

Potential of oncolytic viruses in the treatment of multiple myeloma

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Abstract: Multiple myeloma (MM) is a clonal malignancy of plasma cells that is newly diagnosed in ~30,000 patients in the US each year. While recently developed therapies have improved the prognosis for MM patients, relapse rates remain unacceptably high. To overcome this challenge, researchers have begun to investigate the therapeutic potential of oncolytic viruses as a novel treatment option for MM. Preclinical work with these viruses has demonstrated that their infection can be highly specific for MM cells and results in impressive therapeutic efficacy in a variety of preclinical models. This has led to the recent initiation of several human trials. This review summarizes the current state of oncolytic therapy as a therapeutic option for MM and highlights a variety of areas that need to be addressed as the field moves forward.

Keywords: oncolytic virotherapy, multiple myeloma, review

Introduction

Multiple myeloma (MM) is a clonal malignancy of immunoglobulin-secreting plasma cells. The disease is newly diagnosed in >30,000 people in the US annually, resulting in ~12,000 deaths, making it the second-most common form of hematopoietic malignancy.^{1,2} The disease often evolves slowly through several progressive stages.³ If identified early, patients will often display a nonmalignant precancerous state known as monoclonal gammopathy of undetermined significance.⁴ Over time, this progresses to a malignant but still largely asymptomatic disease called smoldering myeloma, in which clonal plasma cells slowly increase in the bone marrow.^{5,6} Eventually, disease progresses to symptomatic MM, in which malignant cells proliferate rapidly in the bone marrow, causing localized sites of malignancy known as plasmacytomas. While a few patients present with single plasmacytomas,⁷ most develop a systemic disease characterized by multiple distinct tumors, hence the name “multiple myeloma”. Malignant cells in these lesions secrete a variety of factors that cause remodeling of local bone structure, resulting in osteolytic lesions.⁸ This bone remodeling, combined with malignant cells outcompeting normal bone marrow cells for proliferative space, cause the typical symptoms of MM, including elevated levels of calcium in the blood, renal failure, anemia, and bone pain or fractures (frequently referred to as CRAB).²

Historically, treatment for MM patients involved combinations of chemotherapeutic drugs, such as melphalan, cyclophosphamide, and doxorubicin, either with or without radiation. These treatments were frequently able to induce at least partial remissions; however, patients suffered extremely high rates of relapse, typically within 2–3 years. The reason for these relapses was complex and likely involved a variety of factors,

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including the high degree of genomic variability inherent in MM,^{9–11} a tendency for MM cells to display phenotypic plasticity,^{12,13} the relatively undefined nature of tumorigenic MM stem cells,^{14,15} the resistance of these cells to many chemotherapeutic agents,^{16,17} and the interplay between MM cells and their complex tumor microenvironment, which provides a variety of progrowth and pro-survival signals.¹⁸ Due to this poor prognosis, eligible MM patients were often treated with a more aggressive regime involving myeloablative chemotherapy combined with single or tandem autologous stem-cell transplant (ASCT).^{19,20} This improved prognosis; however, the myeloablative conditioning regimes used were associated with severe treatment-related morbidities, including neutropenia and increased chances of infection. This largely limited the usage of ASCT to younger, healthier patients. Additionally, even with ASCT, disease often recurred within 3–5 years. The primary cause of recurrence following ASCT was thought to be residual disease persisting within treated patients.^{21,22} However, studies also found that virtually all ASCT samples were contaminated with low levels of malignant MM cells that were reintroduced to the patient during transplant.^{23,24} The exact impact of these contaminating MM cells on disease relapse remains controversial; however, their reintroduction seems unlikely to be beneficial.

From 2000 to 2005, novel chemotherapeutic agents, including bortezomib,²⁵ thalidomide,²⁶ and lenalidomide,^{27,28} began to improve outcomes for newly diagnosed MM patients. Use of these drugs rapidly increased the median overall survival from 2.5 years in 2000 to over 6.5 years in 2010.^{29,30} Since 2010, a variety of new drugs have also been approved, including carfilzomib,³¹ pomalidomide,³² panobinostat,³³ daratumumab,³⁴ and ixazomib,³⁵ which promise to improve diagnoses even further. Due to these advances, the long-held belief of MM being “incurable” is slowly being challenged.³⁶ Unfortunately, even with the approval of these new drugs, the proportion of patients who achieve durable long-term remissions is still predicted to be <50%.³⁶

