Effective immunotherapeutic strategies require the ability to generate a systemic antigen-specific response capable of impacting both primary and metastatic disease. We have built on our oncolytic vaccinia a granulocyte-macrophage colony-stimulating factor (GM-CSF) strategy by adding recombinant tumor antigen to increase the response in the tumor microenvironment and systemically. In the present study, orthotopic growth of a syngeneic HER2/neu-overexpressing mammary carcinoma in FVB/N mice (NBT1) was associated with increased Gr1+CD11b+ myeloid-derived suppressor cells (MDSCs) both systemically and in the tumor microenvironment. This MDSC population had inhibitory effects on the HER2/neu-specific Th1 immune response. VVneu and VVGMCSF are recombinant oncolytic vaccinia viruses that encode HER2/neu and GM-CSF, respectively. Naïve FVB mice vaccinated with combined VVneu and VVGMCSF given systemically developed systemic HER2/neu-specific immunity. NBT1-bearing mice became anergic to systemic immunization with combined VVneu and VVGMCSF. Intratumoral VVGMCSF failed to result in systemic antitumor immunity until combined with intratumoral VVneu. Infection/transfection of the tumor microenvironment with combined VVGMCSF and VVneu resulted in development of systemic tumor-specific immunity, reduction in splenic and tumor MDSC and therapeutic efficacy against tumors. These studies demonstrate the enhanced efficacy of oncolytic vaccinia virus recombinants encoding combined tumor antigen and GM-CSF in modulating the microenvironment of MDSC-rich tumors.

INTRODUCTION
The goal of tumor immunotherapy is the development of modalities given alone or in combination that result in the generation of an effective systemic tumor-specific immune response by the host that can overcome immune escape mechanisms. The identification of immunogenic targets unique to or overexpressed by tumor cells is critical to achieving this goal. The HER2/neu oncogene encodes human epidermal growth factor receptor 2 (HER2/neu), a member of the epidermal growth factor receptor family of transmembrane tyrosine kinase receptors, which participates in processes including physiology, proliferation and differentiation of various human tissues.1,2 Overexpression of HER2/neu is found in ~20% of invasive breast cancers, and is associated with a more invasive phenotype and a poorer prognosis.3 Development of an active immune response using a vaccine targeting HER2/neu represents an attractive immunotherapeutic strategy for overcoming immune escape mechanisms induced by the tumor microenvironment.

Myeloid-derived suppressor cells (MDSCs), a population of immature myeloid cells that are increased systemically and in the tumor microenvironment of both murine cancer models and human malignancies, are prominent contributors to tumor immune escape.4,5 This heterogeneous population is characterized phenotypically in mice by the cell surface antigens CD11b and Gr-1.6 Gr-1 encompasses two subtypes, Ly-6C and Ly-6G, which have been used to further differentiate MDSCs into CD11b+Ly-6Chigh Ly-6G monocytic (mMDSC) and CD11b+Ly-6C−Ly-6G+ granulocytic (gMDSC) subpopulations, respectively.6,7 Consistent with their heterogeneous phenotype, MDSCs suppress the antitumor immune response through multiple mechanisms.8 MDSCs interfere with lymphocyte proliferation via deprivation of essential amino acids, such as arginine and cysteine.7,9,10 They also mediate oxidative stress via production of reactive oxygen species and peroxynitrate. This leads to nitration of tyrosine in CD8 and the T-cell receptor, ultimately changing the rigidity of the T-cell receptor.11 Furthermore, MDSCs support induction of other immune inhibitory populations such as regulatory T cells (Tregs) through the production of transforming growth factor-β and interleukin-10.12-15 Given these immune-suppressive effects, therapies that can overcome systemic anergy induced by MDSCs have generated great interest.

