Oncolytic herpes simplex virus infects myeloma cells in vitro and in vivo

Jayeepta Ghose, Ohio State University
Ada Dona, City of Hope, Monrovia
Mariam Murtadha, City of Hope, Monrovia
Emine Gulsen Gunes, City of Hope, Monrovia
Enrico Caserta, City of Hope, Monrovia
Ji Young Yoo, University of Texas Health Science Center, Houston
Luke Russell, Ohio State University
Alena Cristina Jaime-Ramirez, Ohio State University
Benjamin Barwick, Emory University
Vikas Gupta, Emory University

Only first 10 authors above; see publication for full author list.

Journal Title: MOLECULAR THERAPY-ONCOLYTICS
Volume: Volume 20
Publisher: CELL PRESS | 2021-03-05, Pages 519-531
Type of Work: Article | Final Publisher PDF
Publisher DOI: 10.1016/j.omto.2021.02.009
Permanent URL: https://pid.emory.edu/ark:/25593/vt0n4

Final published version: http://dx.doi.org/10.1016/j.omto.2021.02.009

Copyright information:

© 2021 The Authors
This is an Open Access work distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License (https://creativecommons.org/licenses/by-nc-nd/4.0/rdf).

Accessed October 10, 2024 6:02 AM EDT
Oncolytic herpes simplex virus infects myeloma cells in vitro and in vivo

Jayeeta Ghose,1,7 Ada Dona,2,7 Mariam Murtadha,2 Emine Gulsen Gunes,2 Enrico Caserta,2 Ji Young Yoo,3 Luke Russell,4 Alena Cristina Jaime-Ramirez,3 Benjamin G. Barwick,5 Vikas A. Gupta,5 James F. Sanchez,2 Douglas W. Sborov,6 Steven T. Rosen,2 Amrita Krishnan,2 Lawrence H. Boise,3 Balveen Kaur,3 Craig C. Hofmeister,5 and Flavia Pichiorri2

1Department of Radiation Oncology, Comprehensive Cancer Center, The Ohio State University, Columbus, OH, USA; 2Department of Hematology and Hematopoietic Cell Transplantation, Judy and Bernard Briskin Center for Multiple Myeloma Research, City of Hope, Monrovia, CA 91016, USA; 3Department of Neurosurgery, McGovern Medical School, University of Texas Health Science Center, Houston, TX, USA; 4Department of Neurological Surgery, The Ohio State University Wexner Medical Center, Columbus, OH, USA; 5Department of Hematology & Medical Oncology, Winship Cancer Institute of Emory University, Atlanta, GA 30307, USA; 6Division of Hematology & Hematologic Malignancies, Department of Internal Medicine, University of Utah, Huntsman Cancer Institute, Salt Lake City, UT, USA

Because most patients with multiple myeloma (MM) develop resistance to current regimens, novel approaches are needed. Genetically modified, replication-competent oncolytic viruses exhibit high tropism for tumor cells regardless of cancer stage and prior treatment. Receptors of oncolytic herpes simplex virus 1 (oHSV-1), NECTIN-1, and HVEM are expressed on MM cells, prompting us to investigate the use of oHSV-1 against MM. Using oHSV-1-expressing GFP, we found a dose-dependent increase in the GFP signal in MM cell lines and primary MM cells. Whereas NECTIN-1 expression is variable among MM cells, we discovered that HVEM is ubiquitously and highly expressed on all samples tested. Expression of HVEM was consistently higher on CD138+/CD38+ plasma cells than in non-plasma cells. HVEM blocking demonstrated the requirement of this receptor for infection. However, we observed that, although oHSV-1 could efficiently infect and kill all MM cell lines tested, no viral replication occurred. Instead, we identified that oHSV-1 induced MM cell apoptosis via caspase-3 cleavage. We further noted that oHSV-1 yielded a significant decrease in tumor volume in two mouse xenograft models. Therefore, oHSV-1 warrants exploration as a novel potentially effective treatment option in MM, and HVEM should be investigated as a possible therapeutic target.

INTRODUCTION

Multiple myeloma (MM) is a plasma cell cancer currently treated with combinations of cereblon-binding drugs (e.g., the immunomodulatory drug thalidomide and its derivatives), proteasome inhibitors (PIs, bortezomib and carfilzomib as examples), CD38-targeted monoclonal antibodies (daratumumab and isatuximab), and steroids. A subset of patients with low-risk disease may live 10–20 years with current therapy, but aggressive myeloma develops resistance quickly, conferring survival of only 1–5 years. Although most patients respond to initial treatment, a pattern of multiple relapses and disease resistance to multi-drug regimens results in an aggressive cancer to which patients succumb.

Oncolytic virus (OV) therapy is a novel treatment strategy defined by tumor cell killing mediated by viruses with high tropism for tumor cells regardless of cancer stage and prior treatment. Interestingly, MM has several features that make it an ideal target for OV, including overexpression of several cell surface proteins that are generally used as viral entry receptors, and mutations in signaling pathways that sensitize MM cells to viral infection.1–4 Genetically engineered oncolytic herpes simplex virus 1 (oHSV-1) is an enveloped, double-stranded DNA virus that specifically targets and kills a myriad of solid tumors, including glioma, melanoma, and breast, prostate, colon, ovarian, and pancreatic cancer. Increased therapeutic efficacy of OV therapies was observed when oHSV-1-modified viruses were used in combination with traditional therapies such as radiotherapy and chemotherapy, providing an attractive strategy to pursue in the clinic.5,6 Moreover, oHSV-1 vectors expressing “suicide” genes (thymidine kinase, cytosine deaminase, rat cytochrome P450) or immune stimulatory genes (e.g., interleukin [IL]-12, granulocyte-macrophage colony-stimulating factor [GM-CSF]) have been constructed to maximize tumor destruction through multimodal therapeutic mechanisms,6 and the virus has been evaluated for safety and efficacy in multiple clinical trials.7 The recent US Food and Drug Administration (FDA) approval of intratumoral injection of talimogene laherparepvec (T-VEC), an oHSV-1 for advanced melanoma, emphasizes its anti-cancer potential.8 The safety of
administering Seprehvir, another modified oHSV-1 (HSV1716), systemically was recently reported in the first intravenous phase 1 trial in cancer patients with extra-cranial solid tumors.