Since two of the major factors contributing to therapeutic failure in MM are tumor-cell heterogeneity and plasticity,^{9–13} alternative therapies for MM that do not rely on the use of targeted small-molecule inhibitors are highly attractive. One such therapy is the use of live replicating viruses specifically to infect and kill malignant MM cells, a strategy known as oncolytic virotherapy (OV).^{37,38} OV functions through two distinct mechanisms.^{39,40} In the first mechanism, known as direct oncolytics, malignant cells are specifically infected with an oncolytic virus. This infection results in the specific elimination of the infected cells through a variety of potential mechanisms, including lytic

viral replication, induction of apoptosis, or cellular necrosis. This specific tumor-cell killing then initiates a second OV mechanism, often referred to as oncolytic immunotherapy, in which the danger signals provided by viral infection combined with the specific killing of tumor cells result in the generation of antitumor immunoresponses. These responses are often typified by large CD8⁺ T-cell responses reactive against both infected and uninfected tumor cells; however, a variety of other immune cells, including CD4⁺ T cells and natural killer cells, have also been shown to play critical roles.^{41–48}

Each oncolytic mechanism provides a significant benefit while also being associated with therapeutic hurdles. Each of these mechanisms provides a significant benefit, while also being associated with therapeutic hurdles. For example, direct oncolytics is extremely rapid and highly efficient in some settings; however, it only results in the elimination of cells that are directly infected with a virus. This places extremely large importance on the delivery of a virus to a high percentage of malignant cells, a significant challenge that remains largely unsolved by the field. In contrast, oncolytic immunotherapy can be completely sterilizing; however, it is much slower to initiate than direct oncolytics and frequently demonstrates clinical benefit in only a fraction of recipients. Successful OV thus requires striking a delicate balance between the rapid but incomplete direct oncolytics and the slow but sterilizing viral immunotherapy.

A number of different oncolytic viruses have been studied for their therapeutic potential against MM. Interestingly, the findings for each virus are often unique in terms of the mechanisms through which specific infection is achieved, how this infection causes elimination of MM cells, and the therapeutic potential of the virus (Table 1). This review summarizes the existing literature for each oncolytic virus that has been studied as a therapeutic against MM, as well as attempting to provide some context for these studies and potential strengths and weaknesses of the work.

Measles

The measles virus (MV) is a medium-sized (150 nm), negative-sense, single-stranded RNA virus from the Paramyxoviridae family. While wild-type MV is extremely pathogenic in humans, attenuated strains, including the attenuated Edmonston B strain on which oncolytic MV is based, have been used safely as vaccines for years.⁴⁹ The initial oncolytic potential of MV was recognized more than 50 years ago;⁵⁰ however, intensive studies into its therapeutic potential began around 2002.⁵¹

Interestingly, unlikely many oncolytic viruses, which are primarily studied as treatments for solid tumors, much of the

Table I Treatment of MM using oncolytic viruses

Virus	Mechanism of MM specificity	Mechanism of MM-cell killing	Potential toxicity	Potential therapeutic uses
Measles virus	Overexpression of viral receptor (CD46)	Lytic viral replication Syncytia formation (?)	Low	Treatment of established disease
Vesicular stomatitis virus	Defects in interferon responses	Lytic viral replication Inhibition of DNA synthesis (?)	Moderate	Treatment of established disease
Reovirus	Overexpression of viral receptor (JAM-A)	Lytic viral replication Apoptosis Autophagy Unfolded protein response (?)	Low	Treatment of established disease Purging of ASCT samples
Adenovirus	Unknown	Lytic viral replication (?)	Low	Treatment of established disease
Vaccinia virus	Engineered	Lytic viral replication (?)	Moderate	Treatment of established disease Generation of MM vaccines
Myxoma virus	MM-specific binding (receptor unknown)	Induction of apoptosis	Low	Treatment of established disease Purging of ASCT samples

Notes: Overview of oncolytic viruses most commonly studied as treatments for MM, as well as mechanisms through which they achieve specific infection of MM cells, mechanisms through which they eliminate infected MM cells, potential for in vivo toxicities, and proposed therapeutic uses; (?) indicates mechanisms that are suspected, but have not yet been demonstrated.

Abbreviations: MM, multiple myeloma; ASCT, autologous stem-cell transplant.

work with oncolytic MV has been focused on hematopoietic malignancies. Therefore, while MV is far from the most commonly used oncolytic virus overall, it does represent the best-studied viral agent for the treatment of MM. Like many oncolytic viruses, MV has been shown specifically to infect both MM cell lines and CD138⁺ cells in MM-patient bone marrow.^{52,53} The specificity of this infection within the hematopoietic compartment appears to be based on an approximately sevenfold increase in expression of the endogenous MV receptor CD46 on malignant MM cells compared to nonmalignant hematopoietic cells.⁵³ In addition, further improvements to the specificity of viral binding have been obtained by fusing the viral glycoprotein H to single-chain variable-fragment antibodies specific to either CD38⁵⁴ or the Wue1 epitope.⁵⁵ Both studies demonstrated improvements in the specificity of MV infection for malignant MM cells in vitro; however, neither strategy appears to have been adopted in other MV studies. Interestingly, while MV has been well studied as a therapeutic agent against MM, the mechanisms responsible for MM elimination following MV infection appear largely unknown. Presumably, lytic viral replication plays a major role; however, MV is also highly fusogenic, and infection results in the formation of large syncytia.^{52,53,56} The relative impact of each of these processes on elimination of infected MM cells thus remains unclear.

