Oncolytic Virotherapy for Solid Tumors

Subjects: Virology

Contributor: Masmudur Rahman , Grant McFadden

Oncolytic viruses (OVs) are one of the most promising novel therapeutics for malignant cancers. They selectively infect and kill cancer cells while sparing the normal counterparts, expose cancer- specific antigens and activate the host immune system against both viral and tumor determinants. Oncolytic viruses can be used as monotherapy or combined with existing cancer therapies to become more potent. OVs have emerged as an exceedingly promising modality for cancer treatment based on their multi-mechanistic advantages against diverse types of cancer: the selective infection and killing of cancer cells, the ability in some cases to target cancer at metastatic sites, the release of tumor-associated antigens, triggering of novel anti-tumor innate and adaptive immune responses, and the activation and recruitment of immune cells into the tumor microenvironment (TME). Furthermore, genetic modification can further enhance the antitumor activity of many OVs and thus increase the suitability for combination with conventional or newer therapies.

Myxoma virus Oncolytic Virus Oncolytic Virotherapy

1. Small Cell Lung Cancer

Lung cancer is the second most common cancer in both men and women. Among the various forms of lung cancers, the two main types are: non-small-cell lung cancer (NSCLC) and small-cell lung cancer (SCLC). In general, about 10–15% of all lung cancers are SCLC. SCLC is considered a very aggressive and lethal malignancy due to its early metastatic dissemination [1]. With SCLC, unfortunately most of the patients are diagnosed late with metastatic disease, and with current treatment options the general 5 year survival rate is only 6%. The SCLC-associated invasive tumor growth and early metastatic dissemination are mainly driven by the mutational inactivation of RB1 and p53. Patients with limited stage (LS) or extensive stage (ES) SCLC are commonly given platinum-based chemotherapy, such as cisplatin and carboplatin, with the non-platinum agent etoposide as first-line treatment [2]. Immunotherapy using an anti-PD1 antibody, prolonged survival compared to standard chemotherapy against NSCLC; however, for SCLC the response rate to immunotherapy remains low, in the range of 15–30% [3]. Various OVs have been tested as potential viral immunotherapies against NSCLC, including VSV, measles virus (MV), VACV and adenovirus [4]. However, only a small number of OVs are in human clinical trials against lung cancer indications. One of the obstacles for OVs is the delivery of sufficient therapeutic virus to the SCLC tumors and metastatic sites.

In vitro, MYXV can productively infect and replicate in both adherent monolayer and nonadherent (i.e., floating spheroid) human SCLC cell lines, whereas normal human lung epithelial cells were essentially nonpermissive to MYXV [5]. In the tested human SCLC cell lines, MYXV infection caused cell death and increased the early release

of ATP, a marker for induced immunogenic cell death (ICD). In addition, like human SCLC cell lines, MYXV also productively infected patient-derived primary SCLC cells collected by bronchoscopy [5]. The oncolytic activity of MYXV against SCLC was then tested in an immunocompetent genetically engineered mouse model (GEMM). In this model, the conditional knockouts of *p53*, *Rb* and *P130* using intratracheal delivery of adenovirus Cre-recombinase (Ad-Cre) led to the development of multiple SCLC tumor foci in the lungs [6]. These murine tumors represented features characteristic of end-stage human lung SCLC. When tested for immune cell infiltration, the lung tumor lesions exhibited minimal $CD45^+$ and $CD3^+$ immune cell populations, which also is reminiscent of the situation with primary human SCLC tumor biopsies [5]. Using this murine SCLC mouse model, syngeneic murine SCLC cell lines that were either adherent or floating spheroid phenotypes were generated and infected with MYXV to test for oncolytic killing. Like human SCLC cell lines, these murine SCLC cell lines were also productively infected with MYXV and showed an enhanced early release of ATP after MYXV infection [5]. Furthermore, a cisplatin-resistant murine SCLC cell line, which was generated by continuous exposure to cisplatin, was also infected by MYXV, suggesting that even drug-relapsed SCLC patients might be treatable with oncolytic MYXV. To test whether MYXV can infect SCLC cells in vivo within the tumor bed in situ in GEMM SCLC mice, the virus was delivered into the lung by intranasal instillation three months after tumor induction via intratracheal Ad-Cre delivery [5]. In the MYXV-treated lungs, MYXV replication was detected three days post-delivery using the expression of FLuc as a reporter, however, the signal was mostly eliminated by seven days post-delivery. More importantly, using this delivery method, MYXV replication was not detected in any other organs, again supporting the safety profile of MYXV. When the tumors were collected after 30 days of viral delivery and stained with $CD45^+$ positive leukocytes, significant increases in immune cell infiltration were observed in the tumor bed [5]. These results suggest that MYXV enhanced the immune-stimulatory responses in vivo in the SCLC tumor bed. Based on these findings, a survival study was performed in this model by intranasal delivery of MYXV as therapy, with or without cisplatin. In this test, MYXV alone, or in combination with cisplatin, significantly enhanced the survival of mice compared to control PBS or treatment with cisplatin alone [5]. These results demonstrate the potential of using MYXV as a virotherapy and immunostimulatory treatment agent for SCLC.

