Cancer immunotherapy with recombinant poliovirus induces IFN-dominant activation of dendritic cells and tumor antigen–specific CTLs

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Tumors thrive in an immunosuppressive microenvironment that impedes antitumor innate and adaptive immune responses. Thus, approaches that can overcome immunosuppression and engage antitumor immunity are needed. This study defines the adjuvant and cancer immunotherapy potential of the recombinant poliovirus/rhinovirus chimera PVSRIPO. PVSRIPO is currently in clinical trials against recurrent World Health Organization grade IV malignant glioma, a notoriously treatment-refractory cancer. Cytopathogenic infection of neoplastic cells releases the proteome and exposes pathogen- and damage-associated molecular patterns. At the same time, sublethal infection of antigen-presenting cells, such as dendritic cells and macrophages, yields potent, sustained type I interferon–dominant activation in an immunosuppressed microenvironment and promotes the development of tumor antigen–specific T cell responses in vitro and antitumor immunity in vivo. PVSRIPO’s immune adjuvancy stimulates canonical innate anti-pathogen inflammatory responses within the tumor microenvironment that culminate in dendritic cell and T cell infiltration. Our findings provide mechanistic evidence that PVSRIPO functions as a potent intratumor immune adjuvant that generates tumor antigen–specific cytotoxic T lymphocyte responses.

INTRODUCTION

The tumor microenvironment favors tumor immune escape by suppressing production, activation, and/or function of antitumor T cells. Therapeutic interception of inhibitory immune receptors (immune checkpoints) that block T cell activation has yielded unprecedented clinical benefit. However, a limitation of immune checkpoint inhibitors, specifically those that target PD1, is their reliance on preexisting antitumor immune responses and the presence of CD8 T cells in the tumor (1). Moreover, resistance to checkpoint blockade can occur through multiple immunosuppressive pathways, particularly when blocking only one of many repressive nodes (2). Thus, strategies to reverse tumor immunosuppression, stimulate antigen presentation, and induce antitumor T cell responses are needed. Tumor antigen–specific approaches, such as dendritic cell (DC) vaccines, aim to elicit antigen-specific antitumor immunity (3). However, such approaches are ultimately limited by profound tumor immunosuppression (4, 5). A reversal of tumor immunosuppression, the activation of antigen presentation, and the generation of antitumor immunity might be achieved by provoking canonical innate antitumor responses within tumors.

Viral targeting of tumors may engage immune responses in a pattern and intensity that mirrors natural infection, including innate immune activation capable of instigating the transition to adaptive immunity. Antiviral effector responses (especially CD8 T cells), geared to stop viral spread by killing infected cells, engage the very mechanisms capable of killing cancer cells. However, the use of viruses for this purpose depends on their ability to effectively stimulate adaptive immunity. This is important, because viruses commonly use elaborate strategies to restrict their inflammatory footprint and prevent antigen presentation as a means to avoid immune rejection and establish long-term host relationships (6). We hypothesized that intratumoral administration of an oncolytic poliovirus (PV) is a rational approach to accomplish these goals due to (i) targeting of CD155 on malignant cells and antigen-presenting cells, such as dendritic cells and macrophages, yields potent, sustained type I interferon–dominant activation in an immunosuppressed microenvironment and promotes the development of tumor antigen–specific T cell responses in vitro and antitumor immunity in vivo. PVSRIPO’s immune adjuvancy stimulates canonical innate anti-pathogen inflammatory responses within the tumor microenvironment that culminate in dendritic cell and T cell infiltration. Our findings provide mechanistic evidence that PVSRIPO functions as a potent intratumor immune adjuvant that generates tumor antigen–specific cytotoxic T lymphocyte responses.

RESULTS

PVSRIPO targets human cancer cells

PVSRIPO is the live-attenuated type 1 (Sabin) PV vaccine containing an internal ribosomal entry site (IRES) from its relative, HRV2 (8). The HRV2 IRES is inherently neuron-incompetent by virtue of cell type–specific ribonucleoprotein complexes intercepting ribosome
recruitment (12, 13). Thus, PVSRIPO does not cause encephalomyelitis or poliomyelitis in primates (14).

Constitutively active Raf–ERK1/2 (extracellular signal–regulated kinase 1/2)–MNK1/2 (mitogen-activated protein kinase–interacting serine/threonine-protein kinase 1/2) signals favor selective PVSRIPO translation and cytotoxicity in cancer cells (15–17). To explore PVSRIPO oncolysis in cancers beyond gliomas (7), we examined the course of PVSRIPO translation and cytopathogenicity in melanoma, prostate, and breast cancer cell lines (Fig. 1). Viral translation (exemplified by 2C and its 2BC/P2 precursors) yields cytopathogenic viral proteases,
whose actions, such as early cleavage of the translation initiation scaffold eIF4G (eukaryotic translation initiation factor 4G), suppress host protein synthesis (Fig. 1A) (18). This coincided with other signs of host cell damage, including poly(adenosine diphosphate–ribose) polymerase (PARP) cleavage. PVSRIPO-induced eIF2α(S51) phosphorylation is likely due to the double-stranded RNA (dsRNA)–activated protein kinase (Fig. 1A). At ≥48 hours post-infection (hpi), host protein synthesis inhibition and cell lysis diminished all proteins (see loss of tubulin loading control at late hpi; Fig. 1A). This cytopathogenicity pattern mirrors PVSRIPO’s effects in glioma cells (Fig. S1A) (7). Flow cytometry analysis of major histocompatibility complex (MHC) class I and CD155 surface expression pre- and post-PVSRIPO confirmed CD155 expression in all cell lines (Fig. 1B). MHC class I was present on all cell lines pre- or post-infection (Fig. 1B). It is unclear whether a decline in surface CD155 after infection of most cell lines (Fig. 1B) reflects global loss of protein from samples or a specific host response.

