

Published in final edited form as:

*Microbes Infect.* 2010 December ; 12(14-15): 1144–1152. doi:10.1016/j.micinf.2010.08.012.

## The immunoregulatory properties of oncolytic myxoma virus and their implications in therapeutics

Jia Liu<sup>a</sup>, Sonia Wennier<sup>a</sup>, and Grant McFadden<sup>a,\*</sup>

<sup>a</sup> Department of Molecular Genetics and Microbiology, University of Florida College of Medicine, P.O. box 100266, Gainesville, FL 32610

### Abstract

Myxoma virus (MYXV) is a poxvirus with a strict rabbit-specific host-tropism for pathogenesis. The immunoregulatory factors encoded by MYXV can suppress some functions of immune effectors from other species. We review their mechanisms of action, implications in therapeutics and the potential to improve MYXV as an oncolytic agent in humans.

### Keywords

myxoma virus; poxvirus; oncolytic virus; immunoregulatory factors

---

Myxoma virus (MYXV) belongs to the *Leporipoxvirus* genus within the *poxviridae* family and is the causative agent for myxomatosis in the European rabbit [1]. MYXV in the wild exhibits a strict host tropism such that it is not pathogenic in any known host species (mouse, human, etc.) other than the European rabbit. Despite this narrow rabbit-specific host range in nature, MYXV has proven to be capable of infecting a wide variety of human cancer cells in culture, and can selectively eliminate cancerous tissues for either xenografted human cancers in mice, or syngeneic murine cancers in rats and mice. In these cases, for both immunocompromised and immunocompetent murine hosts, MYXV replication is completely restricted to tumor tissues and the virus does not propagate to any detectable degree in normal tissues. In stark contrast, within the rabbit host MYXV spreads systemically in a broad spectrum of host tissues and can dismantle essentially all the functional elements of the rabbit innate and acquired immune responses.

It has been documented that MYXV infection rapidly leads to systemic immunosuppression in European rabbits. Depletion of lymphocytes in the draining lymph node has been reported as early as 24 hours after intradermal infection of MYXV [2]. It has also been reported that upon MYXV infection, all T cell subsets (CD4<sup>+</sup>, CD8<sup>+</sup>, and CD4<sup>+</sup>CD8<sup>+</sup> T cells) decreased, while MYXV's effect on B cells was less pronounced [2]. In particular, the CD4<sup>+</sup> T cell subpopulation was affected more severely compared to other T cell subsets. In addition, the ability of lymphocytes to proliferate was also compromised during the course of MYXV infection [3]. This systemic MYXV-induced immunosuppression is utterly unique to infected European rabbits. In contrast, the virus is rapidly cleared by innate immune

---

\*Corresponding author: Grant McFadden, Ph.D, 1600 SW Archer Rd, P.O. Box 100266, Gainesville, FL 32610, U.S.A, Tel: 352-273-6852, Fax: 352-273-6849, grantmcf@ufl.edu.

**Publisher's Disclaimer:** This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

responses in all tested vertebrate hosts outside the lagomorph family of leporid hosts, including mice and humans. Thus, MYXV is considered a safe candidate oncolytic virus for potential human clinical trials. In addition, several of the targeted gene knockout constructs of MYXV, such as vMyx-M135KO and vMyx-M063KO, have lost the ability to be pathogenic even in rabbits while maintaining their oncolytic properties against human cancer cells, and thus represent newer-generation oncolytic candidate MYXV variants that are essentially avirulent for all known vertebrate host species [4].

In recent years, MYXV has been shown to possess oncolytic activity in a variety of preclinical cancer models [5,6]. Studies on the potential immunoregulatory properties of MYXV outside the rabbit host will lead to a better understanding of the immune responses elicited by the host (particularly mouse or human) upon MYXV infection and of their potential modulation in the animal models that are used to evaluate the therapeutic benefits of MYXV-based oncolytic treatments. Similar to the rest of the poxvirus family members, dozens of diverse immunoregulatory factors are encoded by MYXV [1]. In this review, we will focus on MYXV immunoregulatory factors with potential impacts on MYXV-based oncolytic applications as assessed in cellular or animal-based models (Table 1).

### **The impact of type I interferon (IFN) and tumor necrosis factor (TNF)**

The type I IFN pathway plays a crucial role in shaping MYXV host range in pathogenic and non-pathogenic hosts. In primary murine embryonic fibroblasts (pMEFs), within 4 hours after MYXV infection early events during the viral infection triggers the activation of Erk1/2 signaling and drives downstream events including the expression of type I IFN, the activation of STAT1 dependent signaling, IRF3 activation, and the upregulation of IRF7 expression. These induced innate cellular events in response to infection ultimately restrict MYXV replication and spread in murine pMEFs [7]. However, when pMEFs were spontaneously immobilized by serial passages in culture (called iMEFs), the cellular mechanisms responsible for sensing MYXV infection were abrogated, thus preventing the induction of type I IFN and allowing productive MYXV infection [8]. Unlike early-stage pMEFs that are nonpermissive for MYXV replication, in the immortalized iMEFs that have acquired a MYXV-permissive phenotype, in response to other nonviral ligands the activation of IRF3, STAT1 and TLR3 pathways as well as the ability to produce type I IFN, were all normal; however, the activation of Erk1/2 in response to MYXV infection could no longer be detected and type I IFN was never induced by the MYXV infection [8]. The mechanism, by which Erk1/2 is activated during the early stage of MYXV infection in pMEFs, but not iMEFs, remains unclear. It will be intriguing to correlate this ability to recognize MYXV infection to cytoplasmic or pattern recognition sensors that operate in pMEFs but are apparently absent or selectively inactivated in immortalized iMEFs.

In normal primary human cells, the successful inhibition of MYXV replication depends not only on type I IFN, but also on the production of tumor necrosis factor (TNF) in infected cells. In primary human fibroblasts, unlike pMEFs, MYXV infection is partially resistant to inhibition by type I IFN. In order to completely inhibit MYXV viral replication in primary human fibroblasts, both type I IFN and TNF need to be present [9]. The partial resistance of MYXV to type I IFN in primary human fibroblast is likely due to the inhibition of type I IFN-induced phosphorylation of the Janus kinase Tyk2 during infection [10], which consequently prevents the activation of downstream transcription factors (e.g., STAT1). Recently, it has been shown that MYXV replication in human primary fibroblasts is profoundly inhibited by a unique synergistic antiviral state induced upon co-stimulation with TNF plus type I IFN [11]. Importantly, most human cancer cells tested to date are unable to induce this IFN/TNF synergistic state and this defect may contribute to the selective replication of MYXV in a variety of human cancer cells [12]. Note that primary human cells

of different lineages do not respond identically to MYXV infection; for example in primary human macrophages, unlike primary human fibroblasts, MYXV infection causes RIG-I mediated co-stimulation of both TNF and type I IFN expression [9] thereby restricting MYXV replication in these cells. Significantly, when primary human macrophages are mixed with primary human fibroblasts, all the cells become resistant to MYXV because the IFN and TNF induced in the MYXV-infected macrophages render all the cells in the co-culture uniformly resistant to MYXV infection in a paracrine fashion (unpublished observations). The sensing of MYXV infection by RIG-I in primary human macrophages leads to IRF3/IRF7 activation and sustained expression of both TNF and type I IFN [9]. It was also shown that in primary human macrophages, the induction of TNF or type I IFN after MYXV infection was independent of TLR4, MyD88, MDA5 or Trif [9]. Although RIG-I senses MYXV infection and triggers these signaling cascades that lead to the induction of IFN and TNF in primary human macrophages, the MYXV sensors that operate in other classes of primary human cells remains to be defined. Indeed, the innate cellular sensors for cytoplasmic DNA viruses like MYXV may very well turn out to be different for cells of different lineages.