2. Ovarian Cancer

Ovarian cancer (OC) is the sixth most prevalent cancer in women, with a high relapse rate. The five year relative survival rate for all types and stages of ovarian cancer is 47%, making it the most lethal of the gynecologic malignancies (American Cancer Society). OVs, in combination with immunotherapeutic agents such as Immune Checkpoint Inhibitors (ICIs), has shown promise in preclinical models and clinical trials. In a recent study, it was shown that, in select ovarian cancer cell lines, STING-dependent innate immune signaling pathways were defective, allowing cancer cells to become more susceptible to OVs. Surprisingly, the dsRNA activated RIG-I/MDA5 innate immune cytokine production pathway was functional in these cells [7]. Among the OVs, Measles, Adenovirus, VACV and Reovirus have been used in clinical trials [8]. MYXV has been tested in both human EOC cell lines and primary human EOC cells, isolated directly from patient ascites, which are cultured as suspension EOCs or spheroids [9]. In a later study, it was shown that although MYXV infected and reduced the viability of adherent monolayer cultured cells, in 3D spheroids the virus replication was restricted [10]. This is possibly because, in

spheroids, Akt signaling becomes downregulated, which directly affected MYXV replication and oncolytic efficacy [9]. However, when the spheroids were reattached, the activated AKT level increased and also enhanced MYXV infection and oncolytic killing of cells. This was also observed with patient ascites derived EOC cells grown in spheroids, which showed MYXV entry but no cytopathic effects. The direct infection of freshly-collected ascites demonstrated that more than 50% patient samples were sensitive to MYXV-mediated oncolytic cell killing [10]. In a recent study, the combination of MYXV oncolytic virotherapy with chemotherapy cisplatin was tested in a disseminated OC mouse model. In this model, delivery of MYXV first, and subsequent treatments with cisplatin increased, the survival of mice. Another interesting observation was that OC patient ascites-associated $CD14^+$ myeloid cells, when infected in vitro with MYXV, reduced the secretion of IL-10, a signature of the immunosuppressive tumor environment [11].

3. Glioblastoma

Glioblastoma, also known as glioblastoma multiforme (GBM), is one of the most aggressive types of cancers that starts in astrocytes that support nerve cells. The treatments for GBMs are surgery, followed by radiation and chemotherapy. But these treatments are largely ineffective and the median survival time for GBM is only 15 to 16 months [12]. Among the chemotherapy options, temozolomide (TMZ) improves the survival of patients with GBM, however, the development of resistance to GBM is very common and, thus, newer therapies are urgently required. Another barrier to GBM therapy is the intra-tumoral heterogeneity in the tumor-initiating cell population, which quickly develop a resistance to conventional therapies. OVs, in this case, are ideal candidates, as they can overcome the genetic complexity of GBM cell populations. A variety of OVs have been tested against GBM, and several clinical trials are currently ongoing to test them in patients [13]. MYXV has shown potent oncolytic activity in orthotopic preclinical glioma models in which human glioma xenografts were implanted [14]. MYXV was also able to efficiently infect and kill primary human gliomas obtained from surgical specimens cultured ex vivo [14]. In an immunocompetent rat model with Racine gliomas, MYXV, in combination with rapamycin, prolonged survival compared to MYXV alone. This enhanced effect of rapamycin was likely due to reduced type I IFN responses and the infiltration of NK cells and macrophages into MYXV-treated gliomas, suggesting that immunocompetent host modulation of the immune system is required for effective MYXV oncolytic virotherapy [15].

