.8-fold, respectively, after the booster vaccination compared with the results after the second priming vaccination (Fig. 3A). In light of the heterogeneous nature of the patients on this study in terms of cancer type, and number and type of prior therapies, it is difficult to define which type of immune cell responses are most prominent postvaccination. In some patients, immune responses to either CEA or MUC1 declined postvaccination, which is not unexpected due to progression of disease (Supplementary Table S3). In general, however, $IFN\gamma^+$ $CD4^+$ and $CD8^+$ CEA-specific T cells and $IFN\gamma^+$ MUC1-specific $CD4^+$ T cells increased in the majority of patients postvaccination (Supplementary Table S3). CEA-specific CD107a $CD8^+$ T cells also increased postvaccination in 5 of 7 patients (Supplementary Table S3). Polyfunctional TAA responses, defined as $CD4^+$ or $CD8^+$ T cells that express two or more of the markers IFNγ, TNF, IL2, or CD107a, were measured in all patients before and after vaccination. Using the criteria of a >10-fold increase post versus prevaccination, or the presence of >1,000 polyfunctional cells at post per 1×10^6 PBMCs (if negative at pre), polyfunctional T cells specific for MUC1, CEA, or brachyury were generated after BN-CV301 in 58%, 52%, and 33% of patients, respectively (Fig. 3B).

TAA responses were also compared between 4 patients with *KRAS* WT and 6 patients with known/presumed *KRAS* mutations. At week 6, a similar frequency of patients with *KRAS* WT (3/4) and *KRAS* mutations (4/6) developed MUC1-specific T cells after vaccination; however, those with mutated *KRAS* displayed a trend of a greater magnitude (on average 4.9-fold) of MUC1-specific T cells producing cytokine or positive for CD107a compared with those with *KRAS* WT tumors (Fig. 3C). CEA-specific T cells, on the other hand, were generated in a slightly higher frequency of patients with *KRAS* WT (4/4) than *KRAS* mutations (3/6); however, the magnitude of CEA responses generated was similar between these groups. Brachyury T cells were generated in 3 of 4 patients with *KRAS* WT and 3 of 6 patients with *KRAS* mutations, and the two groups of patients displayed a similar magnitude of brachyury-specific T cells.

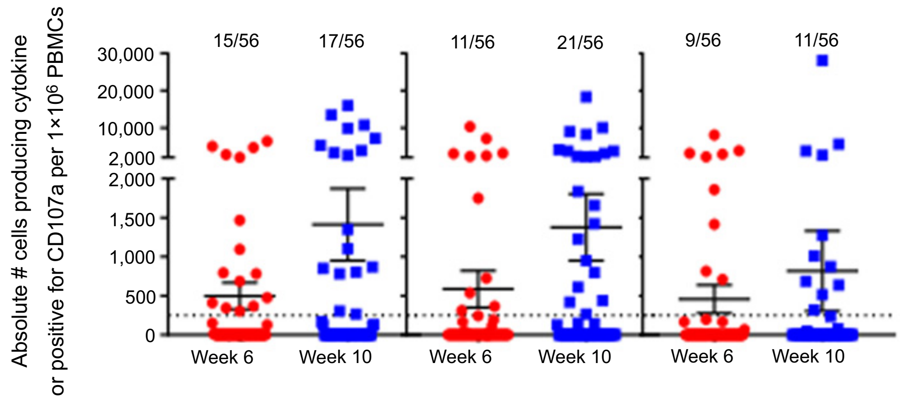
Discussion

The prime-boost regimen of BN-CV301 was well tolerated by all patients and demonstrated prolonged SD for some patients with advanced solid tumors as well as one confirmed PR. Side effects were minimal and were more common with MVA-BN-CV301 prime doses (limited to two doses) than with the FPV-CV301 booster doses. All side effects were self-limited and required only supportive care measures to manage symptoms. The safety profile of BN-CV301 was similar to that observed with the first-generation PANVAC vaccine; however, the use of the replication-defective MVA- and FPV-recombinant vectors eliminates the potential toxicity issues in the use of the replication-competent vaccinia as a prime in the original PANVAC regimen.