Studies from our group were the first to develop and test recombinant vaccinia vectors encoding the immune-enhancing granulocyte-macrophage colony-stimulating factor (GM-CSF) for...
the localized treatment of solid tumors. In preclinical studies, we demonstrated that vaccinia and vaccinia recombinants were effective in infecting/transfecting tumors and, importantly, that despite the immunogenicity of the vaccinia vector, high levels of transfection could be obtained following repeated injections of tumor in mice\(^\text{18}\) and subsequently in patients with recurrent superficial melanoma.\(^\text{19}\) We developed and took clinical VVGMCsf into phase I trials in melanoma.\(^\text{18}\) Subsequent to our studies, this recombinant (JX-594) was shown to have antitumor activity in preclinical models and clinical trials in a number of diseases.\(^\text{20,21}\)

In the present study using orthotopic growth of an aggressive HER2/neu-expressing murine tumor characterized by high levels of the HER2/neu protein, 11 MDSCs in the tumor microenvironment and systemically that suppressed HER2/neu-specific Th1, we show that intratumoral treatment with the oncolytic VVGMCsf is ineffective at reducing tumor growth nor does it lead to the development of a systemic tumor-specific immune response. However, when combined with a neu-encoding vaccinia VNNeu and administered into the tumor microenvironment, mice develop systemic anti-neu immunity, significant reduction in tumoral and systemic MDSC and manifest a major antitumor response. The same virus combination (vaccine) fails to generate a similar response when given systemically in NBT1-bearing mice. We characterize the presence and function of the MDSC response and the resultant cytotoxic T lymphocyte (CTL) and interferon responses to HER2/neu. These results point to the ability of the tumor microenvironment to both promote immune escape and act as an effective vaccination site for tumor antigen-encoding oncolytic viruses that can result in a systemic immune response capable of mediating tumor regression.

**MATERIALS AND METHODS**

**Cell culture**

The HER2/neu-expressing NBT1 mammary tumor cell line was derived by serial cell culture of tumor cells obtained from a spontaneous breast tumor that arose in a FVB/neuT mouse transgenic for a HER2/neu receptor that is systemically that suppressed HER2/neu-specific Th1, we show that intratumoral treatment with the oncolytic VVGMCsf is ineffective at reducing tumor growth nor does it lead to the development of a systemic tumor-specific immune response. However, when combined with a neu-encoding vaccinia VNNeu and administered into the tumor microenvironment, mice develop systemic anti-neu immunity, significant reduction in tumoral and systemic MDSC and manifest a major antitumor response. The same virus combination (vaccine) fails to generate a similar response when given systemically in NBT1-bearing mice. We characterize the presence and function of the MDSC response and the resultant cytotoxic T lymphocyte (CTL) and interferon responses to HER2/neu. These results point to the ability of the tumor microenvironment to both promote immune escape and act as an effective vaccination site for tumor antigen-encoding oncolytic viruses that can result in a systemic immune response capable of mediating tumor regression.

**Construction of recombinant vaccinia virus expressing HER2/neu (VNNeu)**

The rat neu complementary DNA (cDNA) was cloned from pSV2-neuNT (provided by RA Weinberg), ligated into pBluescript (Stratagene, La Jolla, CA, USA) supplemented with 10% fetal bovine serum, 2 μg/ml l-glutamine, 50 IU/ml penicillin/streptomycin and 4 μg/ml dexamethasone. Splenic cell cultures used in immune analyses were carried out using a supplemented RPMI-1640 media as described.\(^\text{21}\) The Sf9 and Sf21 cell lines (Invitrogen) were maintained in Grace’s supplemented medium (Invitrogen) supplemented with 10% fetal bovine serum and 50 IU/ml penicillin/streptomycin. HER2/neu Sf21 cell lysates used for in vitro restimulation of the CTL assay described below were prepared by resuspending cells in RPMI medium with protease inhibitor cocktail (Sigma-Aldrich, St Louis, MO), lysing via four freeze/thaw cycles, sonicating for 2 min and centrifuging for 15 min at 13 500 rcf.