MYXV oncolytic virotherapy was tested against brain tumor-initiating cells (BTICs) for GBM [16]. BTICs were isolated from human gliomas that were cultured under neurosphere conditions and retained stem-cell-like properties. When both TMZ-resistant and -sensitive BTICs were tested, MYXV was able to infect and kill both types of BTIC lines [16]. MYXV infection and replication was further enhanced when BTIC cells were pretreated with rapamycin. The BTIC cell lines were used in a xenograft SCID mouse model. In this model, intratumoral delivery of MYXV, in combination with rapamycin, only provided long-term survival for some of the BTIC lines. This was correlated with the observations that, in the sensitive cells, MYXV infection decreased the expression of stem cell markers both in vitro and in vivo [16]. In contrast, MYXV showed modest activity against patient-derived, brain tumor-initiating cells (BTICs) [17]. In order to improve the oncolytic activity of MYXV against BTICs, a combination of chemotherapeutics with MYXV were tested. In this assay, a library of 73 compounds that are in clinical use or

preclinical development was screened, and led to the identification of additional compounds that worked synergistically with MYXV in vitro [17]. Among the different compounds that were tested, axitinib, rofecaxib and pemetrexed showed the highest effect against BTICs in combination with MYXV [17].

To further study the impact of MYXV virotherapy against malignant glioma, mouse glioma cell lines derived from C57BL/6J NPcis mice (*Trp53*$^{+/-}$/*Nfl*$^{+/-}$), which spontaneously developed high-grade gliomas and recapitulated many clinical phenotypes of the human disease, were tested in syngeneic C57BL/6J mice. These mouse glioma cell lines were susceptible to in vitro MYXV infection, replication and killing [18]. However, intracranial injection of MYXV in the orthotopically grafted mouse gliomas failed to result in viral replication or treatment efficacy, as virus replication was cleared within seven days post-delivery [18]. In order to find out whether type I IFN signaling is involved in the reduction in MYXV replication and activity, an IRF9 knockdown cell line that did not respond to IFN was tested in this model. However, even in this cell-line-derived glioma, the in vivo MYXV virotherapy was not improved, suggesting that an antiviral state independent of glioma IFNα/β signaling might function against MYXV [18]. To further enhance the oncolytic activity of MYXV, a modified MYXV strain lacking the anti-apoptotic M011L (vMyx-M011LKO) was tested against human and mouse BTICs. In both human and mouse BTICs, infection with vMyx-M011LKO virus significantly enhanced the killing of cells by the activation of caspase 3/7 [19]. Human BTICs were tested in xenograft NSG mouse (NOD scid gamma) lacking mature B and T cells. Interestingly, unlike xenograft human BTICs in SCID mice, in NSG mice both wild-type and M11LKO MYXV did not show any survival benefits [19]. This is possibly because SCID mice are not as immunocompromised as NSG mice. In order to test the oncolytic activity of M11LKO MYXV in an immunocompetent mouse model, murine BTIC line mBTIC0309 was engrafted in immunocompetent C57BL/6J mice. In this model, the treatment of murine BTICs with M11LKO MYXV prolonged survival of mice compared to identical cohorts with wild-type MYXV [19]. Again, using the same murine BTIC line in NSG mice, the survival benefit with this MYXV construct was lost, suggesting the critical importance of immune responses against the viral infection, in order to induce clearance of the tumor. Further, in this immunocompetent BTIC model, when TMZ was combined with M11LKO virus, there was further prolonged survival of mice with only a single dose of virus and a subsequent five doses of TMZ [19]. Again, this survival benefit was not observed in NSG mice. In immunocompetent mice, robust activation of caspase 3 and Ki-67 was observed in the brain sections collected from mice treated with a combination of M11LKO and TMZ. However, this activation of caspase was not observed with wild-type or control UV-inactivated virus, suggesting the importance of caspase activation for BTIC tumor clearance in an immunocompetent host.

4. Gallbladder Cancer

Gallbladder cancer (GBC) is rare but the most common biliary tract malignancy. The chances of curing gallbladder cancer are high if it is diagnosed at its earliest stage, but most gallbladder cancers are diagnosed late, after it spreads, due to a lack of early signs and symptoms. Surgery, radiation and chemotherapy are the most common types of treatment for GBC. However, metastatic gallbladder cancer is difficult to cure and the median survival for advanced stage cancer is less than a year [20]. Not many OVs have been tested against GBC. MYXV oncolytic activity has been assessed against human GBC cell lines in vitro and in vivo. MYXV infection of all the tested GBC