However, until very recently, there had not been reports of intravenous oHSV-1 viruses in hematologic malignancies or information on associated potential entry receptors on blood cancer cells. While this manuscript was in communication, two recent reports highlighted that the third-generation oncolytic HSV-1, T-01, could infect and kill human cell lines and primary cells derived from various lineages of hematological malignancies and that the oncolytic effect of T-01 could be augmented by the immunomodulatory drug lenalidomide in the treatment of plasma cell neoplasms. However, we had first reported on the possibility of using HSV-1 in OV therapy of MM. Since herpes virus entry mediator (HVEM, CD270, tumor necrosis factor receptor superfamily member 14 [TNFRSF14]) is over-expressed not only on myeloid and lymphoid cells, but also on primary MM cells and plasma cell leukemias, in this work, we decided to investigate whether this receptor could be used by oHSV-1 to infect and kill MM cells. We show that oHSV-1 can infect and kill several MM cell lines and primary MM cells through viral-mediated induction of programmed cell death via the canonical apoptotic pathway in vitro and in vivo. Aligned with this finding, we show that both MM cell lines and primary MM cells have high expression of HVEM and variable levels of another HSV-1 receptor, NECTIN-1. Furthermore, among several other tumor types, HVEM expression is the highest in MM and is positively correlated with patient survival, supporting the idea that HVEM can be further studied for its potential role as a future candidate for cell therapy and as a prognostic biomarker for MM.

RESULTS

**oHSV-1 infects myeloma cell lines and primary myeloma cells**

To explore whether oHSV-1 could infect MM cells, we used genetically engineered HSV-1 (HSVQ) that is deleted for both copies of viral ICP34.5 and possesses a gene-disrupting insertion of green fluorescent protein (GFP) within the viral UL39 locus encoding for the ICP6 gene. In this study, we have referred to this construct HSVQ as oHSV-1. We treated myeloma cell lines with increasing concentrations of GFP-expressing oHSV-1 and observed a dose-dependent increase in GFP* signal in MM cell lines (MM.1S, LP-1, and NCI-H929) (Figures S1B–S1D) at 24 h post-infection, and a further increase in MM.1S at 48 h (Figure 1A). Efficient infection was observed independently of genetic modifications were treated with RAMBO,16 which expresses human anti-
in the backbone of the oHSV-1 vector (Figure 1B) when MM cell lines were treated with RAMBO,16 which expresses human anti-
angiogenic Vasculostatin-120 (Vstat120) under the viral IE4/5 promoter, and rQnestin34.5,17 which expresses viral ICP34.5 under the nestin promoter in the same attenuated HSVQ (oHSV-1) construct (Figure S1A). All three viruses, namely, HSVQ (oHSV-1), RAMBO, and rQnestin34.5, express GFP under the same viral ICP6 promoter at the UL39 locus.15–17 Furthermore, all MM cell lines tested (U266, RPMI 8226, NCI-H929, and LP-1) displayed a significant GFP* signal even at a low multiplicity of infection (MOI of 0.1) (Figure 1C) at 24 h after treatment. A highly efficient infection was also observed in primary CD138+ MM cells obtained from bone marrow (BM) aspirates but not in the cellular fraction depleted of MM cells (CD138– BM fraction) (Figure 1D). Heat inactivation of the virus denatures its surface proteins and inhibits its interaction with virus receptors on target cells; this process prevented viral entry into MM.1S cells even at a saturating MOI of 2.0 (Figure S1E), suggesting that oHSV-1 infection of myeloma cells is dependent at least partially on surface receptor expression. The cellular receptors NECTIN-1 and HVEM can both mediate oHSV-1 entry in a complex multistep process through binding of the major viral receptor-binding protein glycoprotein D (gD). In our study, we observed reduction in surface NECTIN-1 levels in MM cell lines (MM.1S, RPMI 8226, KMS11, and L363) upon viral treatment for 24 h at an MOI of 0.1 (Figure 1E), indicating NECTIN-1 to be involved in the process of viral uptake.

The HSV-1 receptor HVEM is highly expressed in myeloma cells

To correlate surface expression of receptors with infection efficiency, we next measured NECTIN-1 and HVEM expression in myeloma cells. We observed that, out of the five highly susceptible MM cell lines, NECTIN-1 was highly expressed only in MM.1S and RPMI 8226 cells, whereas it had a considerably lower surface expression in L363, U266, and H929 cells (Figure 2A; Figure S2A). These data are consistent with the fact that PVRIL1, the mRNA that encodes NECTIN-1, was variably expressed in primary myeloma samples, as evident using data from the CoMMpass trial comprising 768 newly diagnosed MM patients (Figure 2B). On the contrary, we found that HVEM is ubiquitously and highly expressed not only in MM cell lines (Figure 2G) but also in primary CD138+ MM cells in all MM samples (3.3–62.7 fragments per kilobase per million reads [FPKM]) (Figure 2D), as evident using data from the CoMMpass trial. As a positive control, expression of B cell maturation antigen (BCMA) (Figure 2E), a known marker of MM that predicts outcome for MM patients and is a targetable antigen for novel therapies, was also similarly measured under such conditions. Aligned with these data, high HVEM surface expression (mean fluorescence index [MFI] 1.664 ± 1.045) was also observed by flow cytometry in CD138+/CD38+ plasma cells, from BM fractions of MM patients (n = 10), but it was practically undetectable (MFI 264.8 ± 103.9, p = 0.002) in the BM CD138− fraction (non-MM cells) (Figures 2F and 2G). Interestingly, in two (RPMI 8226 and H929) out of four MM cell lines we tested, an HVEM-blocking antibody was able to impair oHSV-1 infection of MM cells, an effect that was not observed (RPMI 8226) or only partially observed (NCI-H929) when a NECTIN-1-blocking antibody was used (Figures S2B and S2C), indicating that besides NECTIN-1, HVEM is also involved in the process of viral entry into MM cells. Additionally, gene expression data downloaded from the Genomic Data Commons (GDC; https://portal.gdc.cancer.gov/) for 15 different cancer types (Table S1) also revealed MM to express HVEM to the greatest extent among all cell types tested (Figure 2H). In contrast, NECTIN-1 is not expressed as highly in MM (Figure S2D).
Moreover, Kaplan-Meier analysis of MM patients from the CoMM-pass trial showed that patients with comparatively lower HVEM (TNFRSF14) expression experienced worse overall survival (OS) and progression-free survival (PFS) (Figure S3A). To further understand the reason, we annotated HVEM expression by myeloma subtype defined by gene expression. This test revealed that the Maf (MF), proliferation (PR), and Mmset (MS) gene expression subtypes, which generally have poor outcomes, also expressed lower levels of HVEM, whereas the hyperdiploid (HY) subtype, which is associated with better prognosis, expressed the highest levels of HVEM among the subtypes tested (Figure S3B). In contrast, NECTIN-1 (PVRL1) is not expressed as highly and does not correlate with response to therapy or survival (Figures S3C and S3D).