**Construction of recombinant baculovirus expressing rat HER2/neu**

The rat neu cDNA-coding sequence was assembled in the pFastBac/CT-TOPO recombination vector (Invitrogen). Rat neu-transforming cDNA was excised from a vaccinia recombinant plasmid containing the full-length rat neuNT cDNA sequence\(^\text{22}\) in the vector pSC11/9 using NotI and NotI restriction enzymes. The 4727 bp rat neu DNA was gel purified and then ligated to pBluescript II SK(+)(Stratagene) at NotI/Sal to produce plasmid BSRaceuNT. This neuNT-containing plasmid was used to generate three DNA fragments which, when ligated together, prepared the rat neuNT-coding sequences for ligation into pFastBac/CT-TOPO. pBSraceuNT was first digested with Hincll, which cut at two sites: a Hincll site at residue 1599 and the SalI site at the 3'-end of the neuNT CDNA, residue 4622. Hincll digestion released a 3023 bp fragment, residues 1599–4622, which contains two problematic Ncol sites. The Hincll-cut vector was gel purified, religated and cloned and then digested with Ncol. Filling in the Ncol site, located at position – 19, with T4 DNA polymerase followed by ligation tranformed the Ncol sites into a SalI site. Construction of plasmid BSNeuNsi was digested with both SalI and Sall releasing a 1365 bp fragment and leaving 336 bp of cDNA containing 233 bp of the S'-coding sequence. Plasmid pBSrateuNT was next digested with both Sfl and Sall releasing a 4389 bp fragment, residues 234–4642, which, after gel purification, was cut with Sall to generate a 2775 bp middle fragment comprising residues 300–3008. Lastly, pBSrateuNT was PCR-amplified using primers NTCneu (5'-gaatctacagtctacagcattgacg-3') and NTCneu (5'-gtcgagcacgagtctacagctaggtac-3') to produce a 768-bp fragment of the neu-coding sequence comprising residues 3009–3777. At its 3’ end, this amplicon contained a SalI site for cloning and a final residue codon mutation of GTA to GTG. The NTCneu/NTSal PCR ampiclon—residues 3009–3777—was used without subcloning in a three-way ligation, with the Sfl/ Ncol middle fragment—residues 234–3008—and the plasmid BSNeuNsi— residues 105 to 233—with Sfl/Sall. Following transformation, clone selection and DNA sequencing, a correct full-length neuNT cDNA clone was identified, pBSNeuNsiSal. This plasmid was cut with both Sall and Sfl to release the neuNT cDNA and was treated with mung bean nuclease to remove 3’ and 5’ overhangs, generating a blunt-end cDNA. Treatment with Antarctic alkaline phosphatase removed terminal phosphates allowing neuNT CDNA to be TOPO cloned into pFastBac/CT-TOPO vector. The recombinant bacmid was transfected into the Sf9 cell line to produce P1baculovirus stock expressing HER2/neu (bac HER2/neu), which was further amplified with several rounds of infection of SF21 cells. Bac HER2/ neu was used to infect SF21 cells. Infected HER2/neu SF21 cells were pelleted and used to generate SF21 neu lysate for restimulation and enzyme-linked immunosorbent assay as described below.

**Animal experiments**

Four to 6-week-old FVB/N mice (Jackson Labs, Bar Harbor, ME, USA) were maintained in a high-efficiency particulate air-filtered cage system for at least 1 week prior to use. For in vivo experiments involving tumor-bearing mice, anesthetized FVB/N mice were injected using a 27-gauge tuberculin syringe (Becton Dickinson, Franklin Lakes, NJ, USA) into the right second mammary fat pad with 2 × 10\(^{6}\) NBT1 cells suspended in Hank’s balanced salt solution (Sigma-Aldrich) as previously described.\(^\text{26}\) For in vivo vaccination studies in naive FVB/N mice, mice received 2 subcutaneous (s.c.) or intramammary fat pad (i.m.f.) injections, 2 weeks apart, of 1 × 10\(^{6}\) p.f.u. VwNeu or VβGal using a 27-gauge tuberculin syringe. In in vivo vaccination studies of NBT1 tumor-bearing mice, mice were treated with 2 s.c. or intratumoral (i.t.) injections, 2 weeks apart, of 1 × 10\(^{6}\) p.f.u. VwGMCSF plus 7.5 μg keyhole limpet hemocyanin (KLH) (Sigma-Aldrich) with each injection of 1 × 10\(^{6}\) p.f.u. VwNeu or VβGal. For vaccinations, i.t. injections were made directly into the number 2 fat pad. All i.t. injections were made directly into the mammary fat pad tumors. S.c. injections were placed in the contralateral (left) groin. All viral injections used the same total of 2 × 10\(^{6}\) vaccinia p.f.u. Five animals per group were used for each independent in vivo experiment. Animal experiments were conducted in accordance with protocols approved by the Rutgers Institutional Animal Care and Use Committee.