**PVSRIPO oncolysis releases the cancer cell proteome and dsRNA**

To unravel inflammatory perturbations due to PVSRIPO oncolysis, we examined the release of damage/pathogen-associated molecular patterns (D/PAMPs) and tumor antigens in two cell lines: DM6 melanoma and MDA-MB231 triple-negative breast cancer (Fig. 2). Analyses of cell pellets or supernatants by silver stain revealed that PVSRIPO infection releases the proteome (Fig. 2A), resulting in the appearance of the tumor-associated antigen MART1 (melanoma-associated antigen recognized by T cell 1) (DM6) or CEA (carcinoembryonic antigen) (MDA-MB231) in the supernatant (Fig. 2B). DAMPs, such as heat shock protein (HSP) 60/70/90, were detected in supernatants ≥24 hpi with PVSRIPO (Fig. 2B). HSPs have been shown to mediate antitumor immunity against HSP-bound peptides (19). Another DAMP, HMGBl (high-mobility group box 1 protein) (20), was released only by infected DM6 cells, possibly due to variable expression (21). Loss of loading controls from cell pellets into the supernatant for cytoplasmic (tubulin), nuclear (PARP), mitochondrial (HSP60), and plasma membrane [Na+,K+-dependent adenosine triphosphatase (Na+,K+-ATPase)] subcellular fractions indicates sweeping destruction affecting all cellular compartments (Fig. 2B). We also examined the release of dsRNA from infected cells. dsRNA is a PV replicative intermediate (22) and an agonist for pattern recognition receptors Toll-like receptor 3 (TLR3) and melanoma-derived antigen 5 (MDA5) (23). Dot blots of mock-versus virus-infected cancer cell lysates probed with an anti-dsRNA antibody revealed dsRNA presence in the supernatant of infected cells, indicating that PVSRIPO replication intermediates are released after cell lysis (Fig. 2C). Performing these assays in DU54 glioblastoma cells revealed similar results (fig. S1, B and C). Thus, PVSRIPO-mediated killing of malignant cells exposes host tumor antigens, DAMPs, and PAMPs.

**Fig. 2. PVSRIPO oncolysis releases tumor antigens, dsRNA, and DAMPs.** DM6 and MDA-MB231 cells were infected with PVSRIPO. (A) Silver stain was performed on oncolysates/corresponding supernatants. (B) Immunoblots from oncolysates/corresponding supernatants for tumor antigens (MART1/CEA), DAMPs (HSPs and HMGBl), HSP60 (mitochondrial), Na+,K+-ATPase (membrane-associated), PARP (nucleus), and tubulin (cytoplasm). (C) Immunoblot for dsRNA was performed from oncolysates/corresponding supernatants of DM6 and MDA-MB231 cells (48 hpi); Ponceau S stain serves as the loading control. Loss of total protein late during PVSRIPO infection corresponds with cell lysis. All experiments were repeated twice, and a representative series is shown.
PVSRIPO activates DCs

The presence of PAMPs and DAMPs in PVSRIPO oncolysate may recruit and/or activate APCs and innate cell populations, which could promote adaptive antitumor immunity. Because APCs, particularly DCs, are essential enablers of tumor-specific responses (24, 25), we tested the effects of PVSRIPO oncolysis on human DCs. This is especially relevant, because APCs express CD155 and are susceptible to productive and lethal infection with PV (26). DM6 supernatant was collected 48 hours after mock or PVSRIPO infection (hereafter referred to as “oncolysate”) and coincubated with human monocyte-derived DCs (24 hours). Untreated immature DCs (iDCs) and cytokine cocktail (CC)–treated mature DCs (mDCs) were used as negative and positive controls (27), respectively, to compare DC activation phenotypes. In addition, we tested corresponding mock controls and oncolysates in which PVSRIPO was removed using a 100-kDa cutoff filter before coincubation with DCs (Fig. 3, A and B).

Exposure to the oncolysate (24 hours) increased the percentage of DCs expressing costimulatory/activation markers CD40, CD80 (B7-1), and CD83 (Fig. 3A) and increased the production of cytokines IFN-β and TNF-α (tumor necrosis factor–α) (Fig. 3B). Interleukin-12 (IL-12) induction was modest. Virus removal through the 100-kDa cutoff filter mitigated these effects (Fig. 3, A and B). The costimulatory molecule CD86 (B7-2) is high on both iDCs and mDCs, as previously shown (27), and as expected, none of the treatments affected CD86 expression. A representative flow cytometry analysis illustrating DC phenotype is depicted in fig. S2. Our findings suggest that PVSRIPO oncolysate activates DCs and that the presence of (infectious) virus contributes to this effect. Stimulation of iDCs with PVSRIPO oncolysate or purified virus [adjusted to the same concentrations of plaque-forming units (pfu)] revealed similar levels of IFN-β and TNF-α secretion (Fig. 3C). As shown in Fig. 3B, we observed modest IL-12 production in DC-stimulated PVSRIPO oncolysate; however, IL-12 production was negligible with PVSRIPO alone at 24 hpi (Fig. 3C). Analysis of costimulatory/activation markers at 24 hpi demonstrated that CD40/CD80/CD83 induction was less pronounced with PVSRIPO infection alone compared to exposure to PVSRIPO oncolysate (Fig. 3D), indicating that nonviral oncolysate components contribute to DC activation. Together, our data suggest that DC activation caused by PVSRIPO oncolysis occurs predominately via direct infection, which induces a potent type I IFN response. This is of relevance to cancer immunotherapy, because type I IFN responses are essential for DC function and the transition to adaptive immune responses (28–31).

PVSRIPO infection of DCs induces sustained type I IFN-dominant responses, costimulatory molecule expression, and cytokine production

To decipher the effects of PVSRIPO on DCs over time, we examined markers of cytopathogenicity and activation status after iDC infection.
at MOIs of 1 or 10 (Fig. 4). In contrast to DM6 and MDA-MB231 cancer cells, PVSRIPO did not cause lysis of DCs as measured by lactate dehydrogenase (LDH) release (Fig. 4A; magnified view in fig. S3A), and viral propagation was only marginally productive in DCs (Fig. 4B; magnified view in fig. S3B). Viral translation was active in DCs, evident as detectable viral protein 2C at 8 hpi (Fig. 4C), but declined over time. Despite active viral translation (implying viral protease release), there was no elf4G cleavage, even at an MOI of 10 (Fig. 4C; compare to Fig. 1A). Thus, PVSRIPO infects DCs, but DCs are resistant to viral cytopathogenic damage/host protein synthesis inhibition. An earlier study using the same DC differentiation technique and infection procedure demonstrated efficient viral growth, host protein synthesis shutdown, and DC killing with wild-type PV type 1 (Mahoney) and the type 1 (Sabin) PV vaccine (26). Thus, our data imply that, similar to neuronal lineage cells (32), the foreign HRV2 IRES in PVSRIPO mediates loss of cytopathogenicity and intrinsic host resistance in DCs.

Accordingly, rather than viral cytotoxicity, the most prominent effect of PVSRIPO infection in DCs was a robust, durable type I IFN response, indicated by STAT1(Y701) phosphorylation with STAT1 and IFN-stimulated gene (ISG) induction (IFIT1 and ISG15; Fig. 4C). We observed substantial up-regulation of TAP1 (Fig. 4C), a protein required for MHC class I–restricted antigen processing in cells, likely induced by IFN-β (33). These changes, indicative of an enhanced DC activation status, were accompanied by induction of CD40 (Fig. 4C). Earlier work showed that IFN-β triggers programmed cell death lig-1 (PD-L1) (or B7-H1) expression on APCs (34). Consequently, PVSRIPO infection induced PD-L1 in DCs, in a pattern coinciding with ISG expression/IFN signaling (Fig. 4C).

