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Oncolytic myxoma virus: The path to clinic

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ABSTRACT

Many common neoplasms are still noncurative with current standards of cancer therapy. More therapeutic modalities need to be developed to significantly prolong the lives of patients and eventually cure a wider spectrum of cancers. Oncolytic virotherapy is one of the promising new additions to clinical cancer therapeutics. Successful oncolytic virotherapy in the clinic will be those strategies that best combine tumor cell oncolysis with enhanced immune responses against tumor antigens. The current candidate oncolytic viruses all share the common property that they are relatively nonpathogenic to humans, yet they have the ability to replicate selectively in human cancer cells and induce cancer regression by direct oncolysis and/or induction of improved anti-tumor immune responses. Many candidate oncolytic viruses are in various stages of clinical and preclinical development. One such preclinical candidate is myxoma virus (MYXV), a member of the *Poxviridae* family that, in its natural setting, exhibits a very restricted host range and is only pathogenic to European rabbits. Despite its narrow host range in nature, MYXV has been shown to productively infect various classes of human cancer cells. Several preclinical *in vivo* modeling studies have demonstrated that MYXV is an attractive and safe candidate oncolytic virus, and hence, MYXV is currently being developed as a potential therapeutic for several cancers, such as pancreatic cancer, glioblastoma, ovarian cancer, melanoma, and hematologic malignancies. This review highlights the preclinical cancer models that have shown the most promise for translation of MYXV into human clinical trials.

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1. Introduction

Cancer remains a major public health problem worldwide. For example, one out of four deaths in the United States is due to cancer [1]. Current standards of cancer therapy include resection surgery (if applicable), radiation, chemotherapy, immunotherapy, and/or biological therapy. However, available treatments for many common cancers remain noncurative and in many cases prolong survival rates only in the short term. Therefore, development of more efficacious treatments is much needed in order to significantly prolong the lives of cancer patients and eventually cure a wider spectrum of cancers. One of the newer and promising cancer therapeutic strategies is oncolytic virotherapy, and the first candidate viruses are rapidly approaching licensure in North America and Europe [2]. Successful oncolytic virotherapy in the clinic will possess two closely inter-related properties: the ability to destroy cancer cells directly but also the capacity to enhance acquired immune responses against tumor antigens, *i.e.*, oncolytic virotherapy and anti-tumor immunostimulation are inextricably linked to each other. In general terms, a replication-competent virus, which spares normal tissues but selectively replicates in cancer cells, is used to specifically infect and eliminate cancerous tissues [3]. Viruses with demonstrated oncolytic potential include numerous candidates that are relatively nonpathogenic to humans but have a biologic proclivity to replicate selectively in human cancer cells. Examples of viruses that are in various stages of clinical and preclinical development include measles virus, vesicular stomatitis virus, adenovirus, reovirus, herpes simplex virus and two poxviruses, vaccinia virus and myxoma virus (MYXV) (reviewed in [4–9]). Vaccinia virus, a prototypic member of the *Poxviridae* family, has been widely developed as a vaccination platform, and more recently is being tested as an oncolytic virotherapeutic in Phase II clinical trials for various late stage cancers, including liver cancer and malignancies that metastasize to the liver [4,10–14]. Vaccinia virus, long used in the worldwide vaccination program against smallpox, is of unknown origin in terms of its evolutionary host, but has been tested extensively in humans.

In general, poxviruses infect a wide range of hosts including humans, monkeys, mice, rabbits and insects, but individual members can be highly species-specific in terms of the hosts that they can infect [15,16]. For example, vaccinia virus infects a wide variety of vertebrate hosts whereas MYXV is completely restricted to

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Table 1
Summary of the oncolytic potential of MYXV tested in preclinical animal models of cancer.