In terms of therapy, studies on MV have focused on the treatment of established disease, and efficacy has been shown in both systemically disseminated and localized

plasmacytoma models.^{52,57–63} In these models, MV has shown exceptionally high efficacy, including the ability to induce potentially curative responses in some settings.^{52,57,58} Unfortunately, MV does not naturally infect mice, due to an inability to bind to murine MV receptors.⁶⁴ This has limited the study of oncolytic MV to models in which human MM cells are xenografted into immunodeficient mice. This inherent limitation has resulted in two major translational hurdles that remain to be adequately addressed. First is that MV therapy, particularly following intravenous injection, is highly restricted by existing α MV humoral immunity. Although MM patients do present with slightly decreased α MV-antibody titers compared to healthy controls,⁶⁵ virtually 100% of patients remain α MV-antibody-positive, and these antibodies can potently neutralize MV infection.⁵⁷ In an oncolytic setting, studies have demonstrated that passive transfer of these α MV antibodies into tumor-bearing mice negatively impacts the resulting efficacy of MV therapy;^{57,59} however, whether this passive transfer fully recapitulates a functional humoral response seems questionable. Several methods to overcome this challenge have been attempted, primarily focusing on the use of different carrier cells, including T cells,⁵⁷ macrophages,⁶¹ and irradiated tumor cells,⁵⁹ to deliver the virus to sites of residual MM. Of these, irradiated tumor cells seem to be the most promising; however, this strategy has not yet been adopted clinically, and MV therapy remains largely restricted to patients who present with low α MV titers.⁶⁶ The second major challenge involves the potential impact of

T cells on MV therapy. These cells could either restrict viral therapy by eliminating infected cells prior to viral replication or be induced to generate potent α MM immunoresponses.³⁹ Unfortunately, the use of xenografted, immunodeficient animal models largely eliminates the possible study of how T-cell responses might impact MV therapy.

Despite these unresolved issues, MV represents one of only two oncolytic viruses that have progressed into clinical trials for the treatment of MM.^{66–68} The virus used in these trials is a derivative of the Edmonston B vaccine strain that was modified to include the sodium–iodine symporter (NIS). Inclusion of the *nis* gene causes infected cells to import high levels of radioactive iodine, which allows for both live imaging of infected cells during therapy and improvement of clinical efficacy by increasing accumulation of toxic auger-emitting radioisotopes. Comprehensive preclinical toxicology studies carried out in either MV-susceptible squirrel monkeys or human CD46 transgenic mice⁶⁸ have demonstrated that MV-NIS is safe for use at high doses. Similarly, while a recently completed Phase I trial did identify some potential grade III and grade IV hematological toxicities, including neutropenia, lymphopenia, anemia, and thrombocytopenia, no dose-limiting toxicities following therapy were identified. While this study was not powered for analysis of efficacy, some impressive results, including one complete disease regression, were observed.^{66,67} Overall, the response rate at the highest dose was 36% (4 of 11 patients). MV was detected in the blood following treatment; however, patients in this study rapidly seroconverted, suggesting that α MV humoral immunity could be a major limitation even in patients who initially present as MV-naïve. Unpublished reports have suggested that responding patients in this trial can develop long-term α MM immunological memory, implicating the potential impact of T-cell immunotherapy; however, this possibility has not yet been thoroughly studied. Despite a number of remaining hurdles, however, its established clinical successes currently make MV the most advanced oncolytic candidate for the treatment of MM.

Vesicular stomatitis virus

The vesicular stomatitis virus (VSV) is a small (75–120 nm), enveloped, negative-sense, single-stranded RNA virus from the Rhabdoviridae family. VSV is a severe animal pathogen, particularly in cattle, where it causes pathology virtually indistinguishable from foot-and-mouth disease. Adsorption of VSV to target cells is accomplished through the viral glycoprotein G, which binds to the ubiquitously expressed low-density-lipoprotein receptor. After entry,

VSV displays an extremely rapid replication cycle in which new viral progeny can be generated in as little as 1–2 hours. This replication, however, is rapidly blocked by the presence of functional innate immunoresponses, particularly interferon. This naturally limits VSV replication to cells in which these responses are not present, including a wide array of transformed malignant cells. Due to its extremely rapid replication cycle and natural restriction to cancerous cells, VSV represents one of the more potent direct oncolytic viruses being studied.