We next compared the adjuvant effect of PVSRIPO on DCs with standard methods of maturing and activating human iDCs via TLR stimulation pathways with lipopolysaccharide (LPS), polyinosinic:polycytidylic acid [poly(I:C)], or maturation CC [used in clinical applications of DC-based vaccines (27)]. We treated DCs with PVSRIPO/TLR stimuli over a period of 5 days (Fig. 4, D and E). Compared to TLR stimuli, PVSRIPO infection was distinguished by potent, sustained STAT1(Y701) phosphorylation and ISG induction throughout the 5-day interval (Fig. 4D; repeat data shown in fig. S3C). This coincided with IFN-β release at levels exceeding all other stimuli (Fig. 4E). Poly(I:C) exhibited some IFN-β production, albeit at reduced levels and duration; neither LPS nor CC caused IFN-β production (Fig. 4, D and E) but induced STAT3(Y705) phosphorylation and TNF-α production (fig. S3, D and E). PVSRIPO infection of DCs also led to higher levels and sustained production of IL-12 relative to poly(I:C) or the other stimuli (Fig. 4E). Notably, IL-12 production spiked after 24 hours, which explains modest IL-12 release shown in Fig. 3 (B and C), where analyses were conducted for 24 hours only. CC-stimulated mDCs were not assayed for cytokine production in Fig. 4E, because the cocktail contains TNF-α; we tested them for IFN-β and IL-12 production and confirmed a lack of type I IFN activity (Fig. 4D).

PVSRIPO infects and activates immunosuppressed DCs and macrophages

APC activation is hampered in the immunosuppressive context of the tumor microenvironment. We tested the effects of PVSRIPO and poly(I:C) on iDCs cultured for 24 hours in plain AIM V medium or in cancer cell–conditioned medium [AIM V medium harvested from DM6 melanoma cells after 48 hours in culture (DM6CM)]. DM6CM contained a range of cytokines implicated in APC suppression and other immune cell functions (table S1). iDCs incubated in DM6CM showed reduced basal levels of CD40, CD80, and CD86 compared to iDCs incubated in plain AIM V (Fig. 5A; similar to mock–infected oncolysate in Fig. 3A). Despite this, PVSRIPO infection enhanced the expression of CD40, CD80, and CD86 in DM6CM–exposed iDCs, equivalent to the effects on iDCs cultured in plain AIM V medium (Fig. 5A). In contrast, costimulatory molecule expression upon poly(I:C) treatment was markedly reduced in DM6CM–cultured iDCs compared to AIM V–cultured iDCs (Fig. 5A; representative flow cytometry analysis is shown in fig. S4).

As in previous analyses (Fig. 4E), only PVSRIPO infection of DCs, but not poly(I:C) stimulation, caused potent, sustained IFN-β release (Fig. 5B). DCs cultured in AIM V medium released IL-12 and TNF-α after PVSRIPO infection or poly(I:C) addition (Fig. 5B). In contrast, DCs cultured in DM6CM did not produce appreciable amounts of IFN-β, IL-12, or TNF-α after poly(I:C) treatment but retained IFN-β, IL-12, and TNF-α release after PVSRIPO infection (Fig. 5B). Although PVSRIPO–induced IFN-β and IL-12 production was tempered in DM6CM–cultured DCs, compared to AIM V–cultured DCs, TNF-α production was actually enhanced (Fig. 5B). These data suggest that although DM6CM–suppressed DCs are resistant to poly(I:C) stimulation, they retain sensitivity to PVSRIPO–mediated activation.

To extend these findings, we investigated the effect of PVSRIPO on DM6 cells with and without DC coculture. Regardless of the presence of DCs, infection of DM6 cells with PVSRIPO resulted in complete cell lysis (48 hpi) as indicated by the shift of MART1 (a melanoma-specific antigen) from the cell pellet to the supernatant fraction of infected cultures (Fig. 5C; also shown in Fig. 2B). Retention of the tubulin immunoblot signal after PVSRIPO infection only in DC/DM6 cocultures (48 hpi) was due to the inherent resistance of DCs to PVSRIPO killing. Consistent with type I IFN-dominant DC activation upon PVSRIPO infection, we observed potent up-regulation of CD40, TAP1, and p-STAT1(Y701) in DC/DM6 cocultures (48 hpi) (Fig. 5C). Similar to infection of DCs alone (Fig. 4E), PVSRIPO infection of DC/DM6 cocultures resulted in the release of IFN-β, IL-12, and TNF-α (Fig. 5D). DC/DM6 coculture reduced viral titers in culture supernatants ~70-fold (fig. S5A). These data suggest that (i) DCs remain unharmed in the presence of active PVSRIPO lysis of cocultured cancer cells; (ii) PVSRIPO activates DCs, even in the presence of cancer cell–mediated immunosuppression; (iii) DC coculture does not prevent PVSRIPO from killing tumor cells; and (iv) the principal source for proinflammatory cytokines in DC/DM6 cocultures is PVSRIPO–infected DCs.