**oHSV-1 kills MM cells independent of viral replication**

To test whether oHSV-1 could kill MM cells, we infected MM.1S, U266, and RPMI 8226 cell lines with oHSV-1 at increasing MOI and time. A cell proliferation assay indicated that, compared to control, oHSV-1 could reduce proliferation of all three MM cell lines tested (Figure 3A). Although normal human hematopoietic cells are resistant to oHSV-1 infection, to examine whether direct lytic replication was responsible for oHSV-1-induced eradication of malignant MM cells, we carried out a replication assay as previously described. Surprisingly, it was observed that, although oHSV-1 could efficiently infect and kill all of these cell lines, no viral replication occurred during the process (Figure 3B). To examine whether a structural protein of the oHSV-1 virion is responsible for inducing cell death, we heat-inactivated (HI) oHSV-1 according to a previous protocol. It was observed that HI oHSV-1 failed to induce cell death in MM cells at indicated MOIs and time points (Figure 3C). To determine whether viral particles that are shed into the culture medium trigger cell death, we harvested conditioned medium (CM) in which MM.1S cells were infected with oHSV-1 (MOI of 0.1, 1, and 5) for 10 h, neutralized free virus with 0.4% human immunoglobulin G (IgG), and used this medium to treat freshly seeded MM.1S cells. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assays showed that the CM failed to induce apoptosis in the uninfected, CM-treated MM.1S cells at the indicated time points (Figure 3C).
These observations demonstrated that infection by live viruses is needed to induce cytotoxicity in MM despite the lack of viral replication. Furthermore, to examine whether oHSV-1 infection created sufficient endoplasmic reticulum (ER) stress to lead to apoptosis, we treated MM.1S, U266, and RPMI 8266 cell lines with oHSV-1 at an MOI of 0.1 for 6 h. Immunoblotting showed no significant change in the activation levels of known ER stress response markers PERK and Bip/GRP78 as well as subsequent unfolded protein response (UPR) markers Hsp40 and Hsp90 in infected cells compared to control (Figure 3D), indicating a lack of involvement of ER stress response pathways in the process.

**oHSV-1 kills MM cells through viral-mediated induction of programmed cell death**

Our data suggested that oHSV-1 kills human MM cells via a mechanism that is not dependent on lytic viral replication. There is evidence of replication-independent, abrupt myxoma-induced MM cell death via apoptosis, and oHSV-1 induced apoptosis even in the case of productive viral infection in human gastric cancer cells and HeLa cells. To test apoptosis, we infected MM.1S and U266 cells with oHSV-1 at an MOI of 0.1. The virus-induced cytotoxicity as assayed 24 h post-infection revealed significant induction of both early (annexin+/7-aminoactinomycin D (7-AAD)−/C0) and late (annexin+/7-AAD+) apoptosis by oHSV-1 (Figure 4A). To measure induction of the classical apoptotic pathway, we next assessed caspase-3 cleavage and PARP cleavage in MM.1S, U266, and RPMI 8266 cells under similar conditions. An immunoblot assay displayed cleavage of the apoptotic effector caspase-3, as well as cleavage of PARP, a marker for late-stage apoptosis in all three MM cell lines (Figure 4B). Furthermore, we pre-incubated MM.1S cells with a pan-caspase inhibitor (Z-VAD-FMK) and subsequently infected them with oHSV-1 (MOI of 0.1 for 24 h). Annexin/7-AAD staining revealed that, although 46% of cells were killed by oHSV-1, the percentages of early (annexin+/7-AAD−) and late (annexin+/7-AAD+) apoptotic cells taken together...
were gradually reduced to 39%, 32%, and 25% in the presence of increasing concentrations of the pan-caspase inhibitor (10, 20, and 50 \( \mu \)M Z-VAD-FMK, respectively) (Figure 4C). On the contrary, use of venetoclax (5 \( \mu \)M), an inhibitor of anti-apoptotic BCL2 (B cell leukemia/lymphoma 2) that induces caspase-3 cleavage,29 further potentiated oHSV-1-mediated apoptosis when given to MM.1S and NCI-H929 cells pre-treated with oHSV-1 (MOI of 0.1) (Figures S4A–S4D).

oHSV-1 infects and induces apoptosis in primary myeloma (MM) cells

To assess whether oHSV-1 induces apoptosis in primary MM cells, we infected BM aspirates and peripheral blood (PB) isolated from the same MM patients with oHSV-1 at an MOI of 5. While annexin/7-AAD staining performed 48 h post-infection revealed greater induction of apoptosis (41%) compared to uninfected control (12%) in BM aspirates, a similarly increased induction of apoptosis was not observed in oHSV-1-treated PB samples (17%) compared to control cells (10%) (Figure 5A). A cytotoxicity assay analogously revealed induction of apoptosis in BM aspirates of MM patients at both 24 and 48 h post-infection at MOIs of 5 and 10, in contrast to similarly treated PB isolated from MM patients (Figure 5B). To ascertain whether oHSV-1 induced apoptosis preferentially in the CD138+ malignant plasma cell fraction, we isolated the CD138+ cell population from BM aspirates of MM patients and infected it with oHSV-1 at MOIs of 1 and 10. Cytotoxicity assays revealed that oHSV-1 induced higher cell death in the CD138+ population compared to the CD138− population isolated from BM aspirates (Figure 5C). Next, to determine whether this difference in killing was due to differences in the susceptibility of these subpopulations to infection, we treated BM aspirates, PB, and CD138+ and CD138− cell populations derived from BM aspirates of MM patients with oHSV-1 (MOI of 10) for 48 h. Fluorescence microscopy revealed that oHSV-1 could efficiently infect BM aspirates in comparison to PB (Figure 5D) and the CD138+ fraction derived from BM aspirates in comparison to the CD138− fraction obtained from the same MM patient (Figure 5E), as is also demonstrated in Figure 1D.

oHSV-1 has potent in vivo anti-myeloma efficacy

We next investigated the anti-myeloma effects of oHSV-1 in vivo in two different MM xenograft mice models. Six- to 8-week-old non-obese diabetic (NOD) severe combined immunodeficiency (SCID)
gamma (NSG) mice were subcutaneously injected with $12.5 \times 10^6$ MM.1S or NCI-H929 cells in their right flank. On formation of palpable tumors, they were treated with $10^6$ plaque-forming units (PFU) of oHSV-1 or with saline twice a week for 2 weeks. Figure 6A and 6C show that, while saline-treated tumors grew rapidly, tumor growth in both MM.1S (n = 7, p = 0.00338) and NIH-H929 (n = 7, p = 0.00214) xenograft models was significantly reduced upon treatment with oHSV-1. Figures 6B and 6D show representative images of mice bearing tumors and the tumors extracted from them in both models. These results clearly demonstrate efficient anti-myeloma effects of oHSV-1 in vivo.