Given the crucial role that innate antiviral cytokines like type I IFN plays in determining the host range of MYXV, it is not surprising that this virus encodes several known or predicted modulators of the rabbit versions of type I IFN. Structure studies on the MYXV M156 protein revealed that this protein is a structural mimic of the cellular eIF2 $\alpha$  and a viral pseudosubstrate for PKR, even though its sequence identity with eIF2 $\alpha$  is only 19% [13]. The type I IFN inducible, double-stranded RNA-dependent protein kinase (PKR) is a protein kinase important for cellular responses against viral infections. The N-terminal portion of PKR contains two double stranded (ds) RNA binding motifs. Binding of PKR to ds-RNA produced during a viral infection promotes dimerization and trans-autophosphorylation of the protein that results in the activation of the kinase domain. Activated PKR can induce antiviral responses by: 1) phosphorylation of eIF2 $\alpha$  resulting in the inhibition of both cellular and viral protein synthesis and 2) the activation of NF $\kappa$ B and its downstream targets involved in cellular innate immunity (reviewed in [14]). *In vitro* M156 was shown to be an efficient substrate of PKR and to efficiently compete for phosphorylation with cellular eIF2 $\alpha$ . The VACV K3L protein and the swinepox C8L protein are also viral mimics of eIF2 $\alpha$  and inhibitors of PKR [15,16]. However the protein-protein interactions that lead to the inhibition of PKR by these three viral eIF2 $\alpha$  mimics appear to be distinct and unique to each viral protein. Importantly, of the three eIF2 $\alpha$  viral mimics tested to date only M156 has been reported to be phosphorylated upon binding to PKR [13]. Thus M156 may utilize a different mechanism of PKR inhibition when compared to C8L or K3L. Studies have also shown that the host may also in turn evolve to counteract viral mimicry. In a recent study, using the PKR-K3L model system, it was reported that PKR has evolved and undergone periods of strong positive selection in primates and that these evolutionary mechanisms may help overcome viral mimicry [17]. In fact, mutant PKR proteins have been identified that have decreased binding to K3L and therefore are resistant to its inhibitory effects, but yet still retain their binding affinity for eIF2 $\alpha$  [18]. In the past years, the genomic sequences of naturally occurring strains of MYXV have been obtained. In Californian MYXV isolates, which are more virulent to European rabbits compared to South American strains, M156 has been found to be duplicated [19]. These findings support the prediction that M156 is a virulence factor for MYXV, and suggest that careful analysis of the protein-protein interaction dynamics between M156 and the PKR family members from various host species may be particularly revealing about why MYXV exhibits such strict tropism for rabbits.

MYXV also encodes the M029 protein, a putative homologue of the VACV E3L gene product (with 24% identical to the C terminal 2/3 of E3) that is a known inhibitor of PKR.

VACV E3 inhibits PKR by binding and sequestering dsRNA and by direct interaction with PKR. By binding to dsRNA, E3 also antagonizes the 2'-5'oligoadenylate (2-5OA) synthetase enzyme, another type I IFN inducible enzyme that inhibits protein synthesis during viral infections [20]. OAS binds to dsRNA and produces 2-5OA from ATP, which in turn activates RNase L. Activated RNaseL causes the degradation of both cellular and viral mRNA thereby inhibiting protein translation [21]. In addition, E3 has also been shown to inhibit the activation of IRF-3 and -7 which induce the expression of type I IFN [22].

Thus, MYXV may counteract the type I IFN induced immune responses through the activities of the M156 and M029 proteins. The contributions of these viral proteins to the host range of MYXV remain to be determined. However, in addition to its role in innate immunity, PKR can also control apoptosis, cell growth and differentiation and its activities are regulated by oncogenes. Thus, not surprisingly, cancer cells can exhibit dysregulation of PKR signaling [23]. It remains to be formally tested if M156's interaction with PKR will be relevant to the pathogenesis of MYXV in rabbits or to its host range for cancer cells or if M029 is a true functional homolog for VACV E3.

## Viroceptors

Viroceptors are virus-encoded mimics of immune receptors. For example, MYXV encodes a secreted version of the cellular TNF receptor, called M-T2, but this anti-TNF viroceptor is specific for only rabbit TNF and is unable to bind or inhibit TNF from murine or human sources. Thus, this helps to explain why MYXV replication in primary human cells is so sensitive to inhibition by human TNF.

Other targets of MYXV viroceptor inhibition include IFN $\gamma$  and members of the chemokine pathway. For example, M-T7 of MYXV encodes a secreted IFN $\gamma$  receptor belonging to the vaccinia virus B8R family. M-T7 can efficiently antagonize the anti-viral effects of rabbit IFN $\gamma$  through direct binding in a species-specific manner. As in the case of the MYXV M-T2 TNF inhibitor, the M-T7 IFN $\gamma$  inhibitor cannot bind or inhibit IFN $\gamma$  from human or murine sources. However, M-T7 has another distinct role in preventing immune cell influx to the site of infection by promiscuously binding to the heparin-binding domain of a broad range of chemokines (including members from C, CC, and CXC chemokine subfamilies). This particular inhibitory activity of M-T7, however, operates in a species-independent manner and can target chemokines from rabbit, murine or human sources. M-T7 can disrupt the solid surface of chemokine gradients formed by the attachment of these chemokines to the endothelial wall through their C-terminal heparin-binding domains. This property of M-T7 was exploited to prevent vessel wall plaque generation after angioplasty balloon-mediated vascular injury in a rat model [24] where significantly reduced macrophage influx and post-injury intimal hyperplasia were detected. This demonstrated that the administration of purified M-T7 protein may provide a therapeutic benefit by attenuating harmful inflammatory responses in diseases mediated by systemic inflammation. In another report, in order to prevent ischemia reperfusion, one of the most important risk factors in antigen-independent graft failure, purified M-T7 protein was used to efficiently inhibit leukocyte infiltration in a rat renal transplantation model [25]. In a rat aortic transplantation model of vasculopathy, an inflammatory process leading to transplant failure, purified M-T7 protein administered immediately after transplant surgery effectively inhibited plaque growth and reduced macrophage and T lymphocyte infiltration through the modulation of chemokines from the CC subfamily [26]. Gene therapy involving the intramuscular delivery of a naked plasmid encoding M-T7 was also shown to prevent angiogenesis by reducing the VEGF<sub>164</sub> surrounding an implanted foreign body in addition to attenuating macrophage influx [27]. Thus, M-T7 can not only reduce systemic inflammation but also disrupt the process of angiogenesis by neutralizing VEGF<sub>164</sub>. Furthermore, M-T7 promotes its anti-inflammatory

and anti-plaque growth functions by inhibiting the interaction between chemokines and glycosaminoglycans (GAGs) and not the interaction between chemokines and their corresponding receptor [28]. These robust anti-inflammatory effects of M-T7 translated into therapeutic benefits prolonging survival and reducing inflammatory infiltration in a renal allograft transplantation model [28]. Note that the anti-inflammatory properties of M-T7 are independent of the host species, unlike its ability to inhibit IFN $\gamma$ , which is utterly rabbit-specific.

