6-28-2018

Tanapox: A potential oncolytic virus for the treatment of cancer

Joel Marty
Western Michigan University, joel.r.marty@gmail.com

Abstract

The National Cancer Institute defines cancer as a group of related diseases characterized by unimpeded cell division with metastatic abilities. Cells frequently mutate and potentially become cancerous, but generally these cells are cleared by the immune system. The disease state of cancer arises when the mutated cells evade the immune clearance, which indicates medical intervention is required. Current therapies most commonly utilize chemotherapy, radiation, or a combination of the two, however, alternative therapies are being developed such as immunotherapies and oncolytic viruses (OVs). OVs cause a viral infection, clearing cancerous cells through viral replication mechanisms, such as viral cytolysis, while simultaneously priming the adaptive immune system to better recognize and clear infected cancer cells. OVs are also capable of facilitating changes to the tumor microenvironment (TME), optimizing the immune response. Certain characteristics are required of OVs, such as a large population immunologically naïve to the infection, inability to cause serious disease in humans, and preferential replication in cancerous cells. Tanapoxvirus (TPV) is currently being investigated as an effective OV. Most of the world is immunologically naïve to the virus, which is isolated to the Tana River Valley in Kenya. The infection is defined as self-limiting, and resolves approximately six weeks post-infection independent of treatment. Preferential replication in cancerous cells is not innate to TPV; however, it can be induced by ablation of the *thymidine kinase* gene. The therapeutic benefits of TPV have been observed in *in vivo* nude mouse models utilizing colorectal cancer, triple negative breast cancer (TNBC), as well as melanoma. The TPV/Δ66R/fliC viral recombinant was effective in regression of induced colorectal cancer in a nude mouse model. The 66R ablation

is the *thymidine kinase* gene, which increases preferential replication within cancerous cells, and the *fliC* insertion will stimulate the innate immune response through interactions with Toll-like receptor 5. In TNBC both the TPV/Δ66R/CCL-2 and the TPV/Δ66R/m-IL2 recombinants showed significant regression in induced TNBC tumors on the nude mouse model. These mechanisms function by recruiting macrophages at the site of infection and priming the T cells for increased cancer cell recognition respectively. Finally the TPV/Δ15L recombinant was able to show regression in melanoma tumors in the nude mouse model by preventing the production of interferon-ϒ1 (INF-ϒ1). An in vitro cell assay was conducted and indicated that INF-ϒ1 asserts a greater antiproliferative effect than the type one INFs, so by preventing the production of this cytokine an increase efficiency of viral replication will be observed. Finally, the TPV infection is influenced by the TME through interactions with matrix metalloproteinase-9 (MMP-9). Interactions with MMP-9 prevents viral replication from occurring by an unknown mechanism, and preventing this interaction will increase the therapeutic effects TPV can assert. Future studies will observe the effects of TPV in an immunocompetent mouse model, as well as the potential of TPV as a treatment for pancreatic cancer. This research indicated that TPV has immense potential as an OV for treatment of multiple different types of cancers, and it's therapeutic effects should continue to be investigated.

Tanapox: A potential oncolytic virus for the treatment of cancer

Joel Marty

INTRODUCTION

Cancer is defined, by the National Cancer Institute, as a group of related diseases characterized by uninterrupted, invasive cell growth with the ability to invade other tissues in the body. The National Cancer Institute estimates that in 2018, there will be 1,735,350 new diagnoses of cancer with 609,640 cancer-related deaths in the United States alone. With cancer having such a significant impact, treatment options have been a focal point of medical research. Many different treatment models have been implemented, such as chemotherapy and radiation. However, recent developments have allowed for utilization of the human body, through the immune system, to clear these cancer cells. The immune system is responsible for clearing abnormal cells from the body; however, cancerous cells are able to evade the immune response. Capitalizing on the innate properties of the immune system, researchers have begun to develop new treatment techniques for cancer.

Our body has been imbued with the ability to fight off foreign pathogens, as well as the ability to recognize abnormal self-cells present within the body. The immune system can be divided into two different sections, the innate and the adaptive immune system. The innate immune system is the body's generalized defense to combat disease. No specific pathogen is being targeted by the innate immune system; instead conserved traits in many pathogens are targets for the innate response. For example, lysozyme naturally observed in tears is capable of lysing the cell wall of many different species of bacteria. This type of immune response allows for the body to combat a wide array of pathogens without the time required to produce specialized cell types, as observed in the adaptive immune response.

The adaptive immune response is the body's ability to target and clear specific pathogens from the body. The adaptive immune response can be broken into two divisions, humoral and cellular adaptive immunity. The humoral branch of the adaptive immune system is characterized by recognizing and tagging pathogens for clearance. B cells are the primary cell associated with this process. The presence of the pathogen will stimulate B cells to produce antibodies that will bind to one specific epitope on that specific pathogen. After the infection subsides some B cells will be converted to memory cells, which allows for faster recognition of the pathogen during subsequent infections. The cellular response of the adaptive immune system is facilitated by T cells, and these cells will interact with the antigens presented by other immune cells. This interaction will cause the release of cytolytic proteins, or cytokines to recruit other immune cells, depending on what type of T cell is involved in the interaction. Recent innovations have allowed for potential cancer therapies that utilize the immune response to assist in the clearance of cancerous cells.