DISCUSSION

This work shows that oHSV-1 can infect MM cell lines with high efficiency. HSV-1 receptor density on host cells is directly correlated with virus entry efficacy. The key interaction governing HSV-1 entry into host cells occurs through virus surface gD binding to HVEM, NECTIN-1, or 3-O-sulfated heparan sulfate (3-OS HS), and in some cases NECTIN-2. NECTIN-1 is reported to be the most efficient receptor, and low NECTIN-1 expression levels have been theorized to limit responses to HSV-1-mediated OV therapy in several malignancies. Based on expression patterns, HVEM is thought to act as the principal receptor for HSV on lymphoid cells, with NECTIN-1 on epithelial and neuronal cells. However, cells often express both NECTIN-1 and HVEM receptors, making their individual contributions to viral entry difficult to assess. Although NECTIN-1 appeared to have a dominant effect on determining T-01 viral entry and oncolysis in a broad range of hematological malignancies, including myeloid-, B cell-, and T cell-derived tumors, a closer inspection in this work, specifically of MM cell lines and primary MM samples comprising 768 newly diagnosed MM patients (CoMM-pass trial; ClinicalTrials.gov: NCT01454297), revealed that the oHSV-1 surface receptor HVEM is highly and ubiquitously expressed on the surface of MM cell lines and in primary MM cells both at the mRNA and protein levels. Furthermore, we show that MM expresses HVEM at some of the highest levels of any tumor type, and analysis of MM patient data from the CoMM-pass trial revealed that HVEM expression levels are positively correlated with OS and PFS of MM patients, indicating that the virus receptor may be prognostic. On the contrary, in regard to MM, surface NECTIN-1 expression could not account for...
the efficiency of oHSV-1 infection across all of the MM cell lines tested. Since an HVEM blocking antibody prevented oHSV-1 infection of MM cells, we think that the high levels of HVEM make MM cells highly susceptible to HSV infection. This result is also consistent with the finding that none of the HVEM−/− MM cell lines was susceptible to infection by T-01.10 Thus, this work highlights the potential for oHSV-1 and its receptor HVEM as novel anti-MM treatment approaches. OVls lead to anti-neoplastic effects mainly by (1) induction of immune responses to viral- and/or tumor-specific antigens, (2) direct lysis of cancer cells, and (3) vascular breakdown within the tumor microenvironment. Vaccinia virus and HSV can infect endothelial cells of tumor vasculature, disrupting it and causing indirect death of cells that may not be infected by virus.36,37

Our data demonstrate that, while oHSV-1 treatment had a profound effect on MM growth in vitro, there was no evidence of viral replication and lysis in these cells. We found evidence of OV-induced cell death by activation of ER stress response pathways.38 ER stress response also plays a critical role in the survival of MM cells, and induction of ER stress response pathways occurs in both reovirus-induced MM cell death39 and in the oHSV-1-infected cervical carcinoma cell line HeLa.40 However, MM cell lines treated with oHSV-1 did not show activation of an ER stress response or unfolded protein response (UPR), ruling out this mechanism of cell death in MM. Because our data suggested that oHSV-1 kills MM cells via a mechanism independent of lytic viral replication, we next turned our attention to apoptosis. Although viral infection often leads to an apoptotic response by a cell in order to protect other cells from a similar fate,40 there is evidence of oHSV-1-induced apoptosis even with productive viral infection in human gastric cancer cells27 and HEp2 cells.28 Myxoma induces rapid cellular apoptosis in MM at a rate such that it aborts the virus replication cycle prior to generation of progeny virus.41,42 In this work, MM cell lines infected with oHSV-1 showed similar induction of apoptosis by cleavage of apoptotic effector caspase-3 and PARP, a marker for late-stage apoptosis. Furthermore, the partial rescue from oHSV-1-mediated apoptosis in the presence of a pan-caspase inhibitor (Z-VAD-FMK) in MM cells and further potentiation in the presence of venetoclax, which induces caspase-3 cleavage,29 assert the involvement of caspase-3 in the process. These results suggest that oHSV-1 kills MM cells through a viral-mediated induction of programmed cell death. However, since caspase inhibition alone failed to completely rescue MM cells from oHSV-1-induced cell death, the finding suggests involvement of other mechanisms in the process. Survival of myeloma cells may be influenced by

Figure 5. oHSV-1 infects and induces apoptosis in primary myeloma (MM) cells
(A) Primary MM cells derived from BM aspirates and peripheral blood (PB) of the same MM patients were separately infected with oHSV-1 at an MOI of 5. Forty-eight hours following infection, cells were stained with annexin V/7-AAD to determine the percentage of late apoptotic cells by flow cytometric analysis. (B) Primary MM cells derived from BM aspirates and PB of the same MM patients were separately infected with oHSV-1 at MOIs of 5 and 10. Percentage of viable cells was measured at indicated time points. (C) The CD138+ cell population was isolated from the entire cellular extract derived from BM aspirates of MM patients, and CD138+ and CD138− fractions were similarly treated with oHSV-1 (MOIs of 5 and 10) for 24 and 48 h, and the percentage of viable cells was determined. (D) Primary MM cells derived from BM aspirates and PB of the same MM patients, and also (E) CD138+ and CD138− fractions isolated from BM aspirates, as previously shown in Figure 1D, were separately infected with oHSV-1 at an MOI of 10. Forty-eight hours after infection, cells were observed under a fluorescence microscope for the presence of GFP. Data are reported as mean ± SD of three to four experiments. *p < 0.05, **p < 0.001.
autophagy, and reovirus-mediated oncolysis in MM cells is caused by both induction of apoptosis and autophagy. Autophagic responses of infected tumor cells can either prevent autophagy activation or can induce autophagic cell death in squamous cell carcinoma. Interestingly, the oHSV-1 viral γ34.5 gene contains a Beclin1-binding domain that inhibits the progression of autophagosomes. Hence, similar to other γ34.5 gene-deficient oHSV-1, the oHSV-1 construct used in this study lacking both copies of the viral γ34.5 gene might therefore activate both autophagic and apoptotic cell death in MM cells. Furthermore, it has been recently reported that oncolytic HSV-1, T-01, induces direct oncolysis and immune activation governed by plasmacytoid dendritic cells (pDCs) and natural killer (NK) cells in plasma cell neoplasms.

Additionally, oHSV-1 also induced apoptosis in primary MM cells. It was observed to be higher in the total cellular fraction isolated from BM aspirates of patients with MM, compared to that isolated from PB, and also in the BM CD138+ cellular fraction compared to the BM CD138− cellular fraction. Such differences may be associated with the specific high level of expression of HVEM in MM plasma cells compared to other BM cellular fractions. Thus, on the basis of our observation, it is reasonable to explore the possibility of using surface HVEM as a potential biomarker in MM. Our standpoint is supported by two different xenograft mouse models of MM that indicate potent anti-MM efficacy of oHSV-1 even in vivo. We also speculate that plasma cells may be susceptible to naturally occurring HSV-1 infection, which may result in the observed aberrant interferon signaling, a hypothesis that requires further testing.