Immunologic analyses demonstrated that BN-CV301 was able to generate MUC1- and CEA-specific T cells in most patients (92%) after vaccination, demonstrating the immunogenicity of the BN-CV301 vaccine. In addition, T-cell responses against brachyury, an antigen not encoded in the vaccine, were generated after vaccination, suggesting that BN-CV301 induces

Published OnlineFirst May 20, 2019; DOI: 10.1158/1078-0432.CCR-19-0183

A



B

Scoring criteria of multifunctional TAA response developed post- vs. pre-vaccination	Low 3× pre or if no pre, >100/1×10⁶ cells post	Mid 10× pre or if no pre, >1,000/1×10⁶ cells post	High 100× pre or if no pre, >5,000/1×10⁶ cells post
MUC1	11/12 (92%)	7/12 (58%)	2/12 (17%)
CEA	7/12 (58%)	5/12 (52%)	0/12 (0%)
Brachyury	8/12 (67%)	4/12 (33%)	1/12 (8%)
Any TAA	12/12 (100%)	10/12 (83%)	3/12 (25%)

C

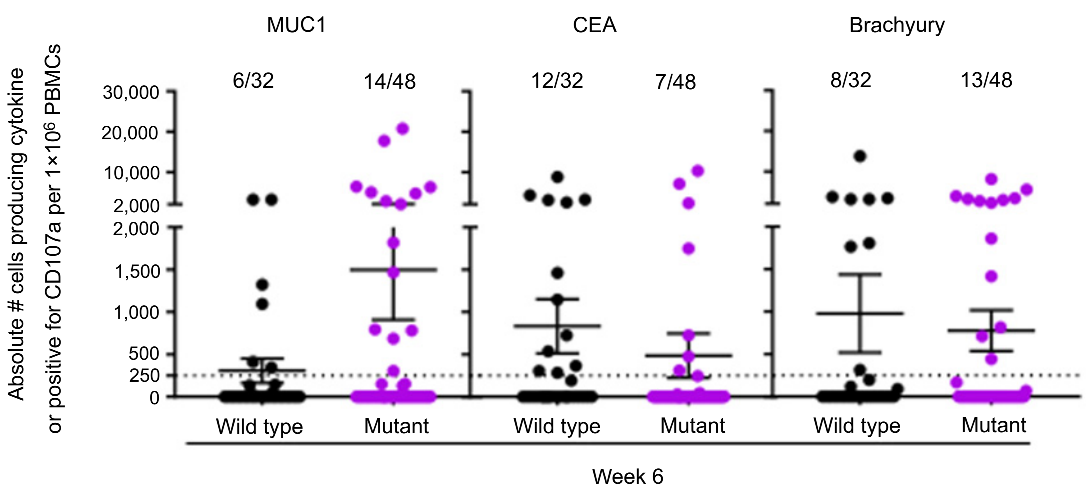


Figure 3.

Magnitude and breadth of combined antigen-specific CD4$^+$ and CD8$^+$ T-cell responses post- (vs. pre) BN-CV301 (MVA-BN-CV301/FPV-CV301) vaccine. **A,** Seven patients were tested and TAA responses compared at both 6 weeks (2 weeks after the second MVA-BN-CV301 prime, red) and 10 weeks (2 weeks after the first FPV-CV301 boost, blue). The absolute number of CD4$^+$ and/or CD8$^+$ T cells producing IFNγ, TNF, or IL2 or positive for the degranulation marker CD107a per 1×10^6 PBMCs plated at the start of the stimulation assay was calculated. Any background signal (obtained with the HLA peptide pool) and any signal obtained prior to vaccination was subtracted [(post-TAA – post-HLA) – (pre-TAA – pre-HLA)]. Each point indicates the magnitude of a cytokine/CD107a measure, with 8 measures assessed per patient (CD8$^+$IFNγ$^+$, CD8$^+$TNF$^+$, CD8$^+$IL2$^+$, CD8$^+$CD107a$^+$, CD4$^+$IFNγ$^+$, CD4$^+$TNF$^+$, CD4$^+$IL2$^+$, and CD4$^+$CD107a$^+$). Frequency of positive measures (>250 CD4$^+$ and CD8$^+$ T cells producing cytokine and/or positive for CD107a) is indicated. **B,** Polyfunctional TAA responses (CD4$^+$ and CD8$^+$ T cells expressing 2 or more of the following: IFNγ, TNF, IL2, or CD107a) were measured before and after any time point postvaccination in all 12 patients. The frequency of patients developing a low, mid, or high magnitude of multifunctional TAA-specific T cells after vaccination at any time point post- versus pre- is indicated. **C,** TAA responses in 4 patients with known WT *KRAS* (black) and 6 patients with known/presumed *KRAS* mutations (purple) were compared at 6 weeks (2 weeks after the second MVA prime). The absolute number of CD4$^+$ or CD8$^+$ T cells producing IFNγ, TNF, or IL2 or positive for the degranulation marker CD107a per 1×10^6 PBMC plated at the start of the stimulation assay was calculated. Each point indicates the magnitude of a cytokine/CD107a measure, with 8 measures assessed per patient. Frequency of positive measures (>250 CD4$^+$ and CD8$^+$ T cells producing cytokine and/or positive for CD107a) is indicated.