cell lines allowed viral replication and cell killing, which was further enhanced with the treatment of rapamycin [21]. The level of activated AKT is one of the determinants of MYXV tropism in these cell lines. In the CD-1 nude mice xenograft model, either MYXV alone or in combination with rapamycin did not show efficacy when compared to a xenograft of human glioma cell line [21]. It was found that, in this model, a higher expression of collagen IV in the GBC tumors physically blocked MYXV intratumoral distribution. Subsequently, it was found that hyaluronan enhanced MMP-9 expression and AKT activation, which eventually also increased the oncolytic activity of MYXV for GBC in vitro and provided a survival benefit for xenograft mice in vivo [21]. In another study, it was shown that, in the same xenograft tumor model, the systemic delivery of MYXV using bone-marrow-derived stem cells (BMSCs) enhanced the oncolytic activity of MYXV [22]. However, further studies will require an improvement in the clearance of the tumor and a long-term survival benefit. The development of the syngeneic GBC mouse model will further allow for testing the efficacy of oncolytic virotherapy.

5. Melanoma

Melanoma is the leading cause of death from skin disease. Failure to diagnose early may lead to the development of metastatic melanoma that spreads from the skin to other parts of the body, such as the lymph nodes, liver, lungs, bones and brain. Metastatic melanoma is much harder to treat and the average five year survival rate for stage 4 metastatic melanoma is only 15–20% [23]. Apart from surgery, radiation, immunotherapy or chemotherapy are used to treat metastatic melanoma. Various OVs have been tested against melanoma [24]. Currently, T-VEC is the only OV approved in the USA and Europe for the treatment of advanced melanoma. T-VEC is a recombinant HSV (type 1) that has been engineered to contain mutations in the viral proteins ICP34.5 and ICP 47, and also to express human GM-CSF [25]. Other OVs tested against melanoma are HSV-1 mutant HF-10, coxsackieviruses (CVA21), Reovirus, VACV and Adenovirus, which all have all been tested in patients under different phases of clinical trial. MYXV was assessed against melanoma using B16F10 mouse melanoma cells in a syngeneic immunocompetent mouse model [26]. MYXV, alone or combination with rapamycin treatment ex vivo, inhibited the development of lung metastasis [26]. MYXV was also tested in a metastatic melanoma brain tumor syngeneic mouse model. In this model, B16.SIY melanoma cells were infused intracranially in C57BL/6 mice. However, multiple injections of MYXV in combination with rapamycin and/or concurrent T-cell immunotherapy were required to improve the overall outcome [27]. This was based on the finding that the presence of cytotoxic lymphocytes in the tumor bed can improve cancer patients' survival and also decrease metastasis [27][28].

OVs can provide a unique platform to express immune-stimulating cytokines directly in the tumor bed. A recombinant MYXV-expressing murine, IL-15 (vMyx-IL-15), was tested in an immunocompetent melanoma model [29]. Intratumoral injection of this IL-15-armed MYXV in a subQ B16F10 melanoma model prolonged the survival of mice compared to the control, unarmed MYXV. Histological analysis of the tumor identified more inflammation in the tumor bed due to the infiltration of neutrophils. Since the co-expression of IL-15 with the α subunit of IL-15 receptor enhances the stability and bioactivity of IL-15, a new recombinant MYXV was constructed, expressing secreted IL-15Rα-IL-15 fusion protein. When this recombinant MYXV was tested in an immunocompetent B16F10 subQ model in wild-type or $RAG^{-/-}$ background mice, tumor growth was attenuated and prolonged the survival of

mice compared to the vMyx-IL-15 or unarmed MYXV [30]. Histological analysis of tumors in C57BL/6 mice showed strong a infiltration of both NK cells and CD8+ T cells in response to vMyx-IL-15Rα-IL-15 virus infection [30].