With regard to MM, VSV has been shown directly to infect and kill both MM cell lines and CD138⁺ cells in MM-patient bone-marrow samples.^{69,70} This is likely due to lytic viral replication; however, the virus has also been shown to inhibit DNA and RNA synthesis rapidly in infected MM cells, which could represent a second potential mechanism of killing.⁷¹ Viral replication is predominantly MM-cell-specific, since signs of infection are typically not observed in most peripheral blood cells, including T cells, B cells, and natural killer cells. However, low-level infection can be seen in some neutrophils, and the virus appears fully infectious to normal monocyte.⁶⁹ Additionally, toxicity studies done in mice using an oncolytic VSV armed with IFN β and NIS demonstrated that in addition to malignant cells, viral RNA could be recovered from the liver and spleen⁷² and fully infectious virus found in the spleen. These studies also demonstrated the potential for intravenous injection to cause systemic inflammatory responses and liver toxicity (measured by ALT and AST). In addition to these “off-target” toxicities, “on-target” infection of meningeal MM deposits has been hypothesized to cause potentially lethal inflammation in the central nervous system.⁷³ Therefore, while unmodified VSV appears naturally oncotropic for MM cells, additional work remains needed to identify ways to reduce toxicity that might be associated with systemic therapy.

Therapeutically, VSV has demonstrated efficacy in MM models following both localized and systemic injections.^{70,74–76} Critically, much of this work has been done in syngeneic models of both localized plasmacytomas and systemically disseminated MM. This work thus allows for analysis of both direct and immunotherapeutic OV mechanisms. Interestingly, while oncolytically-induced immunotherapy is often essential in solid tumor models, the work in MM suggests that this process is largely inhibitory to VSV therapy. Two studies comparing the efficacy of VSV in immunodeficient and immunocompetent models found better therapeutic efficacy in the absence of an immune system.^{70,76} Additionally, in two other models with clear immunotherapeutic involvement,

depletion of CD4⁺ and CD8⁺ T cells resulted in only minor loss of efficacy.^{73,75} These data suggest that VSV-based treatment of MM might be predominantly through a direct oncolytic mechanism. Alternatively, it has been shown that systemic VSV therapy is limited by the presence of α VSV antibodies in the blood,⁷⁷ which cause rapid clearance of free virus by the liver. This concern might be addressable, since chemical modifications of the viral virions using polyethylene glycol (PEG) have been shown to slow viral clearance. The effects of this PEGylation during actual OV of MM, however, have not been demonstrated. Therefore, while the potential efficacy of VSV against MM appears well established, more studies into the exact mechanisms involved remain needed.

Reovirus

Reovirus (ReoV) is an extremely large (600–1,000 nm), nonenveloped, double-stranded RNA virus from the Reoviridae family. While this virus can infect humans, in either the gastrointestinal or respiratory tract, it typically causes only subclinical pathology and is considered safe for clinical use. The oncolytic potential of ReoV in models of both solid tumors and hematological malignancies has been studied for many years,^{78,79} and a clinical grade ReoV (derived from the Dearing strain) is currently available under the brand Reolysin.^{80–83}

In vitro, ReoV has been shown specifically to infect and kill both established MM cell lines^{84–88} and CD138⁺ cells in primary MM bone-marrow samples.^{85,86} The specificity of this infection is likely due to high expression of the ReoV receptor JAM-A on malignant MM cells, since the sensitivity of MM cells to ReoV treatment correlates with their JAM-A expression,^{87,88} elimination of JAM-A from MM cell lines prevents their infection with ReoV, and overexpression of JAM-A significantly increases in the sensitivity of MM cells to ReoV.⁸⁷ In contrast, the mechanism through which MM cells are killed following ReoV infection remains somewhat unclear. Killing is clearly dependent on lytic viral replication;^{85,86} however, viral treatment has also been shown to induce cell death through both apoptotic and autophagic pathways.⁸⁵ Additionally, viral infection significantly impacts the unfolded protein response, which is known to play a critical role in MM-cell survival.⁸⁹ It thus remains to be determined which of these potential mechanisms truly mediates ReoV elimination of infected MM cells.