MYXV encodes an additional secreted chemokine viroceptor, designated M-T1. M-T1 can bind specifically to chemokines of the CC subfamily independently from its heparin binding through the consensus domain at their C terminus [29]. Therefore, it can interrupt the solid CC chemokine gradient and disrupt the trafficking of effector cells towards the site of viral infection in a species independent manner. Treatment with M-T1 protein showed therapeutic effects by reducing early monocyte and CD2<sup>+</sup> lymphocyte cell infiltration that caused vasculopathy in a rat aortic allograft transplant model [26] and in a mouse transplantation model [28]. Thus, M-T1 is an additional example of an anti-immune viroceptor that targets its ligand (in this case, chemokines) in a species-independent fashion.

Cancer often associated with inflammatory and angiogenic events which shape the tumor microenvironment. When evaluating the efficacy of oncolytic viruses (OVs) in this dynamic microenvironment, lymphocyte infiltration (either directed against the virus or targeted against a cancer antigen) and angiogenesis are two important factors whose modulation may profoundly affect the overall therapeutic effects. These factors will also directly affect the ways in which OVs might be genetically modified for the purpose of generating better second generation OVs for specific cancer types. In the case of MYXV as a candidate OV, how to take advantage of the functions of M-T7 and M-T1 will depend on the therapeutic strategy used. One question that arises is whether these viral genes should be deleted from the MYXV genome for the purpose of improving immune cell infiltration and promoting a better anti-tumor immune response. Alternatively, should these MYXV genes be retained in order to inhibit angiogenesis and anti-viral immune responses? These are questions that can be addressed experimentally in the future.

## Serine protease inhibitors (Serpins)

In the MYXV genome, four viral genes encoding Serpin-like proteins have been reported: SERP-1, SERP-2 (a gene related to cowpox crmA that functions as an inhibitor of intracellular caspase 1 and Granzyme B) [30], SERP-3 [31], and M152 (a gene homologous to an intracellular serpin, leupin) [32]. SERP-1 is the most studied among MYXV encoded Serpins and the purified protein has been shown to have significant therapeutic value as a stand-alone drug in the treatment of chronic inflammation associated with surgical trauma or diseases such as angioplasty, transplantation, and rheumatoid arthritis, etc. [33].

SERP-1 is a serine protease inhibitor (Serp) encoded by the MYXV M008.1 gene and is a virulence factor with anti-inflammatory properties. During MYXV infection in European rabbits, SERP-1 is expressed abundantly and undergoes various post-translational modifications (e.g., N-linked glycosylation) prior to being secreted to subsequently antagonize inflammatory responses. When it is circulating systemically in a healthy rabbit, SERP-1 has a half life of 1.3 days and does not seem to accumulate in any particular organ, thus closely resembling the behavior of heparin cofactor II [34]. It can inhibit a broad spectrum of human protease enzymes, including the ones responsible for thrombolytic and thrombotic processes, e.g., urokinase-type plasminogen activator (uPA), tissue-type plasminogen activator (tPA), plasmin, thrombin, and factor Xa, etc. These inhibitory effects of SERP-1 are rendered through its interaction with target proteinases via a reactive center

loop localized at Arg<sup>319</sup>-Asn<sup>320</sup> [35] (P1-P1'), with protease-inhibitor therefore anti-inflammatory functions, and at P2-P7, inhibiting plasmin function, mononuclear cell activation, and thrombosis etc [36]. SERP-1 can directly associate with activated monocytes and T cells and render its inhibition in adherent through modification of filamin and  $\beta$ -integrin in an uPA receptor (uPAR) dependent manner [37]. Purified SERP-1 protein was proven to have effective therapeutic effect in reducing monocyte/macrophage invasion to sites of arterial trauma following a single picogram-to-nanogram bolus dose in numerous animal models including rabbit, rat, and swine [38]. Thus, purified SERP-1 protein inhibits inflammatory cascades in a wide variety of hosts, including humans, and it has entered into human clinical trials to alleviate the systemic inflammation associated with acute myocardial syndromes.

The function of SERP-1 in the context of MYXV oncolysis of tumor tissues has not been evaluated. It can be predicted that the presence of SERP-1 can attenuate the inflammatory effect caused by MYXV infection in the tumor microenvironment, thereby allowing oncolysis to be more effective, but this has not yet been demonstrated experimentally.

## Intracellular immunoregulatory factors expressed by MYXV

### Inhibitors of Toll-like Receptor (TLR) signaling

TLRs are pattern-recognition receptors that when activated in response to pathogen associated molecules trigger the production of type I IFN and other pro-inflammatory cytokines. VACV encodes several proteins known to inhibit TLR signaling: A46, A52, B15, K7 and N1. In the Pfam database of protein families and domains A46, A52, B15 (also known as B14 in VACV WR strains) and K7 are included in one single family termed Pox\_A46, while N1 belongs to the Orthopox\_N1 family [39]. Among these viral TLR inhibitors, A46 is the only one that contains an obvious Toll/Interleukin-1 receptor (TIR) domain. The TIR domain in A46 enables it to interact with several TIR adaptor proteins such as MyD88, MAL (TIRAP), TRIF and TRAM, thereby inhibiting NF $\kappa$ B, MAP kinase and IRF-3 signaling [40,41]. On the other hand, both A52 and K7 inhibit TLR signaling and the activation of NF $\kappa$ B by associating with IRAK2 and TRAF6 [40,42,43]. However, K7 differs from A52 function in that it can also inhibit IRF-3 and -7 activations and the induction of IRF mediated type I IFN by binding to the DDX3 protein [43]. Contrary to A46, A52 appears to activate MAP kinase and IL-10 production [44]. B14 is another VACV that also inhibits TLR signaling by associating with and inhibiting the IKK complex and subsequently the activation of NF $\kappa$ B [45]. N1 is a VACV protein that has been reported to bind the IKK complex thereby inhibiting the activation of NF $\kappa$ B and IRF-3 [46]. However, these findings are controversial [45,47]. Interestingly, A52, B14, K7 and N1 all share a similar Bcl-2 fold [48–50]. Evolutionary analysis of these proteins in different genera of the poxvirus family shows that K7 and A46 are unique to Orthopoxviruses, while A52, B14 and N1 orthologues are present in other genera including Leporipoxvirus. In fact, MYXV encodes orthologous proteins of N1, A52 and B15 named M146, M139 and M003.1, respectively. Interestingly, the MYXV M136 protein has also been shown to be related to the Pox\_A46 family. Orthologues of this MYXV protein are found in Yata-, Capri-, Sui- and Cervidpoxviruses, but not in Orthopoxviruses [51]. To date, no functional studies of these MYXV proteins have been reported and their role in innate immunity and pathogenesis remains to be determined. Activation of TLR signaling in cancer cells may promote proliferation and metastasis, as reviewed elsewhere [52,53]. The influence of all these potential MYXV TLR inhibitors on cancer cells with dysregulated TLR and NF $\kappa$ B signaling pathways may represent strategies for improving tumor growth inhibition and viral oncolysis.