Because tumor–associated macrophages are implicated in tumor immunosuppression (35), we investigated the effects of PVSRIPO infection on macrophages in vitro. Purified human monocytes were differentiated to macrophages with macrophage colony-stimulating factor (MCSF) alone or with MCSF plus transforming growth factor–β (TGF-β), IL-10, and IL-4 (Fig. 5E). The latter induced an immunosuppressive phenotype in vitro (36, 37), associated with reduced basal expression of IFIT1, TAP1, and CD36, a scavenger receptor associated with macrophage activity (38). As in DCs, PVSRIPO infection of macrophages yielded robust type I IFN responses and TNF-α production that was paradoxically enhanced by TGF-β/IL-10/IL-4 treatment (Fig. 5E) and above-threshold IFN-β release coincided with enhanced viral translation and overt cytopathogenicity (elf4G cleavage and tubulin loss) upon prolonged infection (Fig. 5, E and F). Viral translation and cytopathogenicity were not detected in PVSRIPO–infected macrophages derived with MCSF only (Fig. 5E). Treatment of macrophages with combined poly(I:C) and LPS induced IL-1β, IFN-β, and TNF-α responses in MCSF–derived macrophages (Fig. 5, E and F). However, TGF-β/IL-10/IL-4
immunosuppression of macrophages reduced IL-1β, IFN-β, and TNF-α production upon treatment with combined poly(I:C)/LPS at 72 hours (Fig. 5, E and F). Analysis of human monocytes without differentiation revealed similar type I IFN responses to PVSRIPO infection as in MCSF-derived macrophages (fig. S5B). These findings suggest that PVSRIPO infection of macrophages (and monocytes) yields type I
Fig. 5. PVSRIPO-mediated APC activation occurs in immunosuppressive conditions. (A and B) DCs were cultured in the presence of AIM V or DM6 (24 hours) and untreated (mock) or treated with PVSRIPO (MOI, 10) or poly(I:C) (10 μg/ml) (48 hours). (A) Cells were analyzed for activation markers by flow cytometry. Data bars represent the mean of two independent experiments, and error bars denote SEM. Asterisks depict significance as determined by ANOVA protected Tukey’s post hoc test. For representative flow cytometry data, see fig. S4. (B) Supernatant from (A) was tested for proinflammatory cytokine production by ELISA. (C and D) DM6 cells cultured alone or with DCs were mock-treated or infected with PVSRIPO (MOI, 10). (C) Supernatant was tested for lytic release of MART1 by immunoblot; cell pellets were tested for markers of DM6 cells (MART1) and DC activation [CD40, TAP1, p-STAT1(Y701), and STAT1]. (D) Supernatants from (C) were assessed for proinflammatory cytokine production. (E and F) Negatively selected human monocytes were differentiated with MCSF (25 ng/ml) or MCSF + IL-10, TGF-β, and IL-4 (all at 20 ng/ml) for 7 days. Cells were infected with PVSRIPO (MOI, 10) or treated with combined poly(I:C) (10 μg/ml) and LPS (100 ng/ml) as shown. Cell lysates were retained for immunoblot (E), and supernatants were used for ELISA (F). (B, C, and E) Experiments were repeated three times, and representative data are shown. (D and F) Data bars represent the mean of two independent experiments, and error bars indicate SEM.
IFN-dominant activation that is paradoxically enhanced by an immunosuppressive macrophage phenotype.

**PVSRIPO oncolysate–treated DCs induce antigen-specific cytotoxic T cells in vitro**

Our experiments thus far show that PVSRIPO effectively damages tumor cells, releases tumor antigens, and simultaneously induces DC and macrophage activation, including in the context of cancer immunosuppression. Therefore, to test the downstream effects of PVSRIPO tumor cell killing with simultaneous infection and DAMP/PAMP activation of DCs, we examined whether human DCs exposed to PVSRIPO oncolysate pick up released tumor antigen, present it, and prime autologous T cells using an in vitro DC–T cell stimulation assay (Fig. 6A).

Experiments were conducted using human leukocyte antigen (HLA)–A2+ peripheral blood mononuclear cells (PBMCs) obtained from healthy donors (n = 2) and human HLA-A2+ tumor cell lines: SUM149 inflammatory breast cancer, MDA-MB231 triple-negative breast cancer, DM6 melanoma, and LNCaP prostate cancer (Fig. 6B). These cell lines express class I MHC, required for CD8+ T cell recognition of antigens (Fig. 1B). DCs were first treated with supernatant from mock- or PVSRIPO-infected tumor cells (48 hpi), harvested, and then cocultured with autologous T cells (nonadherent fraction of PBMCs).

**Fig. 6. PVSRIPO oncolysate–pulsed DCs generate tumor antigen–specific CTL immunity in vitro.** Primary human DCs coincubated with SUM149, MDA-MB231, LNCaP, or DM6 oncolysate stimulate tumor antigen–specific T cell responses in vitro. (A) Schema of the assay. (B) T cells were cocultured with oncolysate-pulsed autologous DCs, and the stimulated effector T cells were then harvested and tested in a CTL assay against the corresponding tumor cells (red bars), autologous DCs transfected with RNA that encodes for a relevant tumor antigen (black bars; positive control), or autologous DCs transfected with RNA that encodes for an irrelevant tumor antigen (white bars; negative control). Each bar represents mean % specific lysis and SD of triplicate samples. Statistical significance comparing autologous DCs expressing either the relevant or irrelevant tumor antigen for each panel in (B) was calculated using paired two-tailed Student’s t test. A probability of less than 0.05 (P < 0.05) is considered statistically significant. Panel SUM149 DC targets, P = 0.04; panel LNCaP DC targets, P = 0.0008; panel MDA-MB231 DC targets, P = 0.01; panel DM6 DC targets, P = 0.01. EGFR, epidermal growth factor receptor; PSA, prostate-specific antigen.
After 12 to 14 days of DC-T cell coculture in vitro, cells were harvested and assayed for tumor antigen–specific cytotoxic reactivity using a standard 4-hour europium (Eu) release CTL assay (Fig. 6A). To assess CTL reactivity, we cultured T cells (effectors) with the following Eu-labeled target cells: (i) a tumor cell line yielding the PVSRIPO oncolysate used for DC loading, (ii) DCs transfected with mRNA encoding a tumor antigen known to be expressed by the tumor cell line (positive control), or (iii) DCs transfected with mRNA encoding an irrelevant tumor antigen not expressed by the tumor cell line (negative control) (Fig. 6B). The use of autologous antigen-expressing DCs as positive and negative control targets allowed determination of specific CTL reactivity to known tumor-associated antigens as we have done in previous studies (39, 40), by eliminating MHC mismatch between T cells and target cells. CTL-mediated killing of the target cells was assessed by measuring Eu release in the supernatant.

DCs treated with PVSRIPO oncolysate produced CTL responses that effectively lysed the original cancer lines and the positive control (DCs expressing a relevant tumor antigen) but not the negative control (DCs expressing an irrelevant tumor antigen; Fig. 6B). Antigen presentation by PVSRIPO oncolysate–treated DCs did not require the additional maturation step with the CC, which is routinely used (27, 39, 41, 42) to stimulate effector T cells in such in vitro assays. We hypothesized that DC activation/maturation in this instance was due to infection of DCs with PVSRIPO present in the oncolysate. Because the oncolysate represents the entire repertoire of tumor-associated antigens, oncolysate-stimulated T cells likely target multiple tumor antigens, which may explain the greater extent of tumor cell lysis versus target DCs expressing select tumor-associated antigens. Notably, SUM149 breast cancer oncolysate-stimulated T cells did not lyse LNCaP prostate cancer cells and vice versa (fig. S6). Moreover, DCs loaded with supernatant from control lysate (mock-infected cell supernatant) did not stimulate antigen-specific T cells as indicated by minimal lysis of target cells (fig. S6).