Recently researchers have begun using viruses to activate the immune system to target cancer cells. Due to the ability of cancer cells to evade the immune clearing mechanism, they act as optimal sites of viral replication. This allows for the viral replication to proceed uninhibited, producing a larger number of virion particles that are capable of infecting surrounding cancerous cells. The viral infection also has the capability of increasing clearance of cancer cells by promoting interaction of the immune cells. This is facilitated by immune cells recognizing viral antigens present on the surface of infected cancer cells.

CANCER THERAPY THROUGH ONCOLYTIC VIRUSES (OVs)

Only viruses that meet an essential criteria are potential candidates to be utilized in effective oncolytic virotherapy. A crucial requirement of an effective OV is the ability for the virus to preferentially replicate within cancerous cells, while not infecting the healthy cells. This is referred to as the oncospecificity of the virus. Some viruses, such as some species of reovirus, have the innate ability to preferentially replicate within cancerous cells due to imperfections in the antiviral response of these cells (Conrad et al., 2015).

Other OVs can be genetically modified to increase the oncospecificity of the virus. For example, viruses with a DNA genome require phosphorylated nucleotides for replication, and ablation of the viral thymidine kinase gene will increase the oncospecificity of this type of virus. The thymidine kinase gene produces a protein that allows the virus to convert thymidine into a form suitable for incorporation to the viral progeny genome. Without this enzyme, the virus relies on the uptake of a usable thymine nucleotide from the environment for replication. Since cancerous cells are constantly undergoing replication, these cells have high levels of thymidine kinase activity; therefore, these cells will have a surplus of usable nucleotides. Thus, the viral particle will preferentially infect the cancerous cells for usable thymidine, which allows viral replication to occur. This preferential replication in cancerous cells due to thymidine requirement results in an increased oncospecificity of viruses with an ablated thymidine kinase gene (Twumasi et al., 2018).

Other intrinsic factors of a virus that influence its effectiveness as a potential therapeutic agent include mode of transmission, infection mechanisms, and prevalence of the virus. An ideal

OV will not be easily transmissible from the infected individual to others. Therefore, airborne pathogens are not ideal candidates, because of the ease with which other people could become infected. A second trait for an ideal OV is a self-limiting infection, implying a definitive end to the infection, and no incorporation of the viral genome into the host genome. Viral DNA incorporation into the host genome would indicate a persistent infection that would affect the patient for life.

Finally, an ideal characteristic of an OV, is that a majority of the target population for this therapeutic agent is immunologically naïve. The prevalence of the virus in a particular area will generally indirectly correlate with the proportion of the population that is naïve to the infection. OVs tend to originate from a highly centralized area so that a very limited population have been exposed to the viral infection. An individual who has never been exposed to the virus is considered immunologically naïve because the immune system has not interacted with this pathogen before. The naivety allows for the OV to have maximal therapeutic effect before it is cleared by the immune system. However, after a host is exposed to a pathogen, the adaptive immune system produces memory cells that are capable of identifying and clearing harmful matter from the body exponentially faster. This implies that there is a limited use for each OV in one individual, but one individual is capable of benefitting from the therapeutic effects with the use of multiple OVs.

An individual OV is not a cure for cancer, but instead is one option in a wide selection of potential treatments. The array of successful OVs that researchers have been developing come from many different virus families, including the poxvirus family (Chaurasiya et al., 2018). OVs

have been increasingly utilized as a potential treatment for cancer because these viruses are able to combat cancer through numerous modes of action.

Viral Cytolysis

Through natural and engineered processes, viruses can employ a variety of mechanisms to target cancer cells. The first mechanism being the naturally occurring cytolysis that is observed at the end of viral infections. Viral infections will enter either the lytic or lysogenic cycle. The lysogenic cycle is a period of time when the virus incorporates into the host genome, and will be considered dormant, not actively replicating. This incorporation into the genome allows for the virus to infect all of the daughter cells produced by the infected cell due to the incorporation of the viral DNA into the host's genome. Eventually the virus will exit the lysogenic cycle and enter the lytic cycle, which is characterized by active viral replication. Not all viruses are capable of entering the lysogenic cycle, but the lytic cycle can be observed in all viral infections. The end of the lytic cycle is notated by the release of the progeny virion particles from the host cell due to cell lysis. This is one of the innate mechanisms of viral infection that will allow for OVs to assist in clearing cancerous cells from the body.