Type of cancer	Animal model	Tumor establishment	MYXV administration	Outcome (references)
Acute myeloid leukemia (AML)	NSG	Human AML cells in bone marrow xenograft	*Ex vivo*[a]	90% of mice free of human AML cells in BM [40]
Multiple myeloma (MM)	NSG	Human MM cells in bone marrow xenograft	*Ex vivo*[a]	100% of mice free of human MM cells in BM [44]
Pancreatic cancer	NOD/SCID	Human pancreatic cancer cells in intraperitoneal cavity	Intraperitoneal	Reduced tumor burden and prolonged survival [51]
	C57BL/6	Murine pancreatic cancer cells in intraperiotoneal cavity	Intraperitoneal	100% survival when combined with gemcitabine [51]
Glioma	CD-1 nude	Human gliomas in mouse brain	Intratumoral	92% of mice cleared of tumors and cured [66]
	Fischer 344 rats	Racine gliomas in rat brain	Intratumoral	Prolonged survival when combined with Rapamycin [29]
Rhabdoid tumors	CD-1 nude	Human rhabdoid tumor cells in mouse brain	Intratumoral	Reduced tumor mass; 66.7% of mice had long-term survival [71]
		Human rhabdoid tumor cells in hind flank of mice	Intratumoral	Reduced tumor mass [71]
Medulloblastoma	CD-1 nude	Human medulloblastoma in mouse brain	Intratumoral	Prolonged survival; 60% of mice had long-term survival when combined with rapamycin [72]
Melanoma	C57BL/6	Subcutaneous murine melanoma	Intratumoral	Reduced tumor mass [81]
			Intravenous	Decreased development of lung metastasis [81]
	C57BL/6	Murine melanoma	*Ex vivo*[a]	Prevented tumor implantation [81]

[a] *Ex vivo* treatment of samples with MYXV prior to implantation/engraftment.

lagomorphs and is only pathogenic in the European rabbit [17–19]. MYXV is the prototypic member of the *Leporipoxvirus* genus within the *Poxviridae* family [20–22]. The MYXV Lausanne strain genome is 161.8 kbp in size, encoding about 171 genes [23]. The central region of the genome encodes less than 100 genes that are highly conserved in all poxviruses while the terminal genomic regions are enriched for more unique genes that encode immunomodulatory and host-interactive factors that are involved in subverting the host immune system and other anti-viral responses [20,24–26]. A more detailed background on MYXV and its history has been described in recent reviews [21,27].

MYXV causes a lethal disease called myxomatosis in European rabbits (*Oryctolagus cuniculus*) but the virus actually co-evolved within lagomorphs of the *Sylvilagus* genus, such as the Brazilian tapeti [21,28]. In the tapeti, MYXV replicates robustly and transmits efficiently from host-to-host but causes no overt disease [28]. The basis for the extreme virulence of MYXV in the European rabbit, and absence of pathogenesis in the tapeti, is not well understood but the virus is essentially nonpathogenic for any host outside the lagomorph family [17–19,21]. Indeed, the virus fails to replicate to any appreciable extent in any non-rabbit host tested to date, including highly immunodeficient mice [21,29]. MYXV can successfully replicate in rabbits due to the ability of MYXV to escape multiple diverse host innate and adaptive immune responses [20,22,25,26]. Despite its narrow host range in nature, MYXV has been shown to productively infect various classes of human cancer cells due to several factors, including: (I) the failure of most cancer cells to induce appropriate anti-viral responses, such as the synergistic interferon and tumor necrosis factor pathways that efficiently aborts MYXV replication in normal primary human cells [30,31] and (II) the constitutive activation of intracellular pathways related to cellular transformation, such as the phosphorylation of Akt, commonly found in many human cancer cells [32]. A detailed study has shown that MYXV-encoded ankyrin-repeat host range factor, M-T5, interacts with Akt and this interaction is required for the enhanced phosphorylation of Akt [32,33]. Pharmacologic manipulation of Akt activation affects MYXV tropism, indicating a direct correlation between endogenous activated signal transduction pathways and the permissiveness of MYXV to target human cancer cells [34]. Additionally, our laboratory has demonstrated an important role for the IFN signaling in the permissiveness of MYXV to certain specific cell types [35]. Co-treatment of primary human fibroblasts with type I IFN and TNF induces a synergistic antiviral state that aborts MYXV infection [31]. Importantly, a wide spectrum of human cancer cells has been shown to unable to induce the synergistic antiviral state, which may allow for selective/productive MYXV infection in a wide variety of human cancer cells [30].