Can recombinant HSV-1 move forward clinically? HSV-1 is the first approved OV, T-VEC, for the treatment of melanoma via intratumoral injection. HSV spreads locally within the injected tumor and kills tumor cells by in situ necroptosis in the regional lymph nodes. However, detailed analysis of individual lesion response rates showed complete responses in 46% of injected lesions, 30% of uninjected non-visceral lesions, and only 9% of uninjected visceral lesions; evidently, direct infection is important for patients with metastatic disease. MM is a systemic hematologic malignancy with heterogeneous marrow infiltration, which makes intratumoral injection unattractive. Intravenous OV administration is a challenge, as the bloodstream dilutes the virus, circulating antiviral antibodies can remove the agent, and local macrophages sequester viruses before reaching the tumor. Thus, it is imperative to develop strategies to overcome these host immune viral responses. To this end,
cyclophosphamide has been shown to be a suitable immunosuppres-
Sant in animal models and in early clinical trials with measles virus, 
herpes virus, and reovirus.\textsuperscript{52–54} It is noteworthy that cyclophos-
phamide, which is an approved therapeutic for MM,\textsuperscript{55–57} when given in 
a metronomic regimen\textsuperscript{58} sufficient to prolong viral dissemination 
in MM patients may facilitate anti-tumor efficacy\textsuperscript{54} in combination 
with systemic oHSV-1. Moreover, our group was the first to success-
fully deliver Reolysin (pelaorep) as a systemically administered viral 
oncolytic in patients with relapsed MM.\textsuperscript{58} Thus, promising ap-
proaches to deliver viruses intravenously include the use of immuno-
suppressants, the future use of viruses with low seroprevalence in hu-
mans,\textsuperscript{41} and using monocyes to transport OVs to tumors.\textsuperscript{59} Taken 
together, the results obtained in this work provide a platform for 
future exploring the possibility of using oHSV-1 in the treatment of 
MM. The combined use of approaches ensuring improved viral 
dissemination, thereby maximizing the efficacy of HSV-1 oncolytic 
therapy, might represent an available approach for MM when 
conventional treatments fail. Additionally, the highly expressed virus 
receptor HVEM can be further studied for its potential role as a future 
candidate for cell therapy and also as a prognostic biomarker for MM.

MATERIALS AND METHODS

Cell culture

MM cell lines (MM.1S, NCI-H929, RPMI 8226, LP1, U266, L363, and 
KMS11) were purchased from ATCC. The cell lines were cultured in 
RPMI 1640 supplemented with 10% or 2% (prior to infection) fetal 
bovine serum (FBS) (catalog no. 019K8420, Sigma), 100 IU/mL peni-
cillin, and 100 μg/mL streptomycin. African green monkey kidney 
epithelial Vero cells were cultured in DMEM supplemented with 
10% or 2% FBS, 100 IU/mL penicillin, and 100 μg/mL streptomycin.

oHSV-1

Recombinant HSV-1 (rHSVQ1), used and denoted in this work as 
oHSV-1 (Figure S1A), is a first-generation OV deleted with both 
copies of γ34.5 encoding the neurovirulence factor ICP34.5 and 
ICP6 inactivated by insertion of GFP in the UL39 locus encoding 
the large subunit of the viral ribonucleotide reductase as previously 
described.\textsuperscript{15,16} Upon infection in normal cells, γ34.5 gene product 
ICP34.5 targets host shutoff of protein synthesis, enabling the wild-
type virus to replicate in host cells. With deletion of the γ34.5 gene 
and insertional inactivation of viral ICP6 in recombinant, attenuated 
oHSV-1, the virus can only replicate in cancer cells that can comple-
ment these mutations, thereby conferring several important safety ad-

teavantages.\textsuperscript{61–66} Additionally, genetically modified HSV-1 virus con-
structs, namely RAMBO,\textsuperscript{16} which expresses human Vstat120 under 
 viral IE4/5 promoter, and rQnestin34.5, which expresses viral 
ICP34.5 under the regulation of a glioma-specific nestin promoter\textsuperscript{17} 
within the same oHSV-1 backbone (Figure S1A), were used to deter-
ment efficacy of HSV-1 infection of myeloma cells independent of ge-
netic modification.

Primary samples and CD138\textsuperscript{+} plasma cell isolation

Primary samples of blood and BM aspirates from MM patients at The 
Ohio State University were obtained from an Institutional Review 
Board (IRB)-approved protocol (ClinicalTrials.gov: NCT01408225); 
patient samples from Emory University were also collected from an 
IRB-approved protocol (Winship 2226/IRB00057236). BM mononu-
clear cells were isolated using Ficoll gradient centrifugation. CD138\textsuperscript{+} 
plasma cells were purified from all cellular fractions of BM aspirates 
by human whole blood CD138 MicroBeads (catalog no. 140-000-673, 
Miltenyi Biotec) following the manufacturer’s instructions.

Virus propagation and plaque formation assays

Recombinant HSV1 (oHSV-1) was propagated in Vero cells. Three 
days after infection, secreted virus and virus-infected Vero cells 
were harvested, subjected to repeated freeze-thaw cycles, and soni-
cated to release the viruses completely, after which cell debris was 
cleared by centrifugation (4,000 × g, 20 min). Virus containing super-
natant was filtered to remove cell debris and further pelleted by 
 centrifugation at 13,000 × g for 1 h. The virus pellet was dissolved 
in 10 mL of sterile normal saline and purified by 30% sucrose gradient 
ultracentrifugation at 13,000 × g for 90 min using a Sorvall RC-5C 
Plus ultracentrifuge. The titer (PFU/mL) of the resulting virus 
was determined by PFU assay in Vero cells.\textsuperscript{15} For viral infections, 
myeloma cells were treated with oHSV-1 in RPMI 1640 medium with 
2% FBS at the indicated MOI at 37°C and harvested at described 
time points.

Analysis of oHSV-1 infection in cultured myeloma cells

To measure initiation of early viral gene expression or infection, hu-
man myeloma cell lines and primary cells were treated with oHSV-1 
at the indicated MOIs and then analyzed at the indicated time points 
for the expression of GFP using fluorescence microscopy or flow 
cytometry.
Virus replication assay
To measure completion of the viral replication cycle and production of new infectious progeny virus, replication assays were performed. The indicated MM cell lines were infected with oHSV-1 at an MOI of 0.01, corresponding to 500 PFU/mL. Human serum IgG was added between 15 and 16 h of infection. Seventy-two hours after infection, secreted virus and virus-infected MM cells were harvested, subjected to repeated freeze-thaw cycles, sonicated, and centrifuged to obtain supernatant with live viruses. The supernatant was then serially diluted and titrated on Vero cells as described earlier. The number of plaques formed on Vero cells 72 h after infection with the supernatant was compared with that from the initial virus titer used to infect MM cell lines, thereby determining the replication efficacy of the virus within MM cells.

Reagents
Human serum IgG (catalog no. 14506) was purchased from Sigma. Pan-caspase 3 inhibitor Z-VAD-FMK (catalog no. G723A, Promega) was used at concentrations 10, 20, and 50 μM. Venetoclax (catalog no. 4762, Tocris Bioscience) was used at concentration of 5 μM. HVEM antibody (D-5) (catalog no. sc-365971, Santa Cruz) and NECTIN-1 antibody (F-10) (catalog no. sc-271063, Santa Cruz) were used for receptor blocking.