Published OnlineFirst May 20, 2019; DOI: 10.1158/1078-0432.CCR-19-0183

immunologically relevant tumor-cell destruction. Furthermore, we observed a trend indicating a potential dose-related response, resulting in the highest dose being selected for use in future phase II trials. We additionally observed that the magnitude of MUC1-, CEA-, and brachyury-specific immune responses was increased after the FPV-CV301 booster vaccine, supporting the use of a diversified prime-boost approach to generate a TAA immune response. It has been shown that long-lasting polyfunctional T cells can be induced by vaccination and are associated with improved overall survival (30). It is interesting in this study that the majority of patients generated polyfunctional T-cell responses to both CEA and MUC1 postvaccination; moreover, the majority of patients also generated polyfunctional T-cell responses to the cascade antigen brachyury. Finally, we observed a trend in the magnitude of MUC1-specific T-cell responses being enhanced following vaccination to a greater extent in patients with *KRAS* mutations compared with those with *KRAS* WT tumors. The relationship between MUC1-C and *KRAS* mutations has been described previously (31, 32). The immunogenicity demonstrated with the BN-CV301 vaccine described here in advanced and diverse cancer patients in this phase I trial supports the use of this vaccine in combination immunotherapy studies in more homogeneous patients and the safety supports its use in less advanced cancer settings.

With the limitations of the small number of patients treated, a correlation between the dose of vaccine administered and percentage of patients developing an immune response is noted in this trial, consistent with a previous phase I trial studying a similar vaccine construct encoding a different TAA, brachyury (33). In both trials, the three dose levels explored used one, two, and four injection sites with the aim of minimizing the severity of the injection-site reactions, resulting in the activation of an increasing number of lymph node regions. Experimental evidence in mice has shown a significant correlation between the number of draining lymph node areas and the number of induced T cells (34). This intriguing observation raises the possibility of some confounding between a pure dose effect and the number of lymph nodes involved as determinants of the T-cell response magnitude.

Mutations in the *KRAS* proto-oncogene are found in many cancers including colorectal cancers (30%–40%), pancreatic cancers (90%), and NSCLC (25%; refs. 35, 36). Because of our referral pattern, this trial was unintentionally enriched for gastrointestinal tumors. Generally, *KRAS* mutations are associated with worse clinical outcomes and resistance to traditional therapies (35, 37–40). Moreover, despite their prevalence, to the best of our knowledge, no therapeutic agent to date has shown clinical benefit specifically for *KRAS*-mutant cancers.