Together, our findings define a concerted range of activities for PVSRIPO in mediating cancer cytotoxicity, DAMP/PAMP release, and antigen release for uptake by APCs (such as DCs) while simultaneously infecting and activating APCs to bolster costimulatory molecule expression, antigen processing and presentation, and cytokine production. Ultimately, these processes influence the generation of antitumor T cell populations that are capable of lysing cancer cells directly.

**PVSRIPO induces antitumor T cell immunity in an immunocompetent murine melanoma model**

The PVSs are exclusively human pathogens; only old-world primates develop poliomyelitis after experimental infection (chimpanzees are more susceptible than other species). *Enteroviridae* (PV is their flagship) have long been associated with hominids, and the human innate antiviral response is shaped by thousands of years of evolving virus-host relationships during co-speciation (43). Moreover, aggressive rodent syngeneic/xenotransplantation tumor models are notoriously deficient in recapitulating human tumor-stroma relationships (44). Given these shortcomings, we sought to confirm our findings in principle, addressing whether PVSRIPO therapy induces antitumor T cell immunity in an immunocompetent murine tumor model in vivo. We chose B16-F10.9 melanoma–expressing chicken ovalbumin (OVA) as a model antigen (B16-F10.9-OVA); these cells were transfected with (human) CD155 to generate B16-F10.9-OVA-CD155 (the mouse CD155 homolog does not function as a PV receptor), and we used (human) CD155-transgenic (tg) C57Bl/6 mice (45) as tumor hosts. PVSRIPO replication is deficient in murine cells due to inherent host restrictions of the HRV2 IRES, but serial passage of PVSRIPO in CD155-transduced mouse cells selects for mouse-competent variants (46). Using an approach similar to that of Jahan et al. (46), we adapted PVSRIPO by serial passage in explant mouse astrocytoma cells, yielding mRIPO. mRIPO exhibited patterns of viral translation, cytopathogenicity, and IF2α (S51) phosphorylation in the B16-F10.9-OVA-CD155 model resembling PVSRIPO in human melanoma cells (compare Figs. 1A and 7A). In addition, mRIPO infection of bone marrow–derived DCs from CD155-tg mice induced sustained IFN-β production at levels exceeding stimulation with LPS, resembling PVSRIPO infection of human DCs (fig. S7).

B16-F10.9-OVA-CD155 cells were implanted in the flank of CD155-tg C57Bl/6 mice and treated with a single intratumoral injection of control (Dulbecco’s modified Eagle’s medium (DMEM)) or mRIPO (10⁶ pfu). mRIPO therapy delayed tumor growth as measured by tumor volume (Fig. 7B) and increased overall survival (Fig. 7C). Loss of intact PARP and IF2α (S51) phosphorylation after mRIPO treatment was observed in tumor homogenates early after treatment (fig. S8A), mirroring viral cytotoxicity observed in vitro (Figs. 1A and 7A and fig. S1A). To determine whether such therapy produced antitumor T cells, we obtained draining inguinal lymph nodes from mice 7 days after treatment with control or mRIPO. Lymph node cells were restimulated in vitro using tumor antigen–expressing cells. After 5 days, effector cells were harvested and tested for cytotoxicity against EL4 thymoma cells [expressing (i) green fluorescent protein (GFP) (control), (ii) OVA, and (iii) tyrosinase-related protein 2 (TRP-2) (B16 melanoma antigen)] or B16-F10.9-OVA-CD155 cells (Fig. 7D). Effector T cells from control-treated mice exhibited minimal cytotoxicity against the target cells (Fig. 7D). Effector T cells isolated from mRIPO-treated mice killed target cells expressing OVA or TRP-2 or both antigens (B16-F10.9-OVA-CD155 cells) and not EL4 cells expressing GFP, indicating antigen-specific killing (Fig. 7D). Analysis of cytokines in supernatant harvested after 5-day T cell restimulation revealed granulocyte B, IFN-γ, and TNF-α induction only in the mRIPO-treated group (Fig. 7E), which corroborates cytotoxic T cell activity observed in Fig. 7D. To test induction of systemic antitumor responses, we harvested spleens 14 days after intratumoral treatment with control (DMEM) or mRIPO. Splenocytes were cocultured with B16-F10.9-OVA-CD155 cells, and only splenocytes from mRIPO-treated mice released T cell effector cytokines (Fig. 7F). The generation of systemic antitumor T cells was preceded by enhanced ISG expression in tumor lysates and a reduction in viral titers over time (fig. S8, B and C).

To validate the presence of melanoma-specific T cells using another approach, we used a TRP-2–specific tetramer to detect TRP-2–specific CD8 T cells. Tumor-draining inguinal lymph nodes were harvested from control and mRIPO-treated mice and stimulated for 5 days in vitro with tumor antigen–expressing cells. After 5 days, the effector cells were harvested and individual lymph nodes were analyzed using flow cytometry for the presence of CD4, CD8, and TRP-2–specific CD8 T cells. Representative flow cytometry data are shown in fig. S8D. Analysis of lymph node cells after restimulation revealed that mRIPO-treated mice had a higher ratio of CD8 T cells to CD4 T cells, indicating CD8 T cell expansion relative to the control group (Fig. 7G, left and middle panels; T cell numbers are shown in fig. S8E). TRP-2–specific CD8 T cells were detected only in mRIPO-treated mice (Fig. 7G, right panel, and fig. S8E), corroborating data in Fig. 7D that suggest the generation of TRP-2–specific CTLs in mRIPO-treated mice. Together, our data in human and murine systems indicate that PVSRIPO’s cytopathogenic targeting of malignant cells combined with proinflammatory activation of APCs induces productive antitumor T cell responses.

PVSRIPO therapy induces acute neutrophil influx followed by DC and T cell infiltration

Intratumoral administration of mRIPO results in delayed tumor growth, improved survival, and systemic tumor-specific CTLs (Fig. 7). To address events that may contribute to the generation of antitumor CTL responses after mRIPO (versus control) treatment, we analyzed the immune cell content of tumors longitudinally for 6 days. Analysis of mRIPO-treated tumors revealed considerable infiltration of CD45.2+ immune cells as compared to control-treated tumors (Fig. 8A). This was accompanied by early intratumoral release of IFN-α, TNF-α, IL-12, and IFN-γ in response to mRIPO treatment (Fig. 8B).