Attractive features of MYXV as an oncolytic agent include its ability to productively infect various human cancer cells and its consistent safety in all non-rabbit hosts tested, including mice and humans [17–19,21,36]. The work from our laboratory and of others clearly demonstrates that MYXV is an attractive candidate oncolytic virus (reviewed in [22,37,38]), and hence, MYXV is currently being developed as a candidate therapeutic for several cancers, including ovarian cancer, glioblastoma, myeloid leukemia, multiple myeloma (MM), melanoma, and pancreatic cancer. This review updates the current status in the oncolytic potential of MYXV for the treatment of these cancers in preclinical animal models (Table 1) and highlights the projected path toward human clinical trials with this virus.

2. Hematological malignancies: acute myeloid leukemia and multiple myeloma

Hematological malignancies under consideration here include acute myeloid leukemia (AML) and multiple myeloma (MM) that mainly affect adults. Approximately 10% of Americans diagnosed with cancer each year will have one of these blood cancers (National Cancer Institute, Surveillance Epidemiology, and End Results). For high-risk patients with AML, either autologous or allogenic hematopoietic stem cell transplantation has been used to rescue immune function following myeloablative therapy with high dosages of chemotherapeutics. Autologous stem cell transplants are safer than allogeneic transplants but suffer the downside of being potentially contaminated with residual cancer cells from the donor that can potentially re-seed the cancer following engraftment. Various purging strategies have been attempted in the past to selectively kill off cancer cells in contaminated autologous

transplant samples *ex vivo*, while preserving the normal CD34$^+$ stem cell needed for immune reconstitution (reviewed in [37,39]). However, current purging methods do not selectively remove all cancer cells or enrich CD34$^+$ stem cells that would yield transplant samples completely free of contaminating cancer cells, which can still initiate relapse. One suitable purging strategy exploits an oncolytic virus, provided that the candidate virus excels in both properties, namely the ability to recognize and kill cancer cells even at low levels of contamination, and the inability to infect or perturb normal CD34$^+$ hematopoietic stem cells.

Kim et al. reported that MYXV efficiently infects and kills primary leukemic hematopoietic stem and progenitor cells (HSPCs) from patients with acute myeloid leukemia without altering the ability of the normal human CD34$^+$ HSPCs to differentiate into the expected leukocyte lineages *in vitro* [40]. Bone marrow-derived mononuclear cells or selected CD34$^+$ cells derived from normal human donors, following treatment with MYXV *ex vivo*, still efficiently engrafted into the bone marrow of highly immunodeficient NOD/scid/IL2 receptor gamma chain knock out (NSG) mice, indicating that MYXV pretreatment *ex vivo* did not perturb the engraftment/differentiation potential of normal donor human HSPCs. It was recently reported that the completion of the viral replication cycle is not necessary for the prevention of leukemic cell engraftment [41]. It was speculated that binding of virus to the surface of leukemic cells could provoke an early anti-viral response that prevents the engraftment, but other potential explanations are still formally possible.

MM is a malignancy of plasmacytoid cells and is most prevalent in adults. One standard treatment for MM patients is a high-dose chemotherapy followed by autologous hematopoietic stem cell transplantation. While this treatment significantly prolonged survival compared to patients treated with chemotherapy alone, it does not cure the disease and generally leads to relapse [42,43]. Bartee et al. observed that MYXV infected and killed all human MM cell lines tested efficiently in culture by inducing rapid cellular apoptosis [44]. Treatment of human MM cell lines with MYXV prior to xenotransplantation into NSG mice prevented engraftment of the input MM cells [44].