Cell proliferation assay
Myeloma cells (5 × 10³) were seeded in 100 μL of RPMI 1640 medium with 2% FBS per well in 96-well plates in quadruplicate. The cells were infected with live oHSV-1 or with HI (100°C for 1 h) of HSV-1 or with CM of oHSV-1-treated myeloma cells at indicated MOIs and kept incubated for the indicated time points. Cell proliferation was assessed using an aqueous non-radioactive cell proliferation assay kit (Promega).

Flow cytometry
Analysis of in vitro infection and killing of myeloma cell lines and primary cells by GFP-expressing oHSV-1 was done at the indicated time points by staining control and infected cells with V450 annexin V (catalog no. 560506, Becton Dickinson) and 7-AAD (catalog no. 559925, BD Pharmingen) following the manufacturer’s protocol and quantitating GFP̂ and early (annexin’/7-AAD̂’) and late apoptotic cells (annexin V̂/7-AAD’̂) by flow cytometry. Data were analyzed using FlowJo 2.0 (Tree Star, Ashland, OR, USA). Alexa Fluor 647 mouse anti-human HVEM (CD270, catalog no. 56441, BD Pharmingen) and mouse anti-human NECTIN-1 (CD111)-phycoerythrin (PE) (catalog no. 130-103-833, Miltenyi Biotec) were used to determine HVEM and NECTIN-1 expression, respectively, and flow data were analyzed using Kaluza software (Beckman Coulter). To measure HVEM (CD270) expression on plasma cells, cells isolated from BM extracts were stained with anti-CD138 fluorescein isothiocyanate (FITC) (Becton Dickinson), CD38 V450 (Becton Dickinson), and CD270 allophycocyanin (APC) (Becton Dickinson) to distinguish MM cells (CD138⁺, CD38⁺) from all other BM non-MM cells and then analyzed by flow cytometry.

In vitro blocking of HVEM and NECTIN-1 receptors during oHSV-1 infection
1 × 10⁶ MM cells (MM.1S, NCI-H929, RPMI 8266, and L363) were incubated in growth medium with 10 μg/mL HVEM antibody (D-5) (catalog no. sc-365971, Santa Cruz) and/or NECTIN-1 antibody (F-10) (catalog no. sc-271063, Santa Cruz) for 30 min, then infected with an MOI of 0.1 oHSV-1. After 24 h the percentage levels of infected GFP̂ cells, as well as HVEM and NECTIN-1 expression, were assessed by flow cytometry.

Western blotting
Cell lysates were prepared in 1× radioimmunoprecipitation assay (RIPA) lysis buffer, quantitated by the bicinchoninic acid (BCA) method, run on 4%–20% gradient SDS-PAGE gels, and transferred to polyvinylidene fluoride (PVDF) membranes. Membranes were blocked for at least 60 min with 5% nonfat dry milk in TBS-T (25 mM Tris, 150 mM NaCl, 2 mM KCl, 0.1% Tween 20 [pH 7.4]) and incubated overnight at 4°C with the indicated primary antibody diluted (1:1,000) in 2% nonfat dry milk in TBS-T. Appropriate secondary antibody incubations were done at room temperature for 2 h, and blots were treated with chemiluminescent substrate (Pierce Biotechnology) before exposure. For western blots, the primary antibodies used were caspase-3 rabbit Ab (catalog no. 9662S, Cell Signaling Technology), cleaved caspase-3 (catalog no. 9664S, Cell Signaling Technology), PARP rabbit Ab (catalog no. 9542S, Cell Signaling Technology), PERK (catalog no. 5683S, Cell Signaling Technology), Hsp90 rabbit Ab (catalog no. 4877S, Cell Signaling Technology), Hsp94 rabbit Ab (catalog no. 4871P, Cell Signaling Technology), Bip/GRP78 rabbit Ab (catalog no. 3177S, Cell Signaling Technology), GAPDH mouse Ab (catalog no. 97166S, Cell Signaling Technology), and β-tubulin rabbit Ab (catalog no. 2146S, Cell Signaling Technology). The secondary antibodies used were anti-rabbit IgG horseradish peroxidase (HRP)-linked whole Ab (catalog no. NA934, Sigma) and anti-rabbit IgG HRP-linked whole Ab (catalog no. NNA931, Sigma).

Mouse experiments
All animal studies were approved by The Ohio State University Institutional Laboratory Animal Care and Use Committee (IACUC). Mice were housed under a 12-h light/12-h dark cycle with food and water ad libitum. A cohort of 6- to 8-week-old male and female NSG mice was injected subcutaneously with 12.5 × 10⁶ MM.1S or NCI-H929 cells in their right flank. At the time of presence of palpable tumors, the mice were randomly distributed into two experimental groups each and intratumorally (subcutaneously) treated twice a week for 2 weeks with (1) sterile normal saline, 100 μL (control group, n = 7); or (2) oHSV-1, 10⁶ PFU in 100 μL of sterile normal saline (oHSV-1-treated group, n = 7). Tumor measurements (by caliper) were taken three times a week before control mice tumors reached the endpoint volume (30 days after tumor implantation), and mice were humanely euthanized. Because of the characteristic irregular tumor shape using the MM.1S or NCI-H929 cell line, tumor volume (in mm³) was estimated by measuring the long side (mm) times the short side (mm) times the height (mm) of the tumor. The volumes of the
tumors (in mm³) between control and oHSV-1-treated groups were compared to determine the in vivo anti-myeloma efficacy of the virus.

Statistical analysis
For in vitro experiments, data are reported as mean ± SD of three to four experiments. For experiments involving two groups, we have performed a two-tailed unpaired Mann-Whitney test. For experiments involving multiple groups, statistical analysis was conducted by one-way ANOVA followed by a post hoc Tukey’s honestly significant difference (HSD) test (*p < 0.05, **p < 0.001) for pairwise comparisons between groups to determine statistical significance between various pairs of mean. For animal experiments involving two groups, a non-parametric Mann-Whitney test was done (*p < 0.05, **p < 0.001) and the data are shown as mean ± SEM. All statistical analyses were performed using GraphPad software (GraphPad, La Jolla, CA, USA).

SUPPLEMENTAL INFORMATION
Supplemental information can be found online at https://doi.org/10.1016/j.omto.2021.02.009.