While this trial was small ($n = 12$ total with six *KRAS*-mutant, four *KRAS* WT, and two tumors with undetermined *KRAS* status), there appears to be a trend to longer time to progression for patients with *KRAS*-mutant cancers as compared with patients with *KRAS* WT cancers (median time on trial 22 weeks in *KRAS* mutated vs. 6 weeks in *KRAS* WT; $P = 0.076$; Fig. 1B). This is worth noting because anti-PD-L1 therapies to date have shown no efficacy in MSS colorectal cancer, with objective response rates of 0% and a median progression-free survival of 10 weeks (41). This early evidence suggests that a MUC1/CEA vaccine has the potential to produce durable clinical benefit (prolonged SD or PR) in *KRAS*-mutant cancers. To our knowledge, no one to date has described this finding of clinical benefit with a vaccine targeting MUC1 or CEA in this population. Previously, Takahashi and colleagues demonstrated that *KRAS*-mutated tumors have higher MUC1 expression than *KRAS* WT tumors (42). Downregulation of MUC1 expression is associated with reversal of EMT, reversal of an immunosuppressive tumor microenvironment and decreased tumor cell growth in *KRAS*-mutant NSCLC (2, 3, 43). We hypothesize that higher MUC1 expression enabled a more robust immune response to the TAAs contained in the vaccine. Vaccine-induced MUC1 immune responses also trended higher in tumors with *KRAS* mutations compared with those with *KRAS* WT.

Because of the heterogeneity in patient populations and the relatively small number of patients in each trial, it is difficult to draw strong conclusions in comparing the original PANVAC vaccine to BN-CV301. An ELISpot assay was used to measure immune responses to CEA and MUC1 in the PANVAC trials. This assay was capable of measuring only $CD8^+$ responses to HLA-A2 Class I patients using 9-mer peptides. Subsequent to those studies, we have developed a flow cytometry–based assay employing 15-mer peptides; this assay is capable of measuring both $CD4^+$ and $CD8^+$ T-cell responses, regardless of Class I or Class II type (27, 33). Nonetheless, in the pilot study of PANVAC (15), 3 of 8 patients (38%) analyzed developed T-cell responses to CEA, and 4 of 14 patients (29%) developed T-cell responses to MUC1. In two additional studies of PANVAC (13, 14), 5 of 33 patients (15%) developed CEA-specific T-cell responses and 1 of 3 developed MUC1-specific T-cell responses. In this BN-CV301 trial, 11 of 12 patients (92%) developed T-cell responses to CEA, and 11 of 12 patients (92%) also developed T-cell responses to MUC1.

As mentioned previously, the phase Ib component of this trial combines BN-CV301 with pembrolizumab or nivolumab. Following progressive disease on BN-CV301, 2 patients with *KRAS*-mutant, MSS colorectal cancer were transitioned to another trial evaluating an anti-PD-L1 agent and subsequently had prolonged SD. BN-CV301 is hypothesized to induce TAA-specific T cells that migrate to the tumor. Combining BN-CV301 with anti-PD-1/L1 blockade therapy may augment these T-cell–mediated clinical responses. These early clinical data further support the potential clinical benefit of combining BN-CV301 with anti-PD-L1 therapies. Both patients remained on this anti-PD-L1 therapy $\geq$40 weeks, exceeding expectations based upon published data.

In conclusion, the BN-CV301 vaccine can be safely administered to patients with advanced cancer in a prime-boost regimen. Side effects were mild to moderate and self-limited. Immune analysis demonstrated a high level of immunogenicity induced by the BN-CV301 vaccine. The BN-CV301 vaccine may have clinical benefit as monotherapy or in combination with anti-PD-1/L1 agents. A recent study by Massarelli and colleagues (44) has shown the clinical benefit of a therapeutic cancer vaccine with checkpoint inhibition in patients with human papillomavirus–positive cancers. Further trials of the vaccine in combination with other agents are planned on the basis of the results of preclinical data.

Disclosure of Potential Conflicts of Interest

C. Pico Navarro holds ownership interest (including patents) in Bavarian Nordic, Inc. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: C. Pico Navarro, C.R. Heery, J. Schlom, J.L. Gulley
Development of methodology: R.N. Donahue, C. Palena, C.R. Heery, J. Schlom

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M.E. Gatti-Mays, J. Strauss, R.N. Donahue, C. Palena, J. Del Rivero, J.M. Redman, R.A. Madan, J.L. Marté, L.M. Cordes, E. Lamping, A. Orpia, A. Burmeister, J. Schlom, J.L. Gulley

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M.E. Gatti-Mays, J. Strauss, R.N. Donahue, C. Palena, C.R. Heery, J. Schlom, J.L. Gulley

Writing, review, and/or revision of the manuscript: M.E. Gatti-Mays, J. Strauss, R.N. Donahue, C. Palena, J.M. Redman, R.A. Madan, L.M. Cordes, C. Pico Navarro, C.R. Heery, J. Schlom, J.L. Gulley