**CTL assays**

CTL assays were performed as previously described,\(^\text{21,22}\) with several modifications. Briefly, effector cells were prepared from spleenocytes, vaccination site-draining lymph nodes (VSN) or tumor-draining lymph nodes of treated/vaccinated FVB/N mice. Splenocytes or lymph nodes were homogenized and red blood cells were lysed using ammonium chloride buffered (ACK buffer, 0.15 M NaCl, 1.0 mM KHCO\(_3\) and 0.1 mM EDTA), washed with tissue culture medium (TCM) and filtered through a 70-μm nylon mesh (BD Biosciences, San Jose, CA, USA). Effector cells were resuspended at 7 × 10\(^{6}\) cells/ml and cultured with 3 × 10\(^{5}\) cells ml\(^{-1}\) of irradiated (25 Gray) splenocytes from naive FVB/N mice that had been incubated overnight in 50 ml conicals at 37 °C at 4 × 10\(^{5}\) cells ml\(^{-1}\) in TCM+2-mercaptoethanol with 300 μg ml\(^{-1}\) of the RNeU\(_{420-429}\) (PDSRLDLSSFV) (GenScript, Piscataway, NJ, USA) immunodominant epitope of the rat neu protein,\(^\text{23}\) lymphocytic choriomeningitis virus nucleoprotein N\(_{118-126}\) (RPQASGVYM) control

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peptide (Genscript) or indicated cell lysates. Restimulation cultures were in a total of 2 ml of TC19-2-mercaptoethanol in 24-well plates for 5 days at 37 °C, 5% CO₂. On day 3 of restimulation, 50 µl of supernatant was assayed for interferon-γ using an enzyme-linked immunosorbent assay, as described previously.²⁶ On day 5, stimulated effector cells were collected and cultured for 4 h with ³¹Cr-labeled NBT1 cells as targets. Hundred µl of supernatant were removed and ⁵¹Cr release of target cells was measured with a gamma counter (Packard Bioscience, Meriden, CT, USA). Percent-specific lysis was calculated from the formula [(experimental release – spontaneous release) × 100/(maximal release in 1% SDS – spontaneous release)].

Magnetic bead depletion of MDSCs
MDSCs were depleted from the splenocyte population of NBT1 tumor-bearing mice using a mouse MDSC isolation kit (Miltenyi Biotec, Auburn, CA, USA) as per the manufacturer’s instructions. Briefly, 1 × 10⁸ splenocytes were resuspended in 350 µl MACS buffer (phosphate-buffered saline, 0.5% bovine serum albumin and 2 mM EDTA) and blocked for 10 min with 50 µl FcR blocking reagent at 4 °C. Hundred µl of anti-Ly6G-biotin was added and incubated for 10 min at 4 °C. Cells were washed in 10 ml MACS buffer and resuspended in 100 µl MACS buffer. Two hundred µl anti-biotin microbeads were added and incubated at 4 °C for 15 min. Magnetic separation was performed using LS disposable column and MACS Magnetic separator (Miltenyi Biotec). The negative fraction was collected and restimulated as described above. MDSC depletion was validated using flow cytometry.