ACKNOWLEDGMENTS
We are grateful to Domenico Viola for technical support in this work. Research reported in this publication was supported by the generous contributions of the Multiple Myeloma Opportunities for Research and Education (MMORE). This research was supported by the National Cancer Institute of the National Institutes of Health under award number P30CA016058, P30CA033572, and R01CA194742. B.G.B. is supported by developmental funds from the Winship Cancer Institute of Emory University, post-doctoral fellowship PF-17-109-1-TBG from the American Cancer Society, and a Research Fellow Award from the Multiple Myeloma Research Foundation (MMRF). Research reported in this publication included work performed at the City of Hope Liquid Tissue Bank and Analytical Cytometry cores supported by the National Institutes of Health under award number P30CA033572. The content is solely the responsibility of the authors and does not represent the official views of the National Cancer Institute or the National Institutes of Health. The graphical abstract was prepared with the help of BioRender.com.

AUTHOR CONTRIBUTIONS
J.G. performed most of the experiments, A.D. and E.G.G. performed cell line experiments focused on Nectin and HVEM staining and blocking antibodies. M.M. performed work on HSV virus amplification and experiments associated with the effect of HSV + venetoclax in different myeloma cell lines. E.C., J.Y.Y., and L.R. helped perform animal experiments. L.R. and A.C.J.-R. performed flow cytometry on MM cell lines to determine infection. B.G.B. and V.A.G. analyzed the CoMMpass data and performed flow cytometry on myeloma BM samples for HVEM surface expression. J.G., C.C.H., A.D., and F.P. wrote the manuscript. J.F.S. scientifically corrected the manuscript. D.W.S., S.T.R., A.K., L.H.B., and B.K. revised the manuscript. All of the authors have contributed substantially to the analysis and interpretation of data, revising the manuscript for important content, and final approval.

DECLARATION OF INTERESTS
C.C.H. reports grants from Janssen, Bristol-Meyers Squibb, and Oncotherapeutics Biotech, grants and personal fees for membership of a marketing advisory board from Celgene, personal fees for membership of a research advisory board from Karyopharm, personal fees for membership of a research advisory board from Oncopeptides and Imbrium Therapeutics, and personal fees for membership of a regulatory advisory board from Adaptive Biotechnologies, all outside the submitted work. D.W.S. reports grants from Oncotherapeutics Biotech, Bristol-Meyers Squibb, GlaxoSmithKline, and Janssen, all outside the submitted work. L.H.B reports research funding from AstraZeneca and personal fees for membership of advisory boards from AstraZeneca and Genentech.

REFERENCES
1. Bartee, E. (2018). Potential of oncolytic viruses in the treatment of multiple myeloma. Oncolytic Virother. 7, 1–12.
2. Meyers, D.E., Thakur, S., Thirukkumaran, C.M., and Morris, D.G. (2017). Oncolytic virotherapy as an immunotherapeutic strategy for multiple myeloma. Blood Cancer J. 7, 640.
3. Oliva, S., Gambella, M., Boccadoro, M., and Bringen, S. (2017). Systemic virotherapy for multiple myeloma. Expert Opin. Biol. Ther. 17, 1375–1387.
4. Alessandri, F., Menotti, L., Avitabile, E., Appolloni, I., Ceresa, D., Marabbi, D., Campadelli-Fiume, G., and Malatesta, P. (2019). Eradication of glioblastoma by immuno-virotherapy with a retargeted oncolytic HSV in a preclinical model. Oncogene 38, 4467–4479.
5. Bommarettyy, P.K., Aspromonte, S., Zloza, A., Rabkin, S.D., and Kaufman, H.L. (2018). MEK inhibition enhances oncolytic virus immunotherapy through increased tumor cell killing and T cell activation. Sci. Transl. Med. 10, eaar4017.
6. Varghese, S., and Rabkin, S.D. (2002). Oncolytic herpes simplex virus vectors for cancer virotherapy. Cancer Gene Ther. 9, 967–978.
7. Kanai, R., Wakimoto, H., Cheema, T., and Rabkin, S.D. (2010). Oncolytic herpes simplex virus vectors and chemotherapy: are combinatorial strategies more effective for cancer? Future Oncol. 6, 619–634.
8. Rehmam, H., Silk, A.W., Kane, M.P., and Kaufman, H.L. (2016). Into the clinic: talimogene laherparepvec (T-VEC), a first-in-class intratumoral oncolytic viral therapy. J. Immunother. Cancer 4, 53.
9. Streby, K.A., Currier, M.A., Triplett, M., Ott, K., Dishman, D.J., Vaughan, M.R., Ranalli, M.A., Setty, B., Skeens, M.A., Whiteside, S., et al. (2019). First-in-human intravenous Seprehvir in young cancer patients: a phase 1 clinical trial. Mol. Ther. 27, 1930–1938.
10. Ishino, R., Kwase, Y., Kitawaki, T., Sugimoto, N., Oku, M., Uchida, S., Imataki, O., Matsuoka, A., Tsuoka, T., Kawakami, K., et al. (2021). Oncolytic virus therapy with HSV-1 for hematological malignancies. Mol. Ther. 29, 762–774.
11. Oku, M., Ishino, R., Uchida, S., Imataki, O., Sugimoto, N., Todo, T., and Kadowaki, N. (2021). Oncolytic herpes simplex virus type 1 (HSV-1) in combination with lenalidomide for plasma cell neoplasms. Br. J. Haematol. 192, 343–353.
12. Glove, J., Russell, L., Caserta, E., Santanam, S., Jaime-Ramirez, A.C., Viola, D., Krishnan, A., Hofmeister, C.C., Kaur, B., and Pichiorri, F. (2016). Exploring the possibility of using herpes simplex virus in oncolytic virotherapy of multiple myeloma. Blood 128, 4467.
13. Hobo, W., Norde, W.I., Schaap, N., Fredrix, H., Maas, F., Schellen, K., Falkenburg, J.H., Korman, A.J., Olive, D., van der Voort, R., and Dolstra, H. (2012). B and T lymphocyte attenuator mediates inhibition of tumor-reactive CD8⁺ T cells in patients following allogeneic stem cell transplantation. J. Immunol. 189, 39–49.
14. Zhang, N., Yan, J., Lu, G., Guo, Z., Fan, Z., Wang, J., Shi, Y., Qi, I., and Gao, G.F. (2011). Binding of herpes simplex virus glycoprotein D to nectin-1 exploits host cell adhesion. Nat. Commun. 2, 577.

15. Yoo, J.Y., Haseley, A., Bratasz, A., Chiocca, E.A., Zhang, J., Powell, K., and Kaur, B. (2012). Antitumor efficacy of 34.5mNEV: a transcriptionally retargeted and "Vst120"-expressing oncolytic virus. Mol. Ther. 20, 287–297.

16. Hardcastle, J., Kurozumi, K., Dmitrieva, N., Sayers, M.P., Ahmad, S., Waterman, P., Weissleder, R., Chiocca, E.A., and Kaur, B. (2010). Enhanced antitumor efficacy of vasculostatin (Vstat120) expressing oncolytic HSV-1. Mol. Ther. 18, 285–294.

17. Kambara, H., Okano, H., Chiocca, E.A., and Sakiy, S. (2005). An oncolytic HSV-1 mutant expressing ICP34.5 under control of a nestin promoter increases survival of animals even when symptomatic from a brain tumor. Cancer Res. 65, 2832–2839.