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): J. Strauss, E. Lamping, A. Orpia, E. Wagner, J.L. Gulley

Study supervision: M.E. Gatti-Mays, J. Strauss, J. Del Rivero, R.A. Madan, A. Orpia, E. Wagner, C.R. Heery, J. Schlom, J.L. Gulley

Acknowledgments

The authors would like to thank Angie Schwab for her technical assistance with immune assays, Dr. Seth Steinberg for his statistical input, and Debra Weingarten for her editorial assistance. This work was supported by the Intramural Research Program of the Center for Cancer Research, NCI, NIH, and a Cooperative Research and Development Agreement (CRADA) between the NCI and Bavarian Nordic.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received January 16, 2019; revised March 26, 2019; accepted May 16, 2019; published first May 20, 2019.

References

1. Rajabi H, Kufe D. MUC1-C oncoprotein integrates a program of EMT, epigenetic reprogramming and immune evasion in human carcinomas. Biochim Biophys Acta 2017;1868:117–22.
2. Kharbanda A, Rajabi H, Jin C, Alam M, Wong KK, Kufe D. MUC1-C confers EMT and KRAS independence in mutant KRAS lung cancer cells. Oncotarget 2014;5:8893–905.
3. Bouillez A, Adeegbe D, Jin C, Hu X, Tagde A, Alam M, et al. MUC1-C promotes the suppressive immune microenvironment in non-small cell lung cancer. OncoImmunology 2017;6:e1338998.
4. Zeng Y, Zhang Q, Zhang Y, Lu M, Liu Y, Zheng T, et al. MUC1 predicts colorectal cancer metastasis: a systematic review and meta-analysis of case controlled studies. PLoS One 2015;10:e0138049.
5. Kharbanda A, Rajabi H, Jin C, Raina D, Kufe D. Oncogenic MUC1-C promotes tamoxifen resistance in human breast cancer. Mol Cancer Res 2013;11:714–23.
6. Huang X, Sun Q, Chen C, Zhang Y, Kang X, Zhang JY, et al. MUC1 overexpression predicts worse survival in patients with non-small cell lung cancer: evidence from an updated meta-analysis. Oncotarget 2017;8: 90315–26.
7. Khodarev NN, Pitroda SP, Beckett MA, MacDermed DM, Huang L, Kufe DW, et al. MUC1-induced transcriptional programs associated with tumorigenesis predict outcome in breast and lung cancer. Cancer Res 2009;69: 2833–7.
8. Raina D, Kosugi M, Ahmad R, Panchamoorthy G, Rajabi H, Alam M, et al. Dependence on the MUC1-C oncoprotein in non-small cell lung cancer cells. Mol Cancer Ther 2011;10:806–16.
9. Maeda T, Hiraki M, Jin C, Rajabi H, Tagde A, Alam M, et al. MUC1-C induces PD-L1 and immune evasion in triple-negative breast cancer. Cancer Res 2018;78:205–15.
10. Tsang KY, Palena C, Yokokawa J, Arlen PM, Gulley JL, Mazzara GP, et al. Analyses of recombinant vaccinia and fowlpox vaccine vectors expressing transgenes for two human tumor antigens and three human costimulatory molecules. Clin Cancer Res 2005;11:1597–607.
11. Kaufman HL, Kim-Schulze S, Manson K, DeRaffele G, Mitcham J, Seo KS, et al. Poxvirus-based vaccine therapy for patients with advanced pancreatic cancer. J Transl Med 2007;5:60.
12. Heery CR, Ibrahim NK, Arlen PM, Mohebtash M, Murray JL, Koenig K, 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;1:1087–95.
13. Mohebtash M, Tsang KY, Madan RA, Huen NY, Poole DJ, Jochems C, et al. A pilot study of MUC-1/CEA/TRICOM poxviral-based vaccine in patients with metastatic breast and ovarian cancer. Clin Cancer Res 2011;17: 7164–73.