The studies summarized above clearly show that MYXV has the ability to discriminate cancerous myeloid cells from the normal CD34$^+$ HSPCs found within complex bone marrow transplant samples, and is thus an attractive candidate to be exploited as an *ex vivo* purging agent for AML and MM from autologous stem cell transplant specimens. However, the reason MYXV selectively binds and infects so many types of human cancer cells while the virus fails to bind to normal CD34$^+$ HSPCs has not yet been molecularly defined. It is important to next characterize the mechanism by which MYXV selectively purges various classes of cancer cells from autologous bone marrow transplant samples because it will provide a more mechanistic basis for understanding why primary human CD34$^+$ HSPCs cannot bind MYXV, and thus are not infected by this virus.

3. Pancreatic cancer

Pancreatic cancer was reported to be the fourth leading cause of cancer related deaths in the US [1]. Surgical resection is the only potentially curative treatment for patients with pancreatic cancer, although many patients are not candidates for resection because of the late stage of disease diagnosis. Current treatment of pancreatic cancer is resection of tumors, if resectable, followed by radiation and/or chemotherapy with gemcitabine (a nucleoside analog) [45–47]. Unfortunately, current treatments are not sufficiently effective and 5 year survival statistics remain grim. Therefore, it would be beneficial to have new therapies to combine with current therapeutics for an efficient treatment to prolong survival [48].

It is known that activated Akt, an inhibitor of apoptosis, is upregulated in many pancreatic cancer cells and acquired resistance to gemcitabine is associated with increased levels of activated Akt [49]. Given a direct correlation between activated Akt and the permissiveness of cancer cells to MYXV, the abnormal signaling repertoire of pancreatic cancer cells led to the idea of testing the oncolysis of MYXV against human pancreatic cancer cells [32]. Permissiveness of various pancreatic cancer cells to MYXV infection was directly related to the level of endogenous phosphorylated Akt, suggesting that MYXV may represent a potential alternative therapeutics for pancreatic cancer, especially those that are gemcitabine-resistant [49,50].

Detailed studies by Wennier et al. revealed that all five pancreatic cancer cell lines tested were permissive to MYXV *in vitro* [51]. Replication of MYXV within transplanted tumors was also observed when MYXV was intratumorally injected into subcutaneous human or murine cancer cells implanted into immunodeficient or immunocompetent mice, respectively [51]. In an intraperitoneal dissemination (IPD) model of late stage metastatic carcinamatosis, MYXV was found to reduce tumor burden and prolong survival. Therefore, this study demonstrates that MYXV is an effective oncolytic agent in the disseminated pancreatic cancer model. Combined MYXV and gemcitabine therapy enhanced survival more than either treatment alone in this immunocompromised IPD model [51]. Even more dramatically, in the immunocompetent IPD model, sequential treatment with MYXV followed by gemcitabine resulted in 100% survival of all tumor-bearing mice, indicating that MYXV infection of pancreatic cancer cells *in situ* made the tumors more sensitive to gemcitabine therapy. However, an intact immune response appeared to be required for the enhanced survival following the sequential virus-gemcitabine treatment regimen [51].

More work is needed in order to determine the molecular mechanism underlying the clearance of disseminated pancreatic cancer by the MYXV-gemcitabine strategy, and the apparently immunostimulatory mechanisms responsible for the enhanced survival in immunocompetent models. But potential explanations include the improved antigen presentation of tumor epitopes in tumor beds bearing replicating MYXV and/or selective depletion of immune suppressor cells, such as regulatory T cells that dampen the immune responses against pancreatic cancer cells. Indeed, the future for oncolytic virotherapy of pancreatic cancer in general will likely hinge upon the successful integration of viral oncolysis with the subsequent enhancement of anti-tumor immune responses.