References

1. Yang, D.; Denny, S.K.; Greenside, P.G.; Chaikovsky, A.C.; Brady, J.J.; Ouadah, Y.; Granja, J.M.; Jahchan, N.S.; Lim, J.S.; Kwok, S.; et al. Intertumoral Heterogeneity in SCLC Is Influenced by the Cell Type of Origin. Cancer Discov. 2018, 8, 1316–1331.
2. Sun, A.; Durocher-Allen, L.D.; Ellis, P.M.; Ung, Y.C.; Goffin, J.R.; Ramchandar, K.; Darling, G. Initial management of small-cell lung cancer (limited- and extensive-stage) and the role of thoracic radiotherapy and first-line chemotherapy: A systematic review. Curr. Oncol. 2019, 26, e372–e384.
3. Armstrong, S.A.; Liu, S.V. Immune Checkpoint Inhibitors in Small Cell Lung Cancer: A Partially Realized Potential. Adv. Ther. 2019, 36, 1826–1832.
4. Dash, A.S.; Patel, M.R. Viroimmunotherapy of Thoracic Cancers. Biomedicines 2017, 5, 2.
5. Kellish, P.; Shabashvili, D.; Rahman, M.M.; Nawab, A.; Guijarro, M.V.; Zhang, M.; Cao, C.; Moussatche, N.; Boyle, T.; Antonia, S.; et al. Oncolytic virotherapy for small-cell lung cancer induces immune infiltration and prolongs survival. J. Clin. Investig. 2019, 129, 2279–2292.
6. Schaffer, B.E.; Park, K.S.; Yiu, G.; Conklin, J.F.; Lin, C.; Burkhart, D.L.; Karnezis, A.N.; Sweet-Cordero, E.A.; Sage, J. Loss of p130 accelerates tumor development in a mouse model for human small-cell lung carcinoma. Cancer Res. 2010, 70, 3877–3883.
7. de Queiroz, N.; Xia, T.; Konno, H.; Barber, G.N. Ovarian Cancer Cells Commonly Exhibit Defective STING Signaling Which Affects Sensitivity to Viral Oncolysis. Mol. Cancer Res. 2019, 17, 974–986.
8. Hoare, J.; Campbell, N.; Carapuca, E. Oncolytic virus immunotherapies in ovarian cancer: Moving beyond adenoviruses. Porto Biomed. J. 2018, 3, e7.
9. Correa, R.J.; Komar, M.; Tong, J.G.; Sivapragasam, M.; Rahman, M.M.; McFadden, G.; Dimattia, G.E.; Shepherd, T.G. Myxoma virus-mediated oncolysis of ascites-derived human ovarian cancer cells and spheroids is impacted by differential AKT activity. Gynecol. Oncol. 2012, 125, 441–450.
10. Tong, J.G.; Valdes, Y.R.; Barrett, J.W.; Bell, J.C.; Stojdl, D.; McFadden, G.; McCart, J.A.; DiMattia, G.E.; Shepherd, T.G. Evidence for differential viral oncolytic efficacy in an in vitro model of epithelial ovarian cancer metastasis. Mol. Ther. Oncolytics 2015, 2, 15013.
11. Nounamo, B.; Liem, J.; Cannon, M.; Liu, J. Myxoma Virus Optimizes Cisplatin for the Treatment of Ovarian Cancer In Vitro and in a Syngeneic Murine Dissemination Model. Mol. Ther. Oncolytics 2017, 6, 90–99.