Therapeutically, ReoV has been shown to be efficacious in a variety of preclinical MM models. Thirukkumaran et al demonstrated that ReoV is unable to infect normal CD34⁺ bone-marrow progenitor cells and that the virus

can specifically identify low levels of CD138⁺ MM cells contained in mixtures of normal bone-marrow aspirates.^{84,86} This allows the virus to be used as an ex vivo purging agent to prevent MM reintroduction during ASCT while not negatively impacting hematopoietic reconstitution.^{84–86} Additionally, direct viral injection has been shown to be somewhat therapeutically effective against MM in both systemic^{85,88–90} and localized plasmacytoma⁸⁹ models. Unfortunately, the results of viral monotherapy in these studies have frequently been modest, and complete regression has not often been observed. Similarly, while a Phase I clinical trial of Reolysin monotherapy in relapsed MM patients identified no dose-limiting toxicities, only modest efficacy was seen, with the best clinical outcomes being stable disease (observed in 25% of patients).⁸³ These data suggest that the use of ReoV in combination treatments might be needed to increase therapeutic efficacy.

In this context, it is interesting to note that several groups have shown that existing MM therapies, including histone deacetylase inhibitors or the proteasome inhibitor bortezomib, can sensitize MM cells to ReoV infection by increasing the expression of the viral receptor JAM-A.^{87,88} This suggests that ReoV might be more effective in MM patients who have already failed one or more existing therapies. Alternatively, ReoV and histone deacetylase inhibitors have been shown to act synergistically when used together in vivo, suggesting the possible development of rational combination therapies.⁸⁸ Finally, a recently published work has suggested the possibility of using ReoV to improve response rates of MM patients to additional immunotherapeutic treatments, such as PDL1 blockade.⁹⁰ More work, however, is clearly needed to identify and optimize potential combinatorial therapies involving ReoV.

Adenovirus

Adenovirus (AdV) is a medium-sized (90–100 nm), non-enveloped, double-stranded DNA virus from the Adenoviridae family. The term “adenovirus” actually refers to a large number of distinct viral serotypes, many of which display highly distinct pathologies and infectious characteristics. Taken together, AdV infections in humans are extremely common and can account for almost 10% of all respiratory infections. These infections typically present as a “common cold”, although some serotypes are associated with more severe symptoms, including pneumonia. As therapeutic agents, AdVs are one of the best-studied viral families. They have been used in a variety of therapeutic modalities, including lytically replicating oncolytic studies, vaccine studies, and

gene-therapy studies. Due to the substantial breadth of this work, this review focuses on lytically replicating oncolytic studies using AdV.

In the context of MM, AdV has been shown to be highly infectious to both established MM cell lines and CD138⁺ cells from MM-patient bone-marrow samples.^{91,92} Interestingly, while much of the oncolytic work in solid tumors has focused on a single AdV serotype (type 5), work in MM has directly compared the efficacy of a wide range of AdV serotypes, with interesting results. Senac et al found that serotypes 6, 26, and 48 killed MM cells while having only minimal effects of normal peripheral blood mononuclear cells. In contrast, serotypes 11, 35, 40, and 41 displayed the opposite specificity, killing normal peripheral blood mononuclear cells while largely sparing malignant MM cells.⁹¹ This observation was supported by additional research demonstrating that species D AdV, including the previously identified serotypes 26 and 48, was a highly efficient MM killer, while species B AdV, including serotypes 11 and 35, was a highly inefficient killer. This work identified the most commonly used oncolytic serotype (AdV type 5) as only a modest killer of MM cells. This serotype hierarchy appeared to be maintained during in vivo therapy, since serotypes 26 and 45 displayed improved efficacy against established MM tumors in vivo.⁹² Interestingly, unlike many oncolytic viruses, whose preferential infection of MM cells appears to be mediated by receptor specificity, the preferential killing of MM by different AdV serotypes correlated better with replication kinetics than with viral adsorption.

Therapeutically, unmodified AdVs have been shown to be effective against established MM in several studies.^{91–93} Unfortunately, these studies have focused on the treatment of localized human plasmacytomas in immunodeficient animals. Efficacy in more clinically relevant systemic, immunocompetent models has yet to be tested. This is particularly important for AdV-based therapy, given that virtually all patients are exposed to AdV throughout life and many carry potent neutralizing humoral responses against the more common AdV serotypes. In addition to this caveat, it must be noted that the overall efficacy of AdV therapy, even in localized models, has typically been modest, and very few long-term cures have been demonstrated. In order to improve this efficacy, several groups have studied the possibility of “arming” AdV to promote therapy. AdV armed with TRAIL has been shown to display enhanced killing of MM cells in vitro while also demonstrating therapeutic synergy with PI3K or proteasome inhibitors.⁹⁴ Experiments arming AdV with CD40L have also displayed improved killing of MM cells both in

vitro and in vivo.⁹³ Interestingly, while CD40L is a known immunomodulator, the improved efficacy in the latter studies appeared to be mediated by direct induction of apoptosis, and not through enhanced T-cell responses (a possibility that was never examined). Additionally, it is important to note that both experiments arming oncolytic AdV used serotype 5 as their viral backbone. Utilizing similar arming strategies on more potent α MM serotypes has yet to be explored.