**M135**

MYXV encodes an orphan virulence factor designated M135R that operates against unknown host immune targets [54]. It shares similarity to VACV B18R, a known inhibitor of Type I IFN, and is expressed as a cell surface protein in MYXV-infected cells. However, M135 does not bind or inhibit the effects of rabbit IFN $\alpha/\beta$  during MYXV infection, suggesting that its presence is not directly associated with resistance to rabbit type I IFN [54]. Thus, the role of M135 during MYXV infection in rabbits is currently unknown. In addition, M135 was not essential in promoting MYXV resistance to human type I IFN in primary human fibroblasts [10]. Nevertheless, *in vitro* studies have shown that a recombinant MYXV lacking the M135 protein has enhanced oncolytic potential for cultured human glioma cells compared to wild-type MYXV [4] and was suggested as a rabbit-safe candidate for oncolytic application in human cancer therapy.

**M013**

M013 of MYXV is a pyrin domain containing protein and a virulence factor important for the regulation of immune responses and full pathogenicity during MYXV infection in European rabbits [55]. In the human monocyte cell line (THP1), M013's pyrin domain interacted with a cellular pyrin domain containing protein, ASC-1, which is a component of the inflammasome. This interaction inhibited the activation of ASC-1/caspase 1 thereby blocking the cellular pro-inflammatory cytokine response (i.e., the production of IL1 $\beta$  and IL-18) to viral infection. In addition, M013 is capable of directly binding to NF $\kappa$ B1, blocking the nuclear translocation of RelA and consequently inhibiting the NF $\kappa$ B signaling pathway in human THP1 cells [56]. Thus, M013 is able to inhibit both inflammasome activation and NF $\kappa$ B signaling in myeloid cells in a species-independent fashion.

**M130**

Recent reports have shown that the M130 protein of MYXV is a virulence factor required for lethal pathogenesis in European rabbits [57]. The M130 protein is a member of a weakly conserved viral protein family present only in Lepori-, Capri- and Suipoxvirus genera. It is expressed late during virus replication and is primarily found in the cytoplasm of infected cells [57]. A recombinant MYXV lacking the M130 protein (MYXV-M130KO) was attenuated, with 100% of the rabbits surviving the challenge, but no host range or replication defects were noted in cell culture. In addition, initial challenge of rabbits with vMyx-M130KO infection protected them from lethal challenge with wild-type MYXV [57]. Previous reports have suggested that M130 exhibits sequence similarity to the viral transactivating Tat protein of human immunodeficiency virus (HIV) [32,58]. However, M130 appears not to be secreted or to localize to the nucleus [57] and its molecular functions at the present remain unclear.

**M153**

M153 of MYXV is a viral gene containing an N-terminal LAP plant homeodomain (LAP-PHD) motif (a RING finger-like domain). In the absence of MYXV infection, M153 expressed from a plasmid localizes to the endoplasmic reticulum (ER) [59], and in the context of viral infection this protein relocates to endosomes [60]. *In vivo*, M153 functions as a virulence factor, and infection caused by a recombinant MYXV lacking the M153R gene in European rabbits led to a severe mononuclear infiltration contrary to the heterophil infiltration caused by wild-type MYXV at the site of infection [59]. The molecular function of M153 appears to be the down-regulation of MHC class I molecules during infection in a species non-specific manner, resulting in the loss of CD8<sup>+</sup> T cell cytotoxicity [59]. In addition, M153 promoted the lysosomal degradation of the human CD4 molecule, thus preventing its surface presentation [60]. The presence of lysines in the cytoplasmic tails of

substrates appears to be a common feature that correlates with M153-induced down-regulation of surface expression. In fact, M153 functioned as an E3 ligase *in vitro* that promoted ubiquitination of CD4 and possibly other substrates as well [60].

The function of M153 in down-regulating MHC class I, CD4, and possibly other immune signaling surface proteins in a species non-specific manner suggests the possibility of modifying the MYXV genome by deleting this gene. The deletion of M153 from the MYXV genome may potentially enhance the immunostimulatory effect after infecting cancer cells. It is possible that increased lymphocyte activation and infiltration of tumor beds infected with MYXV lacking the M153 gene along with cytotoxic killing of the tumor cells by this virus may lead to further priming of tumor antigens, therefore, elevating the host's immune response against the tumor. However, this might also limit the therapeutic time window for this MYXV knockout virus but the question remains to be investigated.

## Mimicry of diverse cell surface immune recognition molecules

### M128

M128 (also called viral CD47 or vCD47) is a MYXV protein with homology to cellular CD47 proteins or integrin associated proteins (IAP). Members of the Orthopoxvirus, Capripoxvirus, Leporipoxvirus, Suipoxvirus, and Yatapoxvirus genera as well as deerpox virus all encode a putative CD47-like protein [61]. CD47 regulates the immune response by influencing 12 neutrophil recruitment, leukocyte adhesion and migration and dendritic cell (DC) migration, by affecting the differentiation of antigen presenting cells and by regulating apoptosis of immune cells. CD47 is also considered a self antigen and binds thrombospondin 1 (TSP1) thus playing a role in vascular physiology [62]. The M128 is essential for lethal myxomatosis in rabbits but is not essential for virus replication in cell culture [63]. Rabbits inoculated with MYXV lacking the M128 protein survived infection and are protected against a lethal challenge with wild-type MYXV [63]. M128 was also shown to be an inhibitor of macrophage activation and the production of the inducible form of nitric oxide (iNOS), which has potent anti-viral effects [63]. The mechanism by which M128 inhibits iNOS production and the activation of macrophages is still unclear.

### M141

M141 of MYXV is a viral homolog of the cellular CD200 protein (also called vCD200). The interaction of M141 with the CD200 receptor (CD200R) inhibits the activation of immune cells of myeloid-lineage (e.g., macrophages and DCs) [64,65]. During infection of MYXV in European rabbits, expression of M141 on the surface of infected cells suppressed the activation of macrophages in draining lymph nodes and reduced T cell activation in lymph nodes [66]. In addition, M141 was detected as a component of the mature virion and was shown to suppress the activation of infected macrophages in a murine macrophage cell model [67] which may indirectly inhibit the activation of peripheral T cells in wild-type MYXV infected rabbits [66].