Flow cytometry
Flow cytometry for RNeLou20-429 tetramer (provided by the NIAID Tetramer Facility, Atlanta, GA, USA) was conducted as described previously.²¹ Briefly, effectors from treated mice were restimulated for 5 days as described above. Effectors were collected, washed and resuspended in phosphate-buffered saline/5% fetal bovine serum with 0.1% w/v sodium azide at 1 × 10⁸ cells per 100 µl. Cells were stained with anti-CD8a-fluorescein isothiocyanate, anti-CD69 PE-Cy7, anti-Ly6G APC, anti-CD11b PE-Cy7 and anti-Ly6C PE-Cy7 and cultured for 4 h with ⁵¹Cr-labeled NBT1 cells as targets. Hundred µl MACS buffer (phosphate-buffered saline, 0.5% sodium azide at 1 × 10⁸ cells per 100 µl) of supernatant was assayed to determine whether the presence of orthotopic NBT1 tumor affected levels of MDSC in FVB/N mice, we injected FVB/N mice i.m.f. VVneu+VVGMCSF groups compared with controls were significant (***P < 0.01, *P < 0.05). Results are representative of three independent experiments.

Statistical analysis
Results were expressed as mean ± s.e. Significance for experiments with > 2 conditions (P < 0.05) was determined by analysis of variance with the post hoc Tukey multiple comparisons test using the Instat software package (GraphPad Software, La Jolla, CA, USA). Significance for experiments with two conditions was determined using the Student’s T-test, two-tailed with unequal variances in Microsoft Excel (Microsoft, Redmond, WA, USA). Kaplan–Meier survival curves were generated using Prism (GraphPad Software), with significance calculated using the log-rank (Mantel–Cox) test and correction for multiple comparisons using the Bonferroni method.

RESULTS
Vaccination of naive FVB/N mice results in a systemic HER2-specific Th1 response
We hypothesized that vaccination of a naive host with a vaccinia construct expressing HER2/neu would induce an antigen-specific Th1 immune response, regardless of the site of vaccination. We utilized i.m.f. injection or contralateral s.c. injection of VVneu + VVGMCSF in naive FVB/N mice. Two weeks after the final vaccination, splenocytes and draining lymph nodes of the vaccination site (VDN) were restimulated with irradiated splenocytes from naive female FVB/N mice that had been pulsed with immunodominant RNeLou20-429 peptide. (b) NBT1 tumor-specific systemic (spleen) cytotoxic T lymphocyte (CTL) activity against NBT1 target cells after restimulation. Differences of both s.c. and i.m.f. VVneu+VVGMCSF groups compared with controls were significant (***P < 0.01, *P < 0.05). Results are representative of three independent experiments.

MDSCs suppress the systemic antitumor immune response
MDSCs are a heterogeneous population of immature granulocytic and monocytic cells that have inhibitory effects on tumor-specific T-cell activation and function.⁵,⁶,³⁰ Given previous studies demonstrating the importance of a type 1 antitumor response in effective immune therapies,²¹,²⁸ we hypothesized that MDSCs inhibited a systemic antitumor immune response in the NBT1 model. To determine whether the presence of orthotopic NBT1 tumor affected levels of MDSC in FVB/N mice, we injected FVB/N mice with NBT1 cells in the right number 2 mammary fat pad. After 4–5 weeks of tumor growth, we used flow cytometry to measure levels of Gr1, Ly-6G, Ly-6C and CD11b on cells from spleen and tumor. The CD11b⁺Gr1⁺ population was significantly increased in the systemic population of tumor-bearing mice compared with naive mice (Figure 2a). A large population of CD11b⁺Ly-6C⁺Ly6G⁺ cells was detected, a phenotype consistent with granulocytic MDSCs (gMDSC, Figure 2b).

To demonstrate the immune-suppressive nature of the MDSCs induced by NBT1, we used a MDSC magnetic bead isolation kit
(Miltenyi Biotec, Auburn, CA) to deplete Ly6G+ cells from the splenocyte population from NBT1 tumor-bearing mice. We validated depletion of MDSCs from the negative fraction using flow cytometry staining for CD11b, Gr-1, Ly6G and Ly6C (Figure 3a). We then restimulated this MDSC-depleted splenocyte population with irradiated splenocytes from naive female FVB/N mice that had been pulsed with the immunodominant major histocompatibility complex (MHC) class I RNEU[420-429] peptide or HER2/neu protein in Sf21 neu baculovirus-infected lysate, with NP[118-126] peptide and unmodified Sf21 cells used as restimulation controls. Interferon-γ production by MDSC-depleted splenocytes was increased in most conditions compared with pre-depletion controls, with HER2/neu-restimulated effectors significantly increased compared with all other conditions (Figure 3b).