Analyses of tumors before mRIPO treatment (day 0) revealed that myeloid cells (CD45+ CD11b+) were the predominant immune cells in the tumor (Fig. S9). Although monocyte/macrophage populations were...
Fig. 8. mRIPO elicits neutrophil influx followed by DC and T cell infiltration into tumors. B16-F10.9-OVA-CD155 tumors were implanted subcutaneously, and when the tumor volume reached ~100 mm³, they were injected with DMEM (control) or mRIPO. Tumors were harvested after injection as indicated, digested to single-cell suspensions, and analyzed by flow cytometry. (A) Analysis of the percentage of CD45.2⁺ immune cells in the tumor after DMEM (control) or mRIPO treatment. Each bar represents three mice analyzed individually. (B) Cytokine concentrations in tumor homogenates. (C to E) Analysis of tumor-infiltrating neutrophils (C), DCs (D), and T cells (E) at the indicated days after mRIPO injection. (F) Longitudinal analysis of neutrophil, DC, CD4 T cell, and CD8 T cell infiltration is depicted as a percentage of total live cells in the tumor. Each bar represents three mice analyzed individually. Error bars represent SEM. The flow cytometry gating strategy is shown in figs. S9 and S10.
present on the day of treatment, their numbers remained relatively unchanged throughout the course of treatment (fig. S9). We previously reported striking neutrophil infiltration of tumors after PVSRIPO injection in xenograft models of prostate and breast cancer (47). Confirming these findings, mRIPO-treated tumors demonstrated a marked influx of neutrophils (CD45.2+CD11b+CD11c-Ly6C+Ly6G+) (fig. S8C). Neutrophils are a central component of acute inflammatory responses that facilitate the transition from innate to adaptive tumor immunity (48, 49). Neutrophil numbers peaked on days 1 and 2 and declined rapidly thereafter, indicative of a classic acute inflammatory response (fig. S8C). Neutrophil influx was followed by DC (CD45.2+CD11b+CD11c-classII F4/80+) and T cell (CD45.2+CD4+ or CD8+) infiltration into mRIPO-treated tumors (Fig. 8, D to F) on days 5 and 6.

Immune cell infiltration into the tumor occurred in the presence of infectious virus in the tumor (fig. S8C) as in our in vitro oncolysate studies (Figs. 3 to 6). The gating strategy used for analysis of tumor-infiltrating immune cells is shown in figs. S9 and S10. Figure 8F depicts the timeline of neutrophil, DC, CD4 T cell, and CD8 T cell influx over 6 days (data represent the percentage of total live cells in tumor). These data suggest that mRIPO tumor infection sets in motion an inflammatory cascade causing the recruitment of DCs and T cells into the tumor (Fig. 8), which ultimately results in the generation of a productive antitumor T cell response (Fig. 7). This confirms key observations in human systems (Figs. 3 to 6), indicating that combined PVSRIPO oncolysis and APC infection induces type I IFN-dominant responses, antigen presentation activity, and antitumor CTL responses (fig. S11).

**DISCUSSION**

Disruption of the immunological masking of tumors and expansion of the antitumor immune repertoire are eminent objectives for cancer immunotherapy. The inspiration for using PVSRIPO to this end had its origin in specific cytopathogenicity for cells derived from virtually any solid cancer, due to widespread CD155 expression in solid malignancy (7). PVSRIPO was granted “breakthrough therapy designation” by the U.S. Food and Drug Administration/Center for Biologics Evaluation and Research in May 2016, due to promising early clinical results against glioblastoma. Here, we define PVSRIPO’s immunotherapeutic potential, in terms of its ability to engage tumors immunologically and to induce tumor antigen–specific antitumor immunity. Our studies suggest that, in addition to lytic damage to malignant cells, this potential rests on noncytotoxic infection of APCs/DCs.

PVSRIPO infection of primary human DCs did not produce cytopathogenicity or cell killing but induced type I IFN responses that exceeded standard TLR stimulants in potency and duration. This was accompanied by up-regulation of markers of DC antigen-presenting and costimulatory functions. We believe that this pattern of APC stimulation may be specific to PVSRIPO and translation restrictions imparted by the foreign HRV2 IRES because earlier reports indicated productive, cytotoxic propagation and killing of DCs by wild-type PV (26). Such activities of wild-type PV are prototypical for human pathogenic virus-host relationships, where DC targeting is very common and may be a mechanism to prevent the generation of adaptive antiviral immunity by impairing DC function (50). Thus, proinflammatory conditioning of APCs/DCs may be a consequence of PVSRIPO’s recombinant IRES.

Sustained IFN responses induced in DCs by PVSRIPO may be key to engaging the adaptive arm (especially CTLs) of the immune system (28–31). Type I IFNs are critical antitumor immunity-stimulating cytokines because they are essential for DC-mediated rejection of tumors in mice (51). Moreover, type I IFN production by DCs after pathogen encounter is critical for regulating immune responses (52–54). PD-L1 induction after PVSRIPO infection of DCs, an expected consequence of the IFN response, points toward potential nodes of synergy with anti–PD-L1/PD-1 checkpoint inhibition.

The finding that intratumoral PVSRIPO administration induces immune cell infiltration and antitumor CTLs is critical for its clinical application and the selection of rational immunomodulating combinations. PVSRIPO adjuvant effects (infectious PVSRIPO oncolysate) promote DC activation and DC-mediated stimulation of tumor-specific CTLs that recognize target tumor antigens such as CEA, MART1, EGFR, and PSA in assays with human PBMCs.

In an immunocompetent murine model of melanoma, single intratumoral injection of mRIPO delayed tumor growth. This effect was associated with a rapid influx of neutrophils followed by DC and T cell invasion. Hence, neutrophils can impose either tumor-suppressive or tumor-supportive functions (55); however, upon pathogen challenge, neutrophils recruit other immune cell populations, including DCs and T cells (48, 49, 56). We speculate that neutrophil influx follows the release of inflammatory cytokines and chemokines by monocytes and macrophages, which were the primary myeloid cells in the tumor at the time of treatment (48, 57). Presumably, PVSRIPO infects myeloid cells in the tumor, because infectious virus was detected in tumors as late as day 7 (fig. S8C), after DC infiltration into tumors had occurred.

A limitation of this study is the use of murine models and in vitro systems because they lack the complexity and virus-host relationships that dictate the extent and quality of antitumor immunity generated by PVSRIPO therapy in patients. Genetically homogeneous mice and tumors do not recapitulate the range of responses to PVSRIPO infection that may determine antitumor efficacy in patients with heterogeneous genetics and tumor microenvironment composition. In addition, virtually all humans are immunized against polio as children, and consequently, patients have preexisting immunity to polio, which is challenging to recapitulate in mice and outside the scope of current studies. Development of murine models to study this will be needed for future investigations. This study outlines the adjuvant potential of PVSRIPO that drives the generation of antitumor immune responses. PVSRIPO–induced antitumor CTL responses occurred regionally (tumor-draining lymph nodes) and systemically (spleen), were tumor antigen–specific, and were associated with T cell infiltration in PVSRIPO–treated tumors. Such immune engagement and CTL initiation is desirable for current immunotherapies and would conceivably complement other modalities including immune checkpoint blockade or adoptive tumor-infiltrating lymphocyte (TIL/chimeric antigen receptor (CAR) T lymphocyte therapy by enhancing and maintaining TIL/CAR T cell activation.