14. Morse MA, Niedzwiecki D, Marshall JL, Garrett C, Chang DZ, Aklilu M, et al. A randomized phase II study of immunization with dendritic cells modified with poxvectors encoding CEA and MUC1 compared with the same poxvectors plus GM-CSF for resected metastatic colorectal cancer. Ann Surg 2013;258:879–86.
15. Gulley JL, Arlen PM, Tsang KY, Yokokawa J, Palena C, Poole DJ, et al. Pilot study of vaccination with recombinant CEA-MUC-1-TRICOM poxviral-based vaccines in patients with metastatic carcinoma. Clin Cancer Res 2008;14:3060–9.
16. Gatti-Mays ME, Mohebtash M, Madan R, Strauss J, Bilusic M, Jochems C, et al. Durable complete response with PANVAC and trastuzumab in metastatic triple positive breast cancer. J Immunother Cancer 2017; 5(Suppl 2):P440.
17. Greenberg RN, Overton ET, Haas DW, Frank I, Goldman M, von Krempelhuber A, et al. Safety, immunogenicity, and surrogate markers of clinical efficacy for modified vaccinia Ankara as a smallpox vaccine in HIV-infected subjects. J Infect Dis 2013;207:749–58.
18. Overton ET, Stapleton J, Frank I, Hassler S, Goepfert PA, Barker D, et al. Safety and immunogenicity of modified vaccinia Ankara-Bavarian Nordic smallpox vaccine in vaccinia-naive and experienced human immunodeficiency virus-infected individuals: an open-label, controlled clinical phase II trial. Open Forum Infect Dis 2015;2:ofv040.
19. von Sonnenburg F, Perona P, Darsow U, Ring J, von Krempelhuber A, Vollmar J, et al. Safety and immunogenicity of modified vaccinia Ankara as a smallpox vaccine in people with atopic dermatitis. Vaccine 2014;32: 5696–702.
20. Tsang KY, Zaremba S, Nieroda CA, Zhu MZ, Hamilton JM, Schlom J. Generation of human cytotoxic T cells specific for human carcinoembryonic antigen epitopes from patients immunized with recombinant vaccinia-CEA vaccine. J Natl Cancer Inst 1995;87:982–90.
21. Tsang KY, Palena C, Gulley J, Arlen P, Schlom J. A human cytotoxic T-lymphocyte epitope and its agonist epitope from the nonvariable number of tandem repeat sequence of MUC-1. Clin Cancer Res 2004;10:2139–49.
22. Jochems C, Tucker JA, Vergati M, Boyerinas B, Gulley JL, Schlom J, et al. Identification and characterization of agonist epitopes of the MUC1-C oncoprotein. Cancer Immunol Immunother 2014;63:161–74.
23. Sallusto F, Lanzavecchia A. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha. J Exp Med 1994;179:1109–18.
24. Muraro R, Wunderlich D, Thor A, Lundy J, Noguchi P, Cunningham R, et al. Definition by monoclonal antibodies of a repertoire of epitopes on carcinoembryonic antigen differentially expressed in human colon carcinomas versus normal adult tissues. Cancer Res 1985;45:5769–80.
25. Hayes DF, Sekine H, Ohno T, Abe M, Keefe K, Kufe DW. Use of a murine monoclonal antibody for detection of circulating plasma DF3 antigen levels in breast cancer patients. J Clin Invest 1985;75:1671–8.
26. Perey L, Hayes DF, Maimonis P, Abe M, O'Hara C, Kufe DW. Tumor selective reactivity of a monoclonal antibody prepared against a recombinant peptide derived from the DF3 human breast carcinoma-associated antigen. Cancer Res 1992;52:2563–8.
27. Heery CR, Singh BH, Rauckhorst M, Marté JL, Donahue RN, Grenga I, et al. Phase I trial of a yeast-based therapeutic cancer vaccine (GI-6301) targeting the transcription factor brachyury. Cancer Immunol Res 2015; 3:1248–56.
28. Zaremba S, Barzaga E, Zhu M, Soares N, Tsang KY, Schlom J. Identification of an enhancer agonist cytotoxic T lymphocyte peptide from human carcinoembryonic antigen. Cancer Res 1997;57:4570–7.