## Other MYXV host range factors

### M063

M063R belongs to the C7L family of intracellular viral host range factors. However, the M063 protein from MYXV was not able to compensate for the function of the related C7 in a VACV C7L/K1L double knockout virus, suggesting that this MYXV protein is not a functional homolog of the VACV C7 protein [68]. However, M063 was reported to be essential for MYXV replication in rabbit cells *in vitro* and *in vivo* and is thus considered a rabbit-specific host range factor [69]. Interestingly, MYXV lacking M063R is still able to



infect cells from other species as well as, if not better than, wild-type MYXV. The function of M063 has not yet been well defined, but an *in vitro* screen using various knockout MYXVs showed that the MYXV M063R-knockout had superior oncolytic potential compared with other targeted MYXV gene knockouts in cultured human glioma cancer cell lines [4]. Given this report and the fact that this virus is nonpathogenic in any known species (even the European rabbit), its increased safety and its enhanced oncolysis make it an ideal candidate for applications in cancer treatments.

## Summary and perspective: oncolytic potential of MYXV

MYXV is a relatively new candidate oncolytic virus and was first shown to possess oncolytic potential in orthotopic human glioma models in nude mice [70]. In this study, a single dose treatment of MYXV consistently led to significant therapeutic effects, with 87.5% and 100% cure rate in two models, using human glioma cell lines U87 and U251, respectively. In addition, MYXV is also effective in killing *ex vivo* cultured primary glioma cells derived from human patient surgical specimens. It is recognized that intracellular abnormalities in various cell signaling pathways (eg IFN/TNF responses [12] or AKT activation [71]) of cancer cells contributes to MYXV's selective oncolysis. These studies encouraged further exploration of MYXV oncolysis in other cancer models [71], as well as for related anti-cancer applications, including the *ex vivo* purging of cancer initiating cells from bone marrow transplants for patients with acute myeloid leukemia [72], and combination therapy using MYXV as an oncolytic agent along with chemotherapy drugs that may produce synergistic anti-cancer interactions [73].

It is important to better understand the interactions between MYXV, the targeted cancer cells and the cancer microenvironment in order to maximize MYXV's potential therapeutic efficacy. In the clinical setting, a beneficial treatment with a virotherapeutic agent often can only be conducted in a relative short treatment window before the virus gets recognized and eliminated by the immune system of the patient. It is possible that immunosuppressive agents may prolong the oncolytic effects and the therapeutic window of MYXV *in vivo*. How to manipulate the native immunoregulatory factors encoded by MYXV in order to maximize the selective destruction of tumor tissue *in vivo* needs to be further examined. The combination of immunotherapy (e.g., cancer vaccines, therapeutic cytokines or immune cell transplantation) and MYXV virotherapy may very well improve the overall treatment outcome. Finally, we hope to further investigate the specific molecular mechanisms of oncolysis elicited by this virus in different cancer cell types, as a customized treatment strategy to different tumor types may be the ultimate solution for individual cancers. Currently, the optimization of a more effective oncolytic MYXV by modifications of the platform MYXV oncolytic vector (e.g., inserting therapeutic genes) from wild-type backbone or knockout viruses with improved tumor cell selectivity or increased safety, are currently underway in our lab [74].

## Acknowledgments

The authors' lab is supported by NIH grants AI080607 and CA13854, and the Bankhead Coley Foundation.

## References

1. Stanford MM, Werden SJ, McFadden G. Myxoma virus in the European rabbit: interactions between the virus and its susceptible host. *Vet Res* 2007;38:299–318. [PubMed: 17296158]
2. Best SM, Collins SV, Kerr PJ. Coevolution of host and virus: cellular localization of virus in myxoma virus infection of resistant and susceptible European rabbits. *Virology* 2000;277:76–91. [PubMed: 11062038]

3. Jeklova E, Leva L, Matiasovic J, Kovarcik K, Kudlackova H, Nevorankova Z, Psikal I, Faldyna M. Characterisation of immunosuppression in rabbits after infection with myxoma virus. *Vet Microbiol* 2008;129:117–130. [PubMed: 18222052]
4. Barrett JW, Alston LR, Wang F, Stanford MM, Gilbert PA, Gao X, Jimenez J, Villeneuve D, Forsyth P, McFadden G. Identification of host range mutants of myxoma virus with altered oncolytic potential in human glioma cells. *J Neurovirol* 2007;13:549–560. [PubMed: 18097886]
5. Stanford MM, McFadden G. Myxoma virus and oncolytic virotherapy: a new biologic weapon in the war against cancer. *Expert Opin Biol Ther* 2007;7:1415–1425. [PubMed: 17727330]
6. Rahman MM, Madlambayan GJ, Cogle CR, McFadden G. Oncolytic viral purging of leukemic hematopoietic stem and progenitor cells with Myxoma virus. *Cytokine Growth Factor Rev* 2010;21:169–175. [PubMed: 20211576]
7. Wang F, Ma Y, Barrett JW, Gao X, Loh J, Barton E, Virgin HW, McFadden G. Disruption of Erk-dependent type I interferon induction breaks the myxoma virus species barrier. *Nat Immunol* 2004;5:1266–1274. [PubMed: 15502830]
8. Wang F, Barrett JW, Ma Y, Dekaban GA, McFadden G. Induction of alpha/beta interferon by myxoma virus is selectively abrogated when primary mouse embryo fibroblasts become immortalized. *J Virol* 2009;83:5928–5932. [PubMed: 19297474]
9. Wang F, Gao X, Barrett JW, Shao Q, Bartee E, Mohamed MR, Rahman M, Werden S, Irvine T, Cao J, Dekaban GA, McFadden G. RIG-I mediates the co-induction of tumor necrosis factor and type I interferon elicited by myxoma virus in primary human macrophages. *PLoS Pathog* 2008;4:e1000099. [PubMed: 18617992]
10. Wang F, Barrett JW, Shao Q, Gao X, Dekaban GA, McFadden G. Myxoma virus selectively disrupts type I interferon signaling in primary human fibroblasts by blocking the activation of the Janus kinase Tyk2. *Virology* 2009;387:136–146. [PubMed: 19254804]
11. Bartee E, Mohamed MR, Lopez MC, Baker HV, McFadden G. The addition of tumor necrosis factor plus beta interferon induces a novel synergistic antiviral state against poxviruses in primary human fibroblasts. *J Virol* 2009;83:498–511. [PubMed: 18971273]
12. Bartee E, McFadden G. Human cancer cells have specifically lost the ability to induce the synergistic state caused by tumor necrosis factor plus interferon-beta. *Cytokine* 2009;47:199–205. [PubMed: 19640730]
13. Ramelot TA, Cort JR, Yee AA, Liu F, Goshe MB, Edwards AM, Smith RD, Arrowsmith CH, Dever TE, Kennedy MA. Myxoma virus immunomodulatory protein M156R is a structural mimic of eukaryotic translation initiation factor eIF2alpha. *J Mol Biol* 2002;322:943–954. [PubMed: 12367520]
14. Garcia MA, Meurs EF, Esteban M. The dsRNA protein kinase PKR: virus and cell control. *Biochimie* 2007;89:799–811. [PubMed: 17451862]
15. Dar AC, Sicheri F. X-ray crystal structure and functional analysis of vaccinia virus K3L reveals molecular determinants for PKR subversion and substrate recognition. *Mol Cell* 2002;10:295–305. [PubMed: 12191475]
16. Kawagishi-Kobayashi M, Cao C, Lu J, Ozato K, Dever TE. Pseudosubstrate inhibition of protein kinase PKR by swine pox virus C8L gene product. *Virology* 2000;276:424–434. [PubMed: 11040133]
17. Elde NC, Child SJ, Geballe AP, Malik HS. Protein kinase R reveals an evolutionary model for defeating viral mimicry. *Nature* 2009;457:485–489. [PubMed: 19043403]
18. Seo EJ, Liu F, Kawagishi-Kobayashi M, Ung TL, Cao C, Dar AC, Sicheri F, Dever TE. Protein kinase PKR mutants resistant to the poxvirus pseudosubstrate K3L protein. *Proc Natl Acad Sci U S A* 2008;105:16894–16899. [PubMed: 18971339]
19. Labudovic A, Perkins H, van Leeuwen B, Kerr P. Sequence mapping of the Californian MSW strain of Myxoma virus. *Arch Virol* 2004;149:553–570. [PubMed: 14991443]
20. Rivas C, Gil J, Melkova Z, Esteban M, Diaz-Guerra M. Vaccinia virus E3L protein is an inhibitor of the interferon (i.f.n.)-induced 2'-5A synthetase enzyme. *Virology* 1998;243:406–414. [PubMed: 9568039]
21. Silverman RH. Viral encounters with 2',5'-oligoadenylate synthetase and RNase L during the interferon antiviral response. *J Virol* 2007;81:12720–12729. [PubMed: 17804500]