Vaccinia virus

The vaccinia virus (VacV) is a large (200–300 nm), enveloped, double-stranded DNA virus from the Poxviridae family. While the evolutionary host for VacV remains somewhat controversial, it is clear that the wild-type virus is fully infectious in a variety of mammals, including humans, and that systemic infection can result in severe disease symptoms, including death. Due to their use as vaccine platforms to eradicate smallpox, however, attenuated VacV strains have been extensively studied in humans and display relatively good safety profiles. As an oncolytic agent, VacV has been well studied in the context of solid tumors in both preclinical^{95,96} and clinical^{97–102} settings. In particular, recombinant VacV encoding either GM-CSF (known as JX594) or PSA (known as Prostavac) has advanced to large Phase III clinical trials.

While oncolytic VacV is well established as a potential treatment for solid tumors, its use in a hematopoietic setting is much less well studied. This is likely due to the fact that while VacV is extremely lytic, the virus is not naturally oncotropic and requires additional genetic modification to restrict viral replication to malignant cells. In solid tumors, this can be accomplished through a single deletion of the viral *tk* gene. This restricts viral replication to cells with an abundance of thymidine, a state predominately found in rapidly dividing cancer cells. Unfortunately, this restriction is not absolute, and *tk*^{−/−} VacV clones can infect normal tissue, causing severe pathology following system injection.¹⁰³ Therefore, the use of VacV as an oncolytic agent against systemic malignancies, such as MM, requires additional genetic engineering further to restrict viral replication and limit toxicity. A variety of methods to enhance viral specificity have been attempted. One of the most common methods is to delete the *vgf* gene in *tk*^{−/−} clones. VGF stimulates cellular proliferation through activation of EGFR. Its removal in so-called double-deleted VacV further restricts viral replication to rapidly dividing cells, resulting in improved safety profiles.¹⁰⁴ A second approach that has been attempted is actively to restrict viral replication by placing essential viral genes under miRNA restriction. In this approach, several Let7a miRNA-binding

sites are placed within the promoter of the essential viral gene *B5R*. This causes an inhibition of *B5R* expression in normal cells (which express high levels of *Let7a*). In contrast, in MM cells (which fail to express *Let7a*), *B5R* expression occurs at normal levels, thus promoting viral replication only in malignant MM cells.¹⁰³ Attempts have also been made to alter viral binding specifically to increase adsorption of virions in malignant cells.¹⁰⁵ While this is an attractive theoretical approach, the binding determinants of *VacV* are complex and remain poorly understood,¹⁰⁶ making successful retargeting difficult. In constructs that display successful targeting, elimination of infected MM cells appears to be dependent on lytic replication.¹⁰⁴ Interestingly, the efficacy of this elimination seems somewhat controversial. Several groups have suggested that *VacV* is extremely lytic toward infected MM cells, with massive reductions in cellular viability shown within 24 hours after infection.^{103,104,106} In contrast, Lei et al saw only minimal reductions in MM-cell viability following infection with *VacV*.¹⁰⁷ In this work, elimination of infected cells occurred through the induction of apoptosis, and efficient killing required “arming” the oncolytic *VacV* with additional apoptotic modulators, including *miR34a* and *Smac*.

Therapeutically, *VacV* has been used as a treatment for MM in two distinct ways. The first way is the direct treatment of established disease *in vivo*.^{103,104,107} This work has demonstrated that *VacV* has the potential to treat established MM. Unfortunately, it has primarily utilized models of human plasmacytomas established in immunodeficient mice. Anecdotal evidence from a single case report suggests that *VacV* can (at least transiently) reduce systemic MM-tumor burden in a human patient;¹⁰⁸ however, the efficacy of viral treatment against disseminated MM remains largely unclear. The second therapeutic modality in which *VacV* has been studied is the use of the virus to generate α MM-tumor vaccines. In this regard, it has been demonstrated that *VacV* can be used as an adjuvant to increase the magnitude of α MM immunoresponses following vaccination. Animals vaccinated with tumor cells infected *ex vivo* with *VacV* and then lethally irradiated can completely reject secondary challenge with uninfected MM cells,^{109–113} and vaccination of tumor-bearing animals can result in remission of established MM.¹¹⁴ This effect appears to be mediated by cytotoxic T cells, although the exact subset has not been identified.^{111,113} Unfortunately, this methodology requires prevaccination of tumor-naïve individuals with *VacV* to achieve maximal efficacy, which could severely limit its translational potential. Nevertheless, further investigation into this approach might be warranted.