12. Zhang, H.; Wang, R.; Yu, Y.; Liu, J.; Luo, T.; Fan, F. Glioblastoma Treatment Modalities besides Surgery. J. Cancer 2019, 10, 4793–4806.
13. Martikainen, M.; Essand, M. Virus-Based Immunotherapy of Glioblastoma. Cancers 2019, 11, 186.
14. Lun, X.; Yang, W.; Alain, T.; Shi, Z.Q.; Muzik, H.; Barrett, J.W.; McFadden, G.; Bell, J.; Hamilton, M.G.; Senger, D.L.; et al. Myxoma virus is a novel oncolytic virus with significant antitumor activity against experimental human gliomas. Cancer Res. 2005, 65, 9982–9990.
15. Lun, X.; Alain, T.; Zemp, F.J.; Zhou, H.; Rahman, M.M.; Hamilton, M.G.; McFadden, G.; Bell, J.; Senger, D.L.; Forsyth, P.A. Myxoma virus virotherapy for glioma in immunocompetent animal models: Optimizing administration routes and synergy with rapamycin. Cancer Res. 2010, 70, 598–608.
16. Zemp, F.J.; Lun, X.; McKenzie, B.A.; Zhou, H.; Maxwell, L.; Sun, B.; Kelly, J.J.; Stechishin, O.; Luchman, A.; Weiss, S.; et al. Treating brain tumor-initiating cells using a combination of myxoma virus and rapamycin. Neuro-Oncology 2013, 15, 904–920.
17. McKenzie, B.A.; Zemp, F.J.; Pisklakova, A.; Narendran, A.; McFadden, G.; Lun, X.; Kenchappa, R.S.; Kurz, E.U.; Forsyth, P.A. In vitro screen of a small molecule inhibitor drug library identifies multiple compounds that synergize with oncolytic myxoma virus against human brain tumor-initiating cells. Neuro-Oncology 2015, 17, 1086–1094.
18. Zemp, F.J.; McKenzie, B.A.; Lun, X.; Maxwell, L.; Reilly, K.M.; McFadden, G.; Yong, V.W.; Forsyth, P.A. Resistance to oncolytic myxoma virus therapy in nf1(-/-)/trp53(-/-) syngeneic mouse glioma models is independent of anti-viral type-I interferon. PLoS ONE 2013, 8, e65801.
19. Pisklakova, A.; McKenzie, B.; Zemp, F.; Lun, X.; Kenchappa, R.S.; Etame, A.B.; Rahman, M.M.; Reilly, K.; Pilon-Thomas, S.; McFadden, G.; et al. M011L-deficient oncolytic myxoma virus induces apoptosis in brain tumor-initiating cells and enhances survival in a novel immunocompetent mouse model of glioblastoma. Neuro-Oncol. 2016, 18, 1088–1098.
20. Rawla, P.; Sunkara, T.; Thandra, K.C.; Barsouk, A. Epidemiology of gallbladder cancer. Clin. Exp. Hepatol. 2019, 5, 93–102.
21. Weng, M.; Gong, W.; Ma, M.; Chu, B.; Qin, Y.; Zhang, M.; Lun, X.; McFadden, G.; Forsyth, P.; Yang, Y.; et al. Targeting gallbladder cancer: Oncolytic virotherapy with myxoma virus is enhanced by rapamycin in vitro and further improved by hyaluronan in vivo. Mol. Cancer 2014, 13, 82.
22. Weng, M.; Zhang, M.; Qin, Y.; Gong, W.; Tang, Z.; Quan, Z.; Wu, K. Targeting gallbladder carcinoma: Bone marrow-derived stem cells as therapeutic delivery vehicles of myxoma virus. Chin. Med. J. 2014, 127, 2350–2356.
23. Antohe, M.; Nedelcu, R.I.; Nichita, L.; Popp, C.G.; Cioplea, M.; Brinzea, A.; Hodorogea, A.; Calinescu, A.; Balaban, M.; Ion, D.A.; et al. Tumor infiltrating lymphocytes: The regulator of

melanoma evolution. Oncol. Lett. 2019, 17, 4155–4161.

24. Bayan, C.Y.; Lopez, A.T.; Gartrell, R.D.; Komatsubara, K.M.; Bogardus, M.; Rao, N.; Chen, C.; Hart, T.D.; Enzler, T.; Rizk, E.M.; et al. The Role of Oncolytic Viruses in the Treatment of Melanoma. Curr. Oncol. Rep. 2018, 20, 80.
25. Raman, S.S.; Hecht, J.R.; Chan, E. Talimogene laherparepvec: Review of its mechanism of action and clinical efficacy and safety. Immunotherapy 2019, 11, 705–723.
26. Stanford, M.M.; Shaban, M.; Barrett, J.W.; Werden, S.J.; Gilbert, P.A.; Bondy-Denomy, J.; Mackenzie, L.; Graham, K.C.; Chambers, A.F.; McFadden, G. Myxoma virus oncolysis of primary and metastatic B16F10 mouse tumors in vivo. Mol. Ther. J. Am. Soc. Gene Ther. 2008, 16, 52–59.
27. Thomas, D.L.; Doty, R.; Tosic, V.; Liu, J.; Kranz, D.M.; McFadden, G.; Macneill, A.L.; Roy, E.J. Myxoma virus combined with rapamycin treatment enhances adoptive T cell therapy for murine melanoma brain tumors. Cancer Immunol. Immunother. 2011, 60, 1461–1472.
28. Bonaventura, P.; Shekarian, T.; Alcazer, V.; Valladeau-Guilemond, J.; Valsesia-Wittmann, S.; Amigorena, S.; Caux, C.; Depil, S. Cold Tumors: A Therapeutic Challenge for Immunotherapy. Front. Immunol. 2019, 10, 168.
29. Doty, R.A.; Liu, J.; McFadden, G.; Roy, E.J.; MacNeill, A.L. Histological evaluation of intratumoral myxoma virus treatment in an immunocompetent mouse model of melanoma. Oncolytic Virotherapy 2013, 2, 1–17.
30. Tosic, V.; Thomas, D.L.; Kranz, D.M.; Liu, J.; McFadden, G.; Shisler, J.L.; MacNeill, A.L.; Roy, E.J. Myxoma virus expressing a fusion protein of interleukin-15 (IL15) and IL15 receptor alpha has enhanced antitumor activity. PLoS ONE 2014, 9, e109801.