22. Smith EJ, Marie I, Prakash A, Garcia-Sastre A, Levy DE. IRF3 and IRF7 phosphorylation in virus-infected cells does not require double-stranded RNA-dependent protein kinase R or Ikappa B kinase but is blocked by Vaccinia virus E3L protein. *J Biol Chem* 2001;276:8951–8957. [PubMed: 11124948]
23. Clemens MJ. Targets and mechanisms for the regulation of translation in malignant transformation. *Oncogene* 2004;23:3180–3188. [PubMed: 15094767]
24. Liu L, Lalani A, Dai E, Seet B, Macauley C, Singh R, Fan L, McFadden G, Lucas A. The viral anti-inflammatory chemokine-binding protein M-T7 reduces intimal hyperplasia after vascular injury. *J Clin Invest* 2000;105:1613–1621. [PubMed: 10841520]
25. Bedard EL, Kim P, Jiang J, Parry N, Liu L, Wang H, Garcia B, Li X, McFadden G, Lucas A, Zhong R. Chemokine-binding viral protein M-T7 prevents chronic rejection in rat renal allografts. *Transplantation* 2003;76:249–252. [PubMed: 12865819]
26. Liu L, Dai E, Miller L, Seet B, Lalani A, Macauley C, Li X, Virgin HW, Bunce C, Turner P, Moyer R, McFadden G, Lucas A. Viral chemokine-binding proteins inhibit inflammatory responses and aortic allograft transplant vasculopathy in rat models. *Transplantation* 2004;77:1652–1660. [PubMed: 15201663]
27. Boomker JM, Luttkhuizen DT, Veninga H, de Leij LF, The TH, de Haan A, van Luyn MJ, Harmsen MC. The modulation of angiogenesis in the foreign body response by the poxviral protein M-T7. *Biomaterials* 2005;26:4874–4881. [PubMed: 15763267]
28. Dai E, Liu LY, Wang H, McIvor D, Sun YM, Macaulay C, King E, Munuswamy-Ramanujam G, Bartee MY, Williams J, Davids J, Charo I, McFadden G, Esko JD, Lucas AR. Inhibition of chemokine-glycosaminoglycan interactions in donor tissue reduces mouse allograft vasculopathy and transplant rejection. *PLoS One* 2010;5:e10510. [PubMed: 20463901]
29. Seet BT, Barrett J, Robichaud J, Shilton B, Singh R, McFadden G. Glycosaminoglycan binding properties of the myxoma virus CC-chemokine inhibitor, M-T1. *J Biol Chem* 2001;276:30504–30513. [PubMed: 11369757]
30. Turner PC, Sancho MC, Thoennes SR, Caputo A, Bleackley RC, Moyer RW. Myxoma virus Serp2 is a weak inhibitor of granzyme B and interleukin-1beta-converting enzyme in vitro and unlike CrmA cannot block apoptosis in cowpox virus-infected cells. *J Virol* 1999;73:6394–6404. [PubMed: 10400732]
31. Guerin JL, Gelfi J, Camus C, Delverdier M, Whisstock JC, Amardeihl MF, Py R, Bertagnoli S, Messud-Petit F. Characterization and functional analysis of Serp3: a novel myxoma virus-encoded serpin involved in virulence. *J Gen Virol* 2001;82:1407–1417. [PubMed: 11369885]
32. Cameron C, Hota-Mitchell S, Chen L, Barrett J, Cao JX, Macaulay C, Willer D, Evans D, McFadden G. The complete DNA sequence of myxoma virus. *Virology* 1999;264:298–318. [PubMed: 10562494]
33. Richardson J, Viswanathan K, Lucas A. Serpins. the vasculature. and viral therapeutics. *Front Biosci* 2006;11:1042–1056. [PubMed: 16146796]
34. Hatton MW, Ross B, Southward SM, Lucas A. Metabolism and distribution of the virus-encoded serine proteinase inhibitor SERP-1 in healthy rabbits. *Metabolism* 2000;49:1449–1452. [PubMed: 11092510]
35. Nash P, Whitty A, Handwerker J, Macen J, McFadden G. Inhibitory specificity of the anti-inflammatory myxoma virus serpin, SERP-1. *J Biol Chem* 1998;273:20982–20991. [PubMed: 9694848]
36. Dai E, Viswanathan K, Sun YM, Li X, Liu LY, Togonu-Bickersteth B, Richardson J, Macaulay C, Nash P, Turner P, Nazarian SH, Moyer R, McFadden G, Lucas AR. Identification of myxomaviral serpin reactive site loop sequences that regulate innate immune responses. *J Biol Chem* 2006;281:8041–8050. 16. [PubMed: 16407226]
37. Viswanathan K, Richardson J, Togonu-Bickersteth B, Dai E, Liu L, Vatsya P, Sun YM, Yu J, Munuswamy-Ramanujam G, Baker H, Lucas AR. Myxoma viral serpin. Serp-1. inhibits human monocyte adhesion through regulation of actin-binding protein filamin B. *J Leukoc Biol* 2009;85:418–426. [PubMed: 19052145]
38. Lucas A, McFadden G. Secreted immunomodulatory viral proteins as novel biotherapeutics. *J Immunol* 2004;173:4765–4774. [PubMed: 15470015]