Myxoma virus

The myxoma virus (*MyxV*) is a large (200–300 nm), enveloped, double-stranded DNA virus from the *Poxviridae* family that is somewhat related to *VacV*.¹¹⁵ Unlike *VacV*, however, *MyxV* displays a tightly restricted host range specific to lagomorphs (rabbits). No instance of natural *MyxV* infection has ever been documented in any nonrabbit species, and even direct injection of large amounts of the virus does not elicit noticeable pathology in either humans or mice.^{116–118} *MyxV* is a relatively novel oncolytic virus whose therapeutic potential was recognized <10 years ago.¹¹⁹ Since then, it has been investigated in preclinical models of a number of different malignancies, including melanoma,^{42,119,120} rhabdoid tumors,^{121,122} pancreatic cancer,^{123,124} glioma,^{125,126} and gallbladder cancer.¹²⁷

Like many oncolytic viruses, *MyxV* has been shown preferentially to infect and kill both established MM cell lines and CD138⁺ cells found in MM-patient bone-marrow samples.¹¹⁷ This killing appears selective for MM cells, based on a strong preferential binding of the virus to CD138⁺ cells compared to nonmalignant CD138[−] cells found in MM-patient bone marrow.^{117,118} Like *ReoV*, this preferential binding also excludes absorption of *MyxV* to CD34⁺ bone-marrow progenitors, allowing the virus to be used as an *ex vivo* purging agent during ASCT.^{117,118,128,129} Interestingly, unlike the vast majority of OV, killing of MM cells by *MyxV* appears to be independent of lytic viral replication, since no new viral progeny are produced during treatment and replication-incompetent virus is still fully capable to kill infected cells.¹¹⁷ Instead, elimination of *MyxV*-infected MM cells appears to be mediated by the induction of extrinsic apoptosis caused by depletion of cellular apoptotic inhibitors, *Bcl2*, *Mcl1*, *XIAP*, and *survivin*. This depletion results from the rapid decapping of cellular mRNA early in infection.^{130,131} Interestingly, while a truly comprehensive study has never been published, 100% of the primary MM-patient samples studied to date have responded to *MyxV* therapy, suggesting that the unique mechanism through which *MyxV* kills MM cells might overcome some of the challenges associated with MM-cell heterogeneity and resistance.^{106,117}

Therapeutically, many of the studies into *MyxV*'s potential to treat MM have focused on the virus's ability to improve the treatment for MM patients receiving ASCT.^{117,128,129} Bartee et al showed that *MyxV* treatment of MM-patient bone-marrow biopsies resulted in the rapid and specific infection of CD138⁺ cells while sparing the CD34⁺ bone-marrow progenitors.^{117,118} Additionally, treatment of mixtures of human MM cells and bone-marrow progenitors with *MyxV* prior to transplant could

specifically prevent establishment of MM tumors, while still allowing for stem-cell engraftment and hematopoietic reconstitution.¹¹⁷ Due to these results, it has been suggested that MyxV could be used as an ex vivo purging agent to eliminate contaminating MM cells from ASCT samples. It has also been shown that direct intravenous injection of MyxV into immunocompetent mice bearing established, systemic MM can result in both a rapid reduction in tumor burden and potentially curative induction of α MM immunoresponses.⁴³ Unfortunately, while the results of these experiments were striking in some animals, the overall response rates achieved were very low. In order to improve therapy, several groups have studied the possibility of combining ex vivo purging with treatment of established disease.^{128,129} These groups have demonstrated that treatment of ASCT samples with MyxV ex vivo results in the loading of viral particles onto either T cells¹²⁸ or neutrophils,¹²⁹ which has beneficial effects through improving the delivery of the virus in vivo or activating immune cells to improve α MM immunity.

Picornavirus

In addition to the more commonly used viruses listed already, a small number of studies have also demonstrated the oncolytic potential of the Picornaviridae family against MM. For example, it has been shown that MM cells overexpress Coxsackie virus receptors ICAM1 and DAF, rendering them susceptible to infection with the oncolytic Coxsackie virus A21.¹³² Additional work has shown that the efficacy of A21 infection in killing MM cells was high and the virus could be used to treat solid plasmacytomas.^{132,133} However, viral treatment also had a negative impact on bone-marrow progenitor differentiation, suggesting a possible hurdle to translation. Mengovirus (another member of Picornaviridae) has also been shown to infect and kill MM cells; however, in vivo studies with this virus demonstrated only modest efficacy along with relatively high toxicity.¹³⁴ Coxsackie viruses are positive-sense, single-stranded RNA viruses, which means their nucleic acid is directly infectious. In an interesting study, Hadac et al were able to demonstrate that injection of infectious A21 nucleic acid into solid MM plasmacytomas initiated an oncolytic infection with efficacy similar to that seen using intact virus.¹³³ While this is a strategy with somewhat restricted usage, it represents an interesting approach to overcoming some of the challenges that appear to be associated with using intact picornaviruses in the clinic.