18. De Chiara, G., Marcocci, M.E., Civitelli, L., Argnani, R., Piacentini, R., Ripoli, C., Manservigi, R., Grassi, C., Garaci, E., and Palamara, A.T. (2010). APP processing induced by herpes simplex virus type 1 (HSV-1) yields several APP fragments in human and rat neuronal cells. PLoS ONE 5, e13898.

19. Akhtar, J., and Shukla, D. (2009). Viral entry mechanisms: cellular and viral mediators of herpes simplex virus entry. FEBS J. 276, 7228–7236.

20. Stiles, K.M., Mihe, R.S., Cohen, G.H., Eisenberg, R.J., and Krumenacher, C. (2008). The herpes simplex virus receptor nectin-1 is down-regulated after trans-interaction with glycoprotein D. Virology 373, 98–111.

21. Ghermezi, M., Li, M., Vardanyan, S., Harutyunyan, N.M., Gottlieb, J., Berenson, A., Spektor, T.M., Andreu-Vieyra, C., Petraki, S., Sanchez, E., et al. (2017). Serum B-cell adhesion. Nat. Commun. 8, 102.

22. Shukla, D., Liu, J., Blaiklock, P., Shworak, N.W., Bai, X., Eko, J.D., Cohen, G.H., Eisenberg, R.J., Rosenberg, R.D., and Spear, P.G. (1999). A novel role for 3-O-sulfated heparan sulfate in herpes simplex virus 1 entry. Cell 99, 13–22.

23. Huang, Y.Y., Yu, Z., Lin, S.F., Li, S., Feng, Y., and Wong, R.J. (2007). Nectin-1 is a marker of thyroid cancer sensitivity to herpes oncolytic therapy. J. Clin. Endocrinol. Metab. 92, 1965–1970.
pharmacology and toxicology of intravenous MV-NIS, an oncolytic measles virus administered with or without cyclophosphamide. Clin. Pharmacol. Ther. 82, 700–710.

53. Fulci, G., Breymann, L., Gianni, D., Kurozumi, K., Rhee, S.S., Yu, J., Kaur, B., Louis, D.N., Weissleder, R., Caligiuri, M.A., and Chiocca, E.A. (2006). Cyclophosphamide enhances glioma virotherapy by inhibiting innate immune responses. Proc. Natl. Acad. Sci. USA 103, 12873–12878.

54. Qiao, J., Wang, H., Kottke, T., White, C., Twigger, K., Diaz, R.M., Thompson, J., Selby, P., de Bono, J., Melcher, A., et al. (2008). Cyclophosphamide facilitates antitumor efficacy against subcutaneous tumors following intravenous delivery of reovirus. Clin. Cancer Res. 14, 259–269.

55. Steiner, N., Nachbaur, D., König, P., Willenbacher, W., and Gunsilius, E. (2015). Long-term control in a patient with refractory multiple myeloma by oral cyclophosphamide and dexamethasone. Anticancer Res. 35, 2165–2168.

56. Mellqvist, U.H., Lenhoff, S., Johnsen, H.E., Hjorth, M., Holmberg, E., Jullian, G., Tangen, J.M., and Westin, J.; Nordic Myeloma Study Group (2008). Cyclophosphamide plus dexamethasone is an efficient initial treatment before high-dose melphalan and autologous stem cell transplantation in patients with newly diagnosed multiple myeloma: results of a randomized comparison with vincristine, dexamethasone, and dexamethasone. Cancer 112, 129–135.

57. Ito, S., Oyake, T., Murai, K., and Ishida, Y. (2013). Successful use of cyclophosphamide as an add-on therapy for multiple myeloma patients with acquired resistance to bortezomib or lenalidomide. Case Rep. Hematol. 2013, 651902.

58. Sborov, D.W., Nuovo, G.J., Stiff, A., Mace, T., Lesinski, G.B., Benson, D.M., Jr., Efebera, Y.A., Rosko, A.E., Pichiorri, F., Grever, M.R., and Hofmeister, C.C. (2014). A phase I trial of single-agent reovirus in patients with relapsed multiple myeloma. Clin. Cancer Res. 20, 5946–5955.

59. Berkeley, R.A., Steele, L.P., Mulder, A.A., van den Wollenberg, D.I.M., Kottke, T.J., Thompson, J., Coffey, M., Hoenen, R.C., Vile, R.G., Melcher, A., and Ilett, E.J. (2018). Antibody-neutralized reovirus is effective in oncolytic virotherapy. Cancer Immunol. Res. 6, 1161–1173.

60. Barwick, B.G., Neri, P., Bahlis, N.I., Nooka, A.K., Dhopakar, M.V., Jaye, D.L., Hofmeister, C.C., Kaufman, J.L., Gopak, V.A., Auclair, D., et al. (2019). Multiple myeloma immunoglobulin lambda translocations portend poor prognosis. Nat. Commun. 10, 1911.

61. Kanai, R., Zauza, C., Sgubin, D., Antosczyk, S.I., Martuza, R.L., Wakimoto, H., and Rabkin, S.D. (2012). Effect of γ34.5 deletions on oncolytic herpes simplex virus activity in brain tumors. J. Virol. 86, 4420–4431.

62. Gale, M., Jr., and Katze, M.G. (1998). Molecular mechanisms of interferon resistance mediated by viral-directed inhibition of PKR, the interferon-induced protein kinase. Pharmacol. Ther. 78, 29–46.

63. He, B., Gross, M., and Roizman, B. (1997). The gamma134.5 protein of herpes simplex virus 1 complexes with protein phosphatase 1α to dephosphorylate the alpha subunit of the eukaryotic translation initiation factor 2 and preclude the shutoff of protein synthesis by double-stranded RNA-activated protein kinase. Proc. Natl. Acad. Sci. USA 94, 843–848.

64. Farassati, F., Yang, A.D., and Lee, P.W. (2001). Oncogenes in Ras signalling pathway dictate host-cell permissiveness to herpes simple virus 1. Nat. Cell Biol. 3, 745–750.

65. Bezieau, S., Devilder, M.C., Avert-Loiseau, H., Mellerin, M.P., Puthier, D., Pennarun, E., Rapp, M.J., Harousseau, J.L., Moisan, J.P., and Batlle, R. (2001). High incidence of N and K-Ras activating mutations in multiple myeloma and primary plasma cell leukemia at diagnosis. Hum. Mutat. 18, 212–224.

66. Liu, B.L., Robinson, M., Han, Z.Q., Branston, R.H., English, C., Reay, P., McGrath, Y., Thomas, S.K., Thornton, M., Bullock, P., et al. (2003). ICP34.5 deleted herpes simplex virus with enhanced oncolytic, immune stimulating, and anti-tumour properties. Gene Ther. 10, 292–303.