**MATERIALS AND METHODS**

**Study design**

This study sought to define the cancer immunotherapy potential of PVSRIPO, a recombinant PV, using in vitro and in vivo experiments. For in vitro studies, experimental findings were confirmed using multiple cell lines and cells from healthy volunteers (n = 3). Cells were obtained from human subjects after written informed consent using protocols approved by the Duke University Institutional Review Board. For in vivo tumor volume measurements, treatment groups were randomized by cage and tumor volume at the time of treatment to ensure...
equal mean and range of tumor volumes within each group. Measurements were obtained without knowledge of treatment information; however, measurements were recorded by the same person who administered the treatment. To test the antitumor immune response after PVSRIPO therapy in vivo, mice were randomized by tumor size, with equal mean tumor volume between the control and PVSRIPO groups. Information on experimental repeats is presented in the figure legends.

**Cell lines and virus**

DM6 and DM440 cell lines were a gift from D. Tyler (University of Texas, Medical Branch, Galveston, TX). SUM149 cells were a gift from N. Spector (Duke University, Durham, NC). All other cell lines were obtained from the American Type Culture Collection. All lines, except for SUM149, were grown in 10% fetal bovine serum (FBS) in DMEM (Invitrogen); SUM149 cells were grown in 10% FBS in Ham’s DMEM-F12 medium (Lonza). PVSRIPO and mRIPO were grown in HeLa cells as previously described (15) and purified using a 0.45-µm syringe filter followed by concentration and filtration through a 100-kDa filter (Millipore); titers were measured by plaque assay (58). PBMCs and monocytes were obtained and processed as described in Supplementary Materials and Methods.

**Immunoblot, ELISA, LDH release assay, and flow cytometry**

Immunoblots were performed as described previously (15) using antibodies listed in Supplementary Materials and Methods. Dot blots were performed by adding an equal amount (2 µl) of cell pellet lysate or supernatant directly onto a nitrocellulose membrane, air-dried, and processed identically to immunoblots. ELISA was performed using the manufacturer’s recommendations for TNF-α, IL-12, and granulocyte macrophage colony-stimulating factor (all R&D Systems); IFN-γ (Invitrogen); and IFN-β (PBL Biosciences). LDH release assays were performed per the manufacturer’s instructions (Thermo Fisher). DM6-conditioned medium (DM6-CM) was analyzed by cytokine array immunoblot (RayBiotech Inc.), following the manufacturer’s instructions. To analyze DC phenotype, DCs were blocked with anti-human CD16/CD32 Fc block (eBioscience) and stained with the following antibodies: CD83, CD86, CD197 (CCR7), CD80, CD64, HLA-DR (Beckman Coulter), and MHC I (BD Biosciences); antibodies against CD11c, CD14, and CD11b (Beckman Coulter) were used to confirm differentiation and purity. To examine tumor immune cell infiltration, tumors were harvested at the indicated time points. Tumors were minced and incubated in RPMI 1640 containing Liberase (1.67 units/ml; Sigma) and deoxyribonuclease (DNase) (100 U/ml; Sigma). Cells were filtered, blocked with anti-mouse CD16/CD32 Fc block, and stained with the following panels of antibodies and appropriate isotype controls: panel 1, LIVE/DEAD cell stain, Ly6G, Ly6C, CD45.2, CD11c, CD11b, F4/80, and MHC II (BioLegend); panel 2, LIVE/DEAD cell stain, NK1.1, CD11b, CD45.2, CD8, CD4, and B220 (BioLegend). Data were acquired on a CytoFLEX B5-R3-V5 (Beckman Coulter) flow cytometer and analyzed using FlowJo (Tree Star Inc.) software.

**In vitro stimulation of T cells with DCs treated with PVSRIPO oncolysate**

PBMCs were thawed and resuspended in phosphate-buffered saline and treated with DNase I (200 U/ml; Sigma) for 20 min at 37°C. DNase I–treated PBMCs were incubated for 1 hour at 37°C (5% CO2) in a humidified incubator. Nonadherent cells were harvested and stimulated with DCs loaded with PVSRIPO–induced oncolysate at a responder cell/stimulator DC ratio of 10:1 in the presence of IL-7 (25 ng/ml). All stimulations were done in RPMI 1640 with 10% fetal calf serum (FCS), 2 mM l-glutamine, 20 mM HEPES, 1 mM sodium pyruvate, 0.1 mM MEM nonessential amino acids, penicillin (100 IU/ml), streptomycin (100 µg/ml), and 5 x 10^-5 M β-mercaptoethanol (CTL stimulation medium). The responder cell concentration was 2 x 10^6 cells/ml. IL-2 was added at 100 U/ml on day 3 and at 50 U/ml every 4 to 5 days. T cells were maintained at 1 x 10^6 to 2 x 10^6 cells/ml in CTL stimulation medium. In some assays, T cells were restimulated with PVSRIPO oncolysate–loaded DCs at a responder/stimulator ratio of 10:1 after 7 days. T cells were harvested on days 12 to 14, counted, and used as effector cells in a CTL assay.

**In vitro CTL assay**

Tumor cell lines and RNA-electroporated DCs were used as targets. Cells were harvested, washed to remove all traces of medium, and Eu-labeled (see Supplementary Materials and Methods). Ten thousand Eu-labeled targets (T) and serial dilutions of effector cells (E) at varying E/T ratios were incubated in 200 µl of CTL stimulation medium without antibiotics in 96-well V-bottom plates. The plates were centrifuged and incubated (4 hours). Supernatant (50 µl) was harvested and added to enhancement solution (150 µl; Wallac, PerkinElmer) in 96-well flat-bottom plates. Eu release was measured by time-resolved fluorescence using the VICTOR3 Multilabel Counter (PerkinElmer). Specific cytotoxic activity was determined using the following formula: % specific release = ([experimental release – spontaneous release]/[total release – spontaneous release]) x 100. Spontaneous release in target cells was <30% of total release by detergent. Spontaneous release in target cells

was determined by incubating the target cells in medium without T cells. All assays were done in triplicate.