4. Ovarian cancer

Ovarian cancer is the fifth most prevalent cancer in women [1]. Epithelial ovarian cancer (EOC) constitutes approximately 90% of diagnosed ovarian tumors [52]. Lack of clinical symptoms makes early detection problematic and late diagnosis is a major contributor of the poor survival of most ovarian cancer patients. Current standard treatment includes surgery, followed by chemotherapy. Chemotherapy can be initially effective at inducing regression, but most patients subsequently develop chemo-resistant disease. Therefore, the development of new therapeutics is necessary and to date, various virotherapeutics have been applied to EOC [53–59].

MYXV has been shown to productively infect a variety of human EOC cell lines [60]. Additionally, MYXV can infect primary human EOC cells, isolated directly from patient ascites, cultured as suspension EOC or spheroids that mimic EOC dissemination in ascites fluid. Spheroids are multicellular spheres that resemble those observed in patients and are often found in a semi-dormant state with reduced proliferation, and hence, are relatively

chemotherapy-resistant. Infected spheroids had reduced ability to adhere and disperse upon attachment, indicating MYXV has a potential to interfere with EOC secondary metastasis [60]. Importantly, primary patient ascites samples were shown to be susceptible to MYXV infection [60]. This is the first report to show that MYXV is oncolytic to primary EOC cells and to suggest that MYXV can effectively target ovarian cancer stem cells and spheroids residing in the ascites fluids. It will be important to next test MYXV against primary EOC in appropriate animal models that best mimic the human disease.

5. Brain cancer: glioma, rhabdoid, and medulloblastoma cancers

Malignant gliomas are highly invasive and rapidly growing brain tumors [61]. Current treatment of malignant glioma patients includes surgery, radiation, and/or chemotherapy [62]. But the standard of care treatment is not effective and most patients survive for only 1 year on average [62]. To date, a variety of oncolytic viruses have been tested as therapeutics for glioma, and several clinical trials are ongoing to test efficacy in patients [2,63–65]. The oncolytic potential of MYXV for malignant gliomas has been evaluated more extensively in preclinical models than any other types of cancers described in this review.

Six established malignant human glioma cell lines and two racine cell lines were shown to be permissive to MYXV infection and killed, albiet with different efficiencies [66]. A single intratumoral administration of MYXV led to a long-term survival and cure of disease in an orthotopic mice model in which human glioma xenografts were implanted into the putamen [66]. Importantly, MYXV was shown to efficiently infect and kill primary human gliomas obtained from surgical specimens cultured *ex vivo* [66]. Therefore, these data demonstrate that MYXV is a potentially effective virotherapeutic for human gliomas, at least in xenograft mice models.

However, MYXV could only eliminate the tumor mass in the hemisphere in which MYXV was administered [66]. It did not kill distant tumors implanted in the contralateral hemisphere, indicating that MYXV did not appear to efficiently spread to the contralateral hemisphere. Josiah et al. investigated if adipose-derived stem cells (ADSC) are suitable therapeutic vehicles to deliver MYXV to brain tumors since ADSC were susceptible to MYXV and supported productive multi-cycle MYXV infection *in vitro* [67]. Progeny MYXV produced from ADSC cross-infected glioma cells and caused oncolysis of glioma cells, leading to the survival of all mice injected with glioma cells with MYXV-infected ADSC in an orthotopic malignant brain tumor model [67]. Furthermore, multiple injections of MYXV-infected ADSC resulted in long-term survival of mice bearing established orthotopic human malignant gliomas. This is the only report to show that cellular carriers are suitable to be used as therapeutic delivery vehicles for MYXV to cancer tissues.

While MYXV was very effective in eliminating malignant gliomas in the immunocompromised xeno-implanted mice model, MYXV monotherapy did not have a significant effect on the survival of immunocompetent rats bearing racine gliomas [29]. However, survival was markedly prolonged when MYXV was administered in combination with rapamycin. The enhanced effect of rapamycin was due to reduced type I IFN responses and the infiltration of natural killer cells and macrophages into MYXV-treated gliomas [29]. This suggests that host innate immune response barrier(s) might be a major challenge for the effectiveness of virotherapeutic for malignant gliomas, at least in terms of direct oncolytic killing. Whether MYXV replication within a glioma tumor bed might also potentiate anti-glioma immune responses is currently unknown, but would clearly be highly desirable.