Vaccination with VVneu+VVGMCSF into the tumor microenvirnment, but not VVGMCSF alone, results in a systemic HER2-specific CTL response, decreased MDSCs and tumor regression. We asked whether vaccination with HER2/neu-antigen-encoding VVneu and VVGMCSF could overcome MDSC-associated anergy against HER2/neu induced by the NBT1 model. After 2 weeks of
orthotopic NBT1 tumor growth, two doses of VVneu+VVGMCSF+KLH, 2 weeks apart, were administered subcutaneously or by injection into the tumor microenvironment (i.t., Figure 4a). KLH was added to all treatment groups and controls, as previous studies by our group and others had demonstrated its effectiveness as a vaccine adjuvant.21,31,32 Two weeks after the final vaccination, splenocytes and VDN were restimulated with irradiated splenocytes from naive female FVB/N mice that had been pulsed with immunodominant RNEU 420–429 peptide. Spleenocytes from mice vaccinated with i.t. VVneu+VVGMCSF+KLH showed a significantly increased ability to lyse target NBT1 cells after in vitro restimulation when compared with both equivalent s.c. vaccination and i.t. VVBGal+VVGMCSF+KLH (Figure 4b). Similarly, cytolytic activity of the VDN population was highest in mice given i.t. VVneu+VVGMCSF+KLH (Figure 4c). Increased CTL activity was specific to the RNEU420–429 MHC class I epitope, as restimulation with control NP118–126 did not result in increased cytolytic activity (not shown). The percentage of restimulated CD8+ lymphocytes that were RNEU tetramer-positive from both spleen and VDN was significantly higher in mice that were treated with i.t. VVneu+VVGMCSF+KLH than in all other groups (Figure 4d).

MDSC levels were evaluated using flow cytometry. In mice treated with i.t. VVneu+VVGMCSF+KLH, MDSC levels in spleen on day 28 were significantly decreased compared with i.t. vehicle (Figure 5). Systemic MDSC levels were not significantly decreased in mice treated with s.c. VVneu+VVGMCSF+KLH. Notably, tumor-bearing mice treated with i.t. VVBGal+VVGMCSF+KLH exhibited a significant increase in intratumoral, although not systemic, MDSC levels (Figure 5). A potential explanation may be that, in addition to its enhancing effects on antigen presentation, GM-CSF is one of multiple cytokines that support development of MDSCs.30 Primary tumors in mice vaccinated with i.t. VVneu+VVGMCSF+KLH regressed from peak size and were significantly smaller than all other treatment conditions on day 42, including equivalent s.c. VVneu and i.t. VVGMCSF without antigen (Figures 6a–c). S.c. VVneu+VVGMCSF+KLH vaccination or i.t. VVBGal+VVGMCSF+KLH did not inhibit NBT1 growth.

**DISCUSSION**

The studies presented here demonstrate, using an aggressive orthotopic model of HER2/neu-driven mammary tumor (NBT1), that growth of the tumor results in a significant infiltration of intratumor and systemic MDSC, resulting in the lack of
The development of systemic immunity and anergy to peripheral s.c. immunization using a neu-encoding vaccinia vaccine that is effective in naive (non-tumor bearing) mice. Treatment of tumor-bearing mice using i.t. oncolytic VVGMCSF fails to induce systemic immunity nor does it lead to tumor regression. In fact, intratumoral VVGMCSF and resulting GM-CSF expression in tumor leads to a significant expansion of tumor resident MDSC, as suggested by the Vonderheide group. However, when tumor antigen-encoding VVneu is combined with VVGMCSF, thus providing a virally expressed tumor antigen to the tumor microenvironment, the combination results in the generation of systemic neu-specific immunity, a significant reduction in tumor and systemic MDSC and a significant antitumor response. We confirmed the contribution of MDSCs to immune escape in our model using flow cytometry to identify a population of CD11b+Ly-6CintLy-6G+ cells. We also demonstrated that these cells have suppressive effects by finding, despite an overall increased background of IFN-γ in MDSC-depleted cultures, increased HER2/neu-specific IFN-γ production of the systemic splenocyte population after depletion of Ly-6G+ cells.