**Generation of murine cancer cell lines and mRIPO**

Human rhinovirus IRESs are functionally impaired in mouse cells, and mice do not express a functional PV receptor. Thus, we generated mRIPO and human CD155–expressing murine cancer cell lines. A CD155 pcDNA3 expression plasmid was provided by E. Wimmer (Stony Brook University, NY), and pHIV-luciferase was a gift from B. Welm (Addgene plasmid #21375). CD155 was subcloned into pHIV-luciferase, and lentivirus was produced (see Supplementary Materials and Methods). KB158R, a genetically engineered murine astrocytoma line [derived from a mutant Nf1 and Trp53 mouse model (60); a gift from J. Sampson (Duke University, NC)], was lentivirally transduced with CD155 followed by selection [blastcidin (10 μg/ml)]. A stable CD155-expressing clone was identified by immunoblot. The same approach was used to produce B16-F10.9-OVA-CD155. To generate mRIPO, KB158R-CD155–expressing cells were infected with PVSRIP0 and washed three times with serum-free DMEM to remove unbound virus. After 48 hours, supernatant was collected and used to infect HeLa cells to amplify any propagated virus. Amplified virus was added to fresh KB158R-CD155 cells, and this process was repeated 20 times. The resulting virus was prepared and purified as described above and sequenced. This revealed seven genetic adaptations in the 5′ untranslated region of mRIPO, five of which were identical to those observed after serial passage adaptation of a PV/HRV2 IRES construct to mouse competence (46).

**B16-F10.9-OVA-CD155 immunotherapy model**

CD155–tg mice were derived from a homozygous colony provided by J. Pfeiffer (UT Southwestern, TX) and were bred in-house for all studies. For tumor initiation, 5 × 105 B16-F10.9-OVA-CD155 cells were implanted into the right flank of 6- to 8-week-old CD155–tg C57Bl/6 mice. Mice were treated when tumor volumes reached ~50 to 100 mm3. DMEM (control) or mRIPO (1 × 107 to 5 × 107 pfu) was injected in a volume of 20 μl into tumors via a Hamilton syringe. Tumor volume was determined by caliper measurements using the following equation: (L × W × W)/2; survival end point was tumor volume >1000 mm3. All mouse experiments were performed under approved Duke University Institutional Animal Care and Use Committee protocols and represent combined data from two independent experiments.

**In vivo induction of CTLs and CTL assay**

Seven days after treatment of tumor-bearing CD155–tg C57Bl/6 mice with DMEM or mRIPO, tumor-draining inguinal lymph nodes were harvested (n = 4 per group), pooled, processed into a single-cell suspension, and plated in a six-well plate (3 × 106 cells per well). Cells were cultured in CTL stimulation medium (see above) with IL-2 (20 U/ml) for 5 days. Lymph node responder cells were stimulated with 3 × 105 activated B cells expressing TRP-2 (1.5 × 105) and OVA antigen (1.5 × 105). B cell activation is described in Supplementary Materials and Methods. EL4 thymoma cells electroporated with TRP-2–, OVA–, or GFP-encoding mRNA or B16-F10.9-OVA-CD155 cells were used as targets. For tetramer analysis, tumor-draining inguinal lymph nodes (n = 4 per group) were processed and cultured individually in a 24-well plate (5 × 105 cells per well) in CTL stimulation medium. Activated B cells were coincubated with TRP-2 peptide (2.5 μg/ml; amino acids 180 to 188, SVYDFFVWL-H2Kb epitope; 2 hours) and used as stimulators. TRP-2 peptide–pulsed B cells were harvested and washed, and 5 × 104 B cells were cultured with 5 × 105 lymph node responder cells for 5 days. For studies with splenocytes, spleens were harvested 14 days after DMEM or mRIPO treatment; red blood cells were lysed using ammonium chloride tris buffer. Splenocytes (107) were cultured with 2 × 105 B16-F10.9-OVA-CD155 tumor cells in RPMI–10% FCS in a six-well tissue culture plate (48 hours). Supernatant was harvested and analyzed by ELISA.

**Statistical analysis**

Data are summarized as means ± SEM. Statistical significance in immune assays comparing two groups was calculated using paired two-tailed Student’s t test. Statistical analysis was conducted using GraphPad Prism version 6. A probability of <0.05 (P < 0.05) was considered statistically significant. Specific tests were performed as described in the figure legends.

**SUPPLEMENTARY MATERIALS**

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Materials and Methods

Fig. S1. PVSRIP0 cytotoxicity in malignant glioma cell lines.

Fig. S2. Flow cytometry analysis of DC phenotype in Fig. 3.

Fig. S3. Analysis of PVSRIPO infection of DCs.

Fig. S4. Flow cytometry analysis of DC phenotype in Fig. 5.

Fig. S5. Analysis of viral replication in DC/DM6 cocultures and PVSRIP0 infection of human monocytes.

Fig. S6. Tumor antigen production by mRIPO-infected and LPS-treated mouse DCs.

Fig. S7. IFN-β production by mRIPO-infected and LPS-treated mouse DCs.

Fig. S8. Analysis of B16-F10.9-OVA-CD155 tumor homogenate and tumor-draining lymph nodes after mRIPO treatment.

Fig. S9. Analysis of tumor-infiltrating immune cells, myeloid cells, and monocytes/macrophages.

Fig. S10. Analysis of tumor-infiltrating immune cells.

Fig. S11. PVSRIPO-mediated tumor cytotoxicity, DC infection/stimulation, and antitumor immunity.

Table S1. Relevant functions of cytokines/proteins present in DM6 melanoma-conditioned AIM V medium.

References (61–78)

**REFERENCES AND NOTES**


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Competing interests: M.C.B., V.C., D.D.B., M.G., and S.K.N. own intellectual property related to this research, which has been licensed to Istari Oncology Inc. M.G. and D.D.B. are co-founders and equity holders in Istari Oncology Inc. S.K.N., M.C.B., D.D.B., and M.G. are inventors on patent application PCT/US2017/039953 held/submitted by Duke University that covers the composition and methods for activating APCs with chimeric PV. All other authors declare that they have no competing interests.

Data and materials availability: Materials are available to noncommercial researchers through a material transfer agreement with Duke University School of Medicine upon filing a request with S.K.N. or M.G.

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Cancer immunotherapy with recombinant poliovirus induces IFN-dominant activation of dendritic cells and tumor antigen–specific CTLs

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A double virus's double attack on cancer

Oncolytic viruses, which are optimized to kill cancer cells without harming the surrounding normal tissues, have gathered increasing interest in recent years. One such virus is PVSRIPO, an engineered hybrid of poliovirus and rhinovirus that cannot attack neurons but retains cytotoxicity in neoplastic cells, including glioma. Brown et al. determined that PVSRIPO stimulates anticancer immunity by two mechanisms: lysing tumor cells to release a mix of tumor and viral antigens, as well as sublethally infecting antigen-presenting cells and thereby stimulating an interferon-driven immune response in the tumor microenvironment.