29. Witkiewicz AK, McMillan EA, Balaji U, Baek G, Lin WC, Mansour J, et al. Whole-exome sequencing of pancreatic cancer defines genetic diversity and therapeutic targets. Nat Commun 2015;6:6744.
30. Wimmers F, Aarntzen EH, Duiveman-deBoer T, Figdor CG, Jacobs JF, Tel J, et al. Long-lasting multifunctional CD8. Oncoimmunology 2016;5: e1067745.
31. Raina D, Agarwal P, Lee J, Bharti A, McKnight CJ, Sharma P, et al. Characterization of the MUC1-C cytoplasmic domain as a cancer target. PLoS One 2015;10:e0135156.
32. Kufe DW. MUC1-C oncoprotein as a target in breast cancer: activation of signaling pathways and therapeutic approaches. Oncogene 2013;32: 1073–81.
33. Heery CR, Palena C, McMahon S, Donahue RN, Lepone LM, Grenga I, et al. Phase I study of a poxviral TRICOM-based vaccine directed against the transcription factor brachyury. Clin Cancer Res 2017;23:6833–45.
34. Mould RC, AuYeung AWK, van Vloten JP, Susta L, Mutsaers AJ, Petrik JJ, et al. Enhancing immune responses to cancer vaccines using multi-site injections. Sci Rep 2017;7:8322.
35. Phipps AI, Buchanan DD, Makar KW, Win AK, Baron JA, Lindor NM, et al. KRAS-mutation status in relation to colorectal cancer survival: the joint impact of correlated tumour markers. Br J Cancer 2013;108: 1757–64.
36. Zeitouni D, Pylayeva-Gupta Y, Der CJ, Bryant KL. KRAS mutant pancreatic cancer: no lone path to an effective treatment. Cancers (Basel) 2016;8:45.
37. Kim HS, Heo JS, Lee J, Lee JY, Lee MY, Lim SH, et al. The impact of KRAS mutations on prognosis in surgically resected colorectal cancer patients with liver and lung metastases: a retrospective analysis. BMC Cancer 2016; 16:120.
38. Ghidini M, Personeni N, Bozzarelli S, Baretti M, Basso G, Bianchi P, et al. KRAS mutation in lung metastases from colorectal cancer: prognostic implications. Cancer Med 2016;5:256–64.
39. Imamura Y, Morikawa T, Liao X, Lochhead P, Kuchiba A, Yamauchi M, et al. Specific mutations in KRAS codons 12 and 13, and patient prognosis in 1075 BRAF wild-type colorectal cancers. Clin Cancer Res 2012;18: 4753–63.
40. Zdanov S, Mandapathil M, Abu Eid R, Adamson-Fadeyi S, Wilson W, Qian J, et al. Mutant KRAS conversion of conventional t cells into regulatory T cells. Cancer Immunol Res 2016;4:354–65.
41. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, et al. PD-1 blockade in tumors with mismatch-repair deficiency. N Engl J Med 2015; 372:2509–20.
42. Takahashi H, Jin C, Rajabi H, Pitroda S, Alam M, Ahmad R, et al. MUC1-C activates the TAK1 inflammatory pathway in colon cancer. Oncogene 2015; 34:5187–97.
43. Bouillez A, Rajabi H, Pitroda S, Jin C, Alam M, Kharbanda A, et al. Inhibition of MUC1-C suppresses MYC expression and attenuates malignant growth in KRAS mutant lung adenocarcinomas. Cancer Res 2016;76: 1538–48.
44. Massarelli E, William W, Johnson F, Kies M, Ferrarotto R, Guo M, et al. Combining immune checkpoint blockade and tumor-specific vaccine for patients with incurable human papillomavirus 16-related cancer: a phase 2 clinical trial. JAMA Oncol 2019;5:67–73.

Published OnlineFirst May 20, 2019; DOI: 10.1158/1078-0432.CCR-19-0183

American Association for Cancer Research

Clinical Cancer Research

A Phase I Dose-Escalation Trial of BN-CV301, a Recombinant Poxviral Vaccine Targeting MUC1 and CEA with Costimulatory Molecules

Margaret E. Gatti-Mays, Julius Strauss, Renee N. Donahue, et al.

Clin Cancer Res 2019;25:4933-4944. Published OnlineFirst May 20, 2019.