The goal of tumor immunotherapy is the development of modalities given alone or in combination that result in the generation of an effective systemic tumor-specific immune response by the host that can overcome immune escape mechanisms. Original studies from our laboratory using a murine bladder cancer model demonstrated the unexpected expansion of antigen-specific (tetramer positive) CD8+ T cells in the tumor microenvironment, but not systemically. However, when tumor antigen-encoding VVneu was combined with VVGMCSF, the combination resulted in the generation of systemic neu-specific immunity, a significant reduction in tumor and systemic MDSC and a significant antitumor response. We confirmed the contribution of MDSCs to immune escape in our model using flow cytometry to identify a population of CD11b+Ly-6CintLy-6G+ cells. We also demonstrated that these cells have suppressive effects by finding, despite an overall increased background of IFN-γ in MDSC-depleted cultures, increased HER2/neu-specific IFN-γ production of the systemic splenocyte population after depletion of Ly-6G+ cells.

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boosting immunizations were given intratumorally. These initial responses to intratumoral poxvirus-based vaccines have been translated to phase I trials in prostate cancer and by us in pancreatic cancer using antigen-encoding non-replicating fowl-pox vectors.

The strategy of delivering an oncolytic virus to a tumor has long been studied with the overall hypothesis being that virus-mediated oncolysis could have primary antitumor effectiveness and as a sequellae of tumor lysis, antigen released into the tumor microenvironment could lead to a consolidating systemic antitumor response active against metastases. As recently reviewed by Lichty et al., preclinical and clinical studies are ongoing that use a variety of oncolytic vectors and encoded immune regulatory molecules. Our initial studies focused on the
potential of generating systemic immunity using the oncolytic WGMCSF, which would have the potential of lysing tumors, eliciting antigens and enhancing antigen presentation via the GM-CSF. This agent studied by others as JX-594 was subsequently characterized as to its bioavailability, and antitumor and immune responses.19,20,38 Of particular note in these studies, JX-594 was shown to preferentially persist and replicate in the tumor microenvironment following intravenous administration in preclinical models and in patients.19,20 Given that accessibility of tumor for local administration is a limitation to the use of these constructs (OncoVEXGM-CSF) have also demonstrated local infection alone, and supports future approaches combining this with added administration.19,20 These combined studies support the conclusion of the preclinical and clinical studies combining the oncolytic virus with other modalities such as chemotherapy and immune checkpoint inhibitors are also under development and ongoing by a number of investigators.41,42

In conclusion, the studies presented here provide support for delivering to the tumor microenvironment a combination of virally encoded tumor antigen and immunomodulating GM-CSF, both encoded by the oncolytic vaccinia vector. Our studies demonstrate the requirement for antigen delivered to the microenvironment in overcoming MDSC-based immune escape and subsequent development of a systemic antitumor immune response in our model. Our and others’ studies have proven the feasibility of using DNA viral vectors to deliver multiple gene constructs to the tumor microenvironment via direct injection18,20,38 and in some cases following intravenous administration.19,20 These combined studies support the conclusion that this approach has the potential of impacting tumor alone, and supports future approaches combining this with added immune targeted and antitumor approaches.

CONFLICT OF INTEREST
Dr Lattime is an inventor of the patented recombinant Vaccinia-GMCSF that has been licensed to Sillajen and is being studied as JX-594. As such, he derives royalties and licensing fees from the Thomas Jefferson University where the patent is held. The remaining authors declare no conflict of interest.

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