Rhabdoid tumors are rare aggressive neoplasms that occur in infants and children under 2 years old. They have been described as kidney tumors but they can develop in various tissues, including the liver, lung, and brain [68–70]. Current available treatments are unsatisfactory for rhabdoid tumors. Wu et al. found that MYXV productively infected and killed four human rhabdoid tumor cell lines *in vitro* [71]. Intratumoral administration of MYXV to human rhabdoid tumors xeno-implanted either in the hind flank or in the brain of CD-1 nude mice reduced the tumor mass [71]. MYXV treatment dramatically prolonged the survival of mice bearing human rhabdoid tumors in the brain, indicating that MYXV can efficiently eliminate rhabdoid brain tumors [71]. However, the efficacy of MYXV for malignant rhabdoid tumors in immunocompetent models remains to be determined.

Unlike rhabdoid tumors, medulloblastoma is the most common malignant brain tumor in children but it is very difficult to treat due to its invasive nature. Lun et al. observed that nine of the ten medulloblastoma cell lines tested were permissive to MYXV and MYXV oncolysis was enhanced by treating cells with rapamycin prior to infection. This enhancement was correlated with increased levels of endogenous activated Akt [72]. Intratumoral injection of MYXV prolonged the survival of mice bearing human medullablastoma. Consistent with *in vitro* observations, administration of rapamycin in conjunction with MYXV enhanced MYXV oncolysis [72]. These data indicate that MYXV will be an effective virotherapeutic agent for the treatment of human medulloblastoma and that combined MYXV and rapamycin therapies will improve the oncolytic potential of MYXV. Again, the efficiency of MYXV for malignant medulloblastoma in immunocompetent models, where host immune response may affect the oncolysis of MYXV, has not been tested yet. More work is needed to determine the mechanisms by which MYXV kills human medulloblastoma and the mechanisms as to how rapamycin enhances oncolysis of MYXV.

6. Melanoma

Melanoma is a particularly dangerous type of skin cancer and is the leading cause of death from skin disease [1]. Malignant cutaneous melanoma can further develop into metastatic melanoma brain tumors. Patients with metastasized melanoma currently have very poor prognoses [73]. One current promising method of treating metastatic melanoma is adoptive transfer of tumor-specific T cells [74]. While the treatment is still at the investigational stage, recurrence occurs due to various mechanisms: for example, the escape of antigen-loss variant tumor cells from the killing of antigen-specific T cells [75–79]. Combination therapies with other various treatment modalities might be more efficacious at preventing tumor recurrence. For example, the oncolytic potential of vesicular stomatitis virus has been tested in treating melanoma [80].

Stanford et al. tested the permissiveness of murine melanoma cell lines to MYXV infection *in vitro* and found a correlation between the permissiveness and the level of endogenous phosphorylated Akt [81]. It was shown that pre-treatment of B16F10 melanoma cells with MYXV prevented the cells from developing primary tumors and lung metastasis in a syngeneic immunocompetent mouse model. In mice bearing established primary subcutaneous tumors, intratumoral injection of MYXV decreased the tumor size. Additionally, systemic intravenous administration of MYXV in series inhibited the development of lung metastasis [81]. Rapamycin, an immunosuppressant that also inhibits growth of tumors and prevents angiogenesis, enhanced the effectiveness of MYXV in the B16F10 lung metastasis mouse model by attenuating the neutralizing antibodies against MYXV from prior administrations [81]. These data demonstrate that MYXV is a promising

virotherapeutic for melanoma-derived lung metastasis in immunocompetent animal tumor models, with improved efficacy when combined with rapamycin treatment.