39. Finn RD, Tate J, Mistry J, Coghill PC, Sammut SJ, Hotz HR, Ceric G, Forslund K, Eddy SR, Sonnhammer EL, Bateman A. The Pfam protein families database. *Nucleic Acids Res* 2008;36:D281–288. [PubMed: 18039703]
40. Bowie A, Kiss-Toth E, Symons JA, Smith GL, Dower SK, O'Neill LA. A46R and A52R from vaccinia virus are antagonists of host IL-1 and toll-like receptor signaling. *Proc Natl Acad Sci U S A* 2000;97:10162–10167. [PubMed: 10920188]
41. Stack J, Haga IR, Schroder M, Bartlett NW, Maloney G, Reading PC, Fitzgerald KA, Smith GL, Bowie AG. Vaccinia virus protein A46R targets multiple Toll-like-interleukin-1 receptor adaptors and contributes to virulence. *J Exp Med* 2005;201:1007–1018. [PubMed: 15767367]
42. Harte MT, Haga IR, Maloney G, Gray P, Reading PC, Bartlett NW, Smith GL, Bowie A, O'Neill LA. The poxvirus protein A52R targets Toll-like receptor signaling complexes to suppress host defense. *J Exp Med* 2003;197:343–351. [PubMed: 12566418]
43. Schroder M, Baran M, Bowie AG. Viral targeting of DEAD box protein 3 reveals its role in TBK1/IKKepsilon-mediated IRF activation. *Embo J* 2008;27:2147–2157. [PubMed: 18636090]
44. Maloney G, Schroder M, Bowie AG. Vaccinia virus protein A52R activates p38 mitogen-activated protein kinase and potentiates lipopolysaccharide-induced interleukin-10. *J Biol Chem* 2005;280:30838–30844. [PubMed: 15998638]
45. Chen RA, Ryzhakov G, Cooray S, Randow F, Smith GL. Inhibition of IkkappaB kinase by vaccinia virus virulence factor B14. *PLoS Pathog* 2008;4:e22. [PubMed: 18266467]
46. DiPerna G, Stack J, Bowie AG, Boyd A, Kotwal G, Zhang Z, Arvikar S, Latz E, Fitzgerald KA, Marshall WL. Poxvirus protein N1L targets the I-kappaB kinase complex. inhibits signaling to NF-kappaB by the tumor necrosis factor superfamily of receptors. and inhibits NF-kappaB and IRF3 signaling by toll-like receptors. *J Biol Chem* 2004;279:36570–36578. [PubMed: 15215253]
47. Cooray S, Bahar MW, Abrescia NG, McVey CE, Bartlett NW, Chen RA, Stuart DI, Grimes JM, Smith GL. Functional and structural studies of the vaccinia virus virulence factor N1 reveal a Bcl-2-like anti-apoptotic protein. *J Gen Virol* 2007;88:1656–1666. [PubMed: 17485524]
48. Aoyagi M, Zhai D, Jin C, Aleshin AE, Stec B, Reed JC, Liddington RC. Vaccinia virus N1L protein resembles a B cell lymphoma-2 (Bcl-2) family protein. *Protein Sci* 2007;16:118–124. [PubMed: 17123957]
49. Graham SC, Bahar MW, Cooray S, Chen RA, Whalen DM, Abrescia NG, Alderton D, Owens RJ, Stuart DI, Smith GL, Grimes JM. Vaccinia virus proteins A52 and B14 Share a Bcl-2-like fold but have evolved to inhibit NF-kappaB rather than apoptosis. *PLoS Pathog* 2008;4:e1000128. [PubMed: 18704168]
50. Kalverda AP, Thompson GS, Vogel A, Schroder M, Bowie AG, Khan AR, Homans SW. Poxvirus K7 protein adopts a Bcl-2 fold: biochemical mapping of its interactions with human DEAD box RNA helicase DDX3. *J Mol Biol* 2009;385:843–853. [PubMed: 18845156]
51. Gonzalez JM, Esteban M. A poxvirus Bcl-2-like gene family involved in regulation of host immune response: sequence similarity and evolutionary history. *Virol J* 2010;7:59. [PubMed: 20230632]
52. Huang B, Zhao J, Unkeless JC, Feng ZH, Xiong H. TLR signaling by tumor and immune cells: a double-edged sword. *Oncogene* 2008;27:218–224. [PubMed: 18176603]
53. Li X, Jiang S, Tapping RI. Toll-like receptor signaling in cell proliferation and survival. *Cytokine* 2010;49:1–9. [PubMed: 19775907]
54. Barrett JW, Sypula J, Wang F, Alston LR, Shao Z, Gao X, Irvine TS, McFadden G. M135R is a novel cell surface virulence factor of myxoma virus. *J Virol* 2007;81:106–114. [PubMed: 17065210]
55. Johnston JB, Barrett JW, Nazarian SH, Goodwin M, Ricciuto D, Wang G, McFadden G. A poxvirus-encoded pyrin domain protein interacts with ASC-1 to inhibit host inflammatory and apoptotic responses to infection. *Immunity* 2005;23:587–598. [PubMed: 16356857]
56. Rahman MM, Mohamed MR, Kim M, Smallwood S, McFadden G. Co-regulation of NF-kappaB and inflammasome-mediated inflammatory responses by myxoma virus pyrin domain-containing protein M013. *PLoS Pathog* 2009;5:e1000635. [PubMed: 19851467]