Final words

Review of the existing literature clearly demonstrates the potential for OV to be used as a viable therapy for MM

patients. A large number of viruses have been shown specifically to infect MM cells, with infection resulting in the rapid elimination of these cells through a variety of mechanisms. Preclinical studies conducted with most of these viruses have typically resulted in at least stable disease or partial disease remission, and early results from clinical studies in human patients appear to be promising. However, the field also suffers from several obvious issues, which must be addressed. First and foremost is the use of appropriate preclinical models to study the mechanisms and efficacies of OV. With the notable exception of VSV, the majority of work studying OV treatment of MM has been conducted in immunodeficient models of single subcutaneous plasmacytoma. While these models represent a technically easy starting point, they largely fail to recapitulate the clinical realities of MM in two major ways. First, due to the nature of oncolytic virus particles, the challenges associated with treatment of systemic versus localized disease are very different. In particular, delivery of a virus through the bloodstream to sites of systemic disease is known to be a major translational hurdle. Since MM typically presents as a systemic disease, this hurdle should be addressed in any preclinical study. Unfortunately, the use of subcutaneous plasmacytoma models largely bypasses this issue, possibly skewing results in a more favorable manner. Second, the immune system is known to impact OV efficacy significantly in a variety of both positive and negative ways. For example, serum complement and existing antiviral humoral responses can inhibit viral infectivity.^{135–137} In contrast, much of the efficacy of OV is now thought to be mediated through the induction of antitumor T-cell-mediated immunotherapy.³⁹ The use of immunodeficient animal models largely precludes studies into how these issues might affect MM therapy. In particular, as immunotherapy becomes more clinically prevalent, the propensity of OV to induce α -tumor T-cell responses raises the possibility that OV can be used to improve the response of MM to other immunotherapeutic agents, such as PD1- or CTLA4-blocking antibodies. To date, these antibodies have proven only modestly successful as single agents against MM;^{138–140} however, given their success in other malignancies, this line of study still appears conceptually attractive. Importantly, a variety of immunocompetent MM models have been developed in recent years that can be used to address these issues.^{141,142} While none of these models perfectly recapitulates the clinical realities of MM, they are all clearly better suited to preclinical studies on OV than subcutaneous immunodeficient models.

The second issue that arises during a review of the literature is the need to examine the impact of disease

heterogeneity on OV. A number of studies have shown that oncolytic viruses can infect and typically kill a high percentage of CD138⁺ cells in MM-patient bone marrow. However, the number of samples used in these studies is often small (typically data from only one to three patients is shown), and the methodologies used (often flow cytometry or immunofluorescence) are typically unable to detect small numbers of resistant cells. One of the major challenges in treating MM is the heterogeneity of disease, both between patients and within single patients, which increases the likelihood of resistant clones developing following therapy. While OV theoretically has the potential to overcome these challenges, a demonstration of this ability is notably lacking in the current literature. The field would thus benefit from a comprehensive study of the efficacy of OV therapy on a large number of patient samples using sensitive techniques to study efficacy versus the development of resistance.

Finally, as is often the case in the field of OV, the literature reveals a set of studies that are often disjointed and unconnected. Multiple lines of research that appear promising within a single virus are never combined, nor are these lines of research adopted in other viral platforms. Additionally, the results of combining OV with more typical MM standards of care, such as chemotherapy, radiation, or monoclonal antibodies, are generally lacking. This latter point is especially critical, in order to place OV in the context of other MM therapies. For example, should OV treatment be used as frontline therapy, or is it more suited for relapsed or refractory patients? Does OV synergize with existing MM treatments? If so, which one? At some point, if the field is to advance from preclinical work to meaningful human trials, the “optimal” genetic modifications must be identified, synergistic combinatorial modifications must be introduced, and therapy must be integrated into current standards of care. In conclusion, a number of significant issues must still be resolved before OV for MM is to become truly clinically viable. However, for a field that should probably be considered in its infancy, the results appear extremely promising, and more work is clearly warranted.

Disclosure

The author reports no conflicts of interest in this work.

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