As stated above, adoptive transfer of activated antigen-specific T cells alone was not sufficient to cure disease because tumors recur from the antigen-loss variant tumor cells that evade the killing of antigen-specific T cells [82]. Adoptive transfer of activated antigen-specific T cells has been shown to prolong the survival of C57BL/6 and C57BL/6 RAG1$^{-/-}$ mice infused intracranially with B16.SIY melanoma cells [82]. Thomas et al. tested the efficiency of combined MYXV virotherapy and adoptive T cell therapy in treating metastatic melanoma brain tumor in pre-clinical syngeneic mouse model. Intratumoral injection of MYXV led to specific infection of tumor cells with the virus in the brain [82]. It has been shown that intraventricular administration of MYXV exhibits transient expression of viral proteins in a small population of normal neural stem cells, ependymal and subventricular cells, in mouse brain [83]. However, there was no evidence of those cells undergoing apoptosis despite the occurrence of early stage replication of MYXV [83]. These studies suggest that MYXV is safe for use as virotherapeutic agent for the treatment of brain tumors [29,66,82,83].

Whereas pre-treatment of B16.SIY with MYXV prior to infusion increased survival of mice, long-term survival was not achieved [82]. This suggests that there exists anti-viral mechanism(s) that prevent the spread of virus *in vivo* and/or the virus was cleared rapidly due to the recruitment of immune cells to the site of viral replication. It was found that the levels of IFNβ and TNF were significantly increased in response to virus, indicating that viral replication maybe blocked due to the cytokine responses, therefore, preventing the virus spread [82]. It was indeed shown that injection of neutralizing antibodies against IFNβ along with MYXV and adoptive 2C T cells transfer significantly prolonged survival of mice compared to those receiving MYXV injection and 2C T cells, demonstrating that IFNβ induced in response to MYXV dampened the MYXV oncolytic potential [82]. Including rapamycin increased the survival of established tumor-bearing mice, as rapamycin treatment reduced the local IFNβ production. Therefore, these data indicate the beneficial effect of combined immunotherapy, virotherapy, and rapamycin in treating metastatic melanoma brain tumors.

7. Conclusions and future perspectives

MYXV is a relatively new oncolytic virus candidate compared to other viruses being evaluated for oncolytic properties in clinical trials. Based on the literature reviewed here, it is clear that MYXV possesses significant oncolytic potential for many types of cancers, particularly AML, MM, pancreatic cancer, and gliomas, in the pre-clinical models tested to date. Since it is well known that all animal models of cancer suffer various degrees of variance from true human cancers *in situ*, and the oncolytic efficacy of any virus in either immunocompetent or immunodeficient models does not necessarily predict their oncolytic behavior in humans, it will be important to next conduct properly controlled human clinical trials to test MYXV oncolytic potential to investigate if any one virus is superior to others for specific type of target cancers, and which clinical indication is best suited for each candidate virus.

In certain cases, engineered oncolytic viruses have been demonstrated to exhibit improved oncolytic potential [84–86]. In the case of MYXV, a panel of targeted gene knockout mutant viruses has been constructed and the functions of the various gene products have been studied [87]. Many of these mutant viruses have lost the ability to infect certain cultured cells (*i.e.*, host-range restricted), and yet, they still have the ability to infect human cancer cells *in vitro* [88]. These single-gene deletion MYXV mutants are nonpathogenic for all known vertebrate hosts, including rabbits, and represent attractive candidates for the testing of MYXV as an oncolytic agent in human clinical trials in the future. Recently, it was reported that a recombinant MYXV expressing the F11L gene from vaccinia virus makes larger plaques when tested on primary rabbit cornea cell monolayers in culture, indicating that the virus spread from cell-to-cell more efficiently than the wild type MYXV [89]. It would be informative to examine if the MYXV recombinant expressing F11 has improved oncolysis and dissemination within various cancer tissues *in situ.*

Conflict of interest

The authors declare no conflict of interest.

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