57. Barrett JW, Werden SJ, Wang F, McKillop WM, Jimenez J, Villeneuve D, McFadden G, Dekaban GA. Myxoma virus M130R is a novel virulence factor required for lethal myxomatosis in rabbits. *Virus Res* 2009;144:258–265. [PubMed: 19477207]
58. Barrett JW, Cao JX, Hota-Mitchell S, McFadden G. Immunomodulatory proteins of myxoma virus. *Semin Immunol* 2001;13:73–84. [PubMed: 11289802]
59. Guerin JL, Gelfi J, Boullier S, Delverdier M, Bellanger FA, Bertagnoli S, Drexler I, Sutter G, Messud-Petit F. Myxoma virus leukemia-associated protein is responsible for major histocompatibility complex class I and Fas-CD95 down-regulation and defines scrapins, a new group of surface cellular receptor abductor proteins. *J Virol* 2002;76:2912–2923. [PubMed: 11861858]
60. Mansouri M, Bartee E, Gouveia K, Hovey Nerenberg BT, Barrett J, Thomas L, Thomas G, McFadden G, Fruh K. The PHD/LAP-domain protein M153R of myxomavirus is a ubiquitin ligase that induces the rapid internalization and lysosomal destruction of CD4. *J Virol* 2003;77:1427–1440. [PubMed: 12502858]
61. Seet BT, Johnston JB, Brunetti CR, Barrett JW, Everett H, Cameron C, Sypula J, Nazarian SH, Lucas A, McFadden G. Poxviruses and immune evasion. *Annu Rev Immunol* 2003;21:377–423. [PubMed: 12543935]
62. Isenberg JS, Roberts DD, Frazier WA. CD47: a new target in cardiovascular therapy. *Arterioscler Thromb Vasc Biol* 2008;28:615–621. [PubMed: 18187671]
63. Cameron CM, Barrett JW, Mann M, Lucas A, McFadden G. Myxoma virus M128L is expressed as a cell surface CD47-like virulence factor that contributes to the downregulation of macrophage activation in vivo. *Virology* 2005;337:55–67. [PubMed: 15914220]
64. Barclay AN, Wright GJ, Brooke G, Brown MH. CD200 and membrane protein interactions in the control of myeloid cells. *Trends Immunol* 2002;23:285–290. [PubMed: 12072366]
65. Hoek RM, Ruuls SR, Murphy CA, Wright GJ, Goddard R, Zurawski SM, Blom B, Homola ME, Streit WJ, Brown MH, Barclay AN, Sedgwick JD. Down-regulation of the macrophage lineage through interaction with OX2 (CD200). *Science* 2000;290:1768–1771. [PubMed: 11099416]
66. Cameron CM, Barrett JW, Liu L, Lucas AR, McFadden G. Myxoma virus M141R expresses a viral CD200 (vOX-2) that is responsible for down-regulation of macrophage and T-cell activation in vivo. *J Virol* 2005;79:6052–6067. [PubMed: 15857991]
67. Zhang L, Stanford M, Liu J, Barrett C, Jiang L, Barclay AN, McFadden G. Inhibition of macrophage activation by the myxoma virus M141 protein (vCD200). *J Virol* 2009;83:9602–9607. [PubMed: 19570854]
68. Meng X, Chao J, Xiang Y. Identification from diverse mammalian poxviruses of host-range regulatory genes functioning equivalently to vaccinia virus C7L. *Virology* 2008;372:372–383. [PubMed: 18054061]
69. Barrett JW, Shun Chang C, Wang G, Werden SJ, Shao Z, Barrett C, Gao X, Belsito TA, Villeneuve D, McFadden G. Myxoma virus M063R is a host range gene essential for virus replication in rabbit cells. *Virology* 2007;361:123–132. [PubMed: 17184804]
70. Lun X, Yang W, Alain T, Shi ZQ, Muzik H, Barrett JW, McFadden G, Bell J, Hamilton MG, Senger DL, Forsyth PA. Myxoma virus is a novel oncolytic virus with significant antitumor activity against experimental human gliomas. *Cancer Res* 2005;65:9982–9990. [PubMed: 16267023]
71. Stanford MM, Shaban M, Barrett JW, Werden SJ, Gilbert PA, Bondy-Denomy J, Mackenzie L, Graham KC, Chambers AF, McFadden G. Myxoma virus oncolysis of primary and metastatic B16F10 mouse tumors in vivo. *Mol Ther* 2008;16:52–59. [PubMed: 17998900]
72. Kim M, Madlambayan GJ, Rahman MM, Smallwood SE, Meacham AM, Hosaka K, Scott EW, Cogle CR, McFadden G. Myxoma virus targets primary human leukemic stem and progenitor cells while sparing normal hematopoietic stem and progenitor cells. *Leukemia* 2009;23:2313–2317. [PubMed: 19865109]
73. Lun X, Alain T, Zemp FJ, Zhou H, Rahman MM, Hamilton MG, McFadden G, Bell J, Senger DL, Forsyth PA. Myxoma virus virotherapy for glioma in immunocompetent animal models: optimizing administration routes and synergy with rapamycin. *Cancer Res* 2010;70:598–608. [PubMed: 20068158]

74. Liu J, Wennier S, Reinhard M, Roy E, MacNeill A, McFadden G. Myxoma virus expressing interleukin-15 fails to cause lethal myxomatosis in European rabbits. *J Virol* 2009;83:5933–5938. [PubMed: 19279088]

Table 1

## Immunoregulatory factors of myxoma virus

Category	MYXV gene product	Function	Therapeutic implications
PKR signaling related	M156	A structural homolog of cellular eIF2 $\alpha$ and a viral pseudosubstrate for PKR	Not tested
	M029	Truncated homolog of VACV E3, a PKR inhibitor	Not tested
Viroceptors	M-T2	A rabbit-specific TNF viroceptor	Not tested
	M-T7	<ul style="list-style-type: none"> <li>A viroceptor for rabbit IFN<math>\gamma</math></li> <li>A species-nonspecific inhibitor of immune cell influx by binding to heparin-binding domain of chemokines (members from C, CC, CXC subfamilies)</li> </ul>	<ul style="list-style-type: none"> <li>Purified M-T7 protein can attenuate inflammatory responses in systemic inflammation disorders.</li> <li>It can also inhibit leukocyte infiltration after transplantation and prevent transplant failure.</li> </ul>
	M-T1	A species-nonspecific viroceptor for chemokines of CC subfamily through C-terminal consensus domain	M-T1 can inhibit immune cell infiltration into damaged tissue and can prevent transplant failure.
Serine protease inhibitors (Serpins)	SERP-1	A broad spectrum protease inhibitor in a species non-specific manner	Purified SERP-1 inhibits inflammatory cascades and has undergone clinical trial for acute myocardial syndrome.
Intracellular immunoregulatory factors	M135	A virulence factor in rabbit pathogenesis of myxoma virus. Expressed on the cell surface during infection but its function is still unknown.	Myxoma virus with M135R gene knockout exhibits improved oncolysis for human glioma cancer cells <i>in vitro</i> .
	M013	In human monocytes, M013 binds to ASC-1 and NF $\kappa$ B1 to inhibit inflammasome activation and NF $\kappa$ B signaling.	Not tested
	M130	A virulence factor with unknown function	Not tested
	M153	<ul style="list-style-type: none"> <li>An E3 ligase that promotes host target ubiquitination and degradation (e.g., CD4)</li> <li>Downregulation of MHC I during infection</li> </ul> <p>Functions of M153 are species non-specific.</p>	Not tested.
Mimicry of cell surface immune recognition molecules	M128 (vCD47)	A cell surface inhibitor of macrophage activation with unknown mechanism	Not tested
	M141 (vCD200)	<ul style="list-style-type: none"> <li>Inhibits the activation of immune cells with myeloid lineage through interaction with CD200 receptor.</li> </ul>	Not tested

Category	MYXV gene product	Function	Therapeutic implications
		<ul style="list-style-type: none"><li>A component of the myxoma virus virion, it suppresses the activation of murine macrophages during infection <i>in vitro</i>.</li></ul>	
Others	M063	MYXV with M063R gene knockout loses the ability to infect rabbits. M063 is a rabbit-specific host range factor of unknown function.	M063R knockout MYXV has been shown to have superior oncolytic effect to human glioma cancer cells <i>in vitro</i> .