Priming the Adaptive Immune System

Although the immune system has the power to vanquish tumor cells, cancer cells are able to evade the adaptive immune system. However, with OV therapy, cells infected with the virus display antigenic markers to notify the immune system that a viral infection is underway within the cell. The display of these antigens interacts with the adaptive immune cells and initiates the clearing of the infected cells. To increase the rate of clearance of the infected cells, different

transgenes, such as pro-apoptotic and immunostimulatory transgenes, can be introduced to the genome of an OV (Chaurasiya et al., 2018). The insertion of these transgenes will cause the gene product to be formed during viral replication. These protein products trigger cancer cell apoptosis or signal immune system activity, which is how these genes assert their effect during the infection. Activation of the adaptive immune system will also initiate the production and release of cytokines, which are able to recruit other immune cells to the site of infection. The production of cytokines attracts the T cells to intratumor locations, which has been associated with better health outcomes for the patient (Twumasi et al., 2018). To fully assert their effects, the adaptive immune cells must still overcome the immune suppression that is observed in the tumor microenvironment (TME) due to the tumor (Twumasi et al., 2018).

Changes to TME

OV infection will cause the secretion of inflammatory cytokines that are capable of TME remodeling and allow for the adaptive immune cells to function within the TME longer and cause more tumor necrosis (Martin and Bell, 2018). Aside from triggering the immune response, viruses also can evade the immune system, which allows for increase in replication time and a subsequent increased viral load. A larger viral load increases the number of free virus particles, released after cell lysis, that are able to infect surrounding tumor cells. As part of this process, the OV can utilize some of the immunosuppressive characteristics of the tumor to its benefit. Most cancerous cells do not respond to apoptotic signaling, which is beneficial to allow for maximal viral replication without premature cell clearance due to the immune response (Chaurasiya et al., 2018). Not only do cancerous cells create an immunosuppressive

environment, but they are also capable of evading adaptive immune responses by inhibiting the communication between cancerous cells and immune cells. Infection by some OVs can reverse this phenomenon, and allow for immune cells to better recognize cancerous cells and initiate clearing these cells from the body by presentation of viral antigens during infection (Twumasi et al., 2018). OVs are such a powerful tool because not only does the initial viral infection begins lysis of the tumor cells, but the virus also primes the adaptive immune system to increase the recognition and clearance rate of the cancerous cells.

TANAPOX VIRUS AS A POTENTIAL OV

The *Poxviridae* family is a very promising family of viruses to utilize for future oncolytic virotherapies. Poxviruses are stable, allowing for these viruses to freely travel in the blood to infect a wide array of cells, and initiate rapid cell lysis. Furthermore, poxviruses cause a self-limiting infection (Mundi et al, 2014). Poxviruses are also considered large genome viruses, which allows for more ablation and insertion of genes without interfering with genes responsible for viral replication within the cell. These genetic modifications can play many roles as noted earlier, but most importantly allow for an increase in oncospeceficity that is not native to the Poxvirus family (Conrad et al., 2015).

Tanapox virus (TPV), a species of the Poxvirus family, is a strong candidate as an OV partially due to the large proportion of the population of the world naïve to infection by this virus. The TPV species is located primarily in equatorial Africa, specifically to the Tana River Valley. In the 1970's, a study was conducted that indicated that TPV was isolated by comparing the serum

concentrations of anti-TPV antibody from individuals from the Tana River valley and Tanzania. The individuals from the Tana River valley showed varying concentrations of neutralizing antibodies present within their blood, whereas those from Tanzania had very little to no antibodies present (Manson-Bahr and Downie, 1973). This conclusion indicates that most of the population outside of the Tana River Valley will be immunologically naïve for the TPV infection. This trait appears to have persisted over time, and is still observed today. Only four known cases of TPV infection have occurred in the United States, most of which occurred in research labs utilizing the virus (Dhar et al., 2004). TPV infections are characterized by the formation of one or two small nodules, fever, backaches, and severe headaches; these symptoms resolve within a couple weeks (Dahr et al., 2004). TPV replication is limited to the location of the nodules, because of decreased replication efficiency in higher temperatures observed at the bodies core; this may be associated with the self-limiting characteristic of the TPV infection (Nazarian et al., 2007). Furthermore, TPV is transmitted through contact with an open lesion, and therefore will have limited ability to infect other individuals (Dahr et al., 2004). These characteristics make TPV a promising virus for OV therapy. The next steps in development of TPV as a therapeutic agent is the demonstration of the efficacy of TPV in tumor infection and size regression.

Tanapox Priming the Immune System

Specifically, TPV has been utilized in *in vivo* models of treatments for multiple cancer types. Significant tumor size reduction was observed in colorectal cancer, triple negative breast cancer, and melanoma using *in vivo* models treated with TPV recombinants. Since the poxviruses generally lack oncospecificity, to facilitate the infection of tumor cells, the abalation of the

thymidine kinase gene, 66R, was performed for recombinant TPV strains. For a colorectal cancer study conducted by Conrad et al. (2015), the 2L gene was also ablated as this gene plays a role in reducing inflammation and facilitates evasion of the immune response by infected cells. With the 2L gene ablated, inflammation is observed at the site of infection, as well as an increase in recognition of infected cells by the adaptive immune system. In attempts to increase the clearance of the cancerous cells, the *fliC* transgene was introduced to the TPV genome, producing a Δ66R/Δ2L/fliC strain of the TPV. The *fliC* gene codes for the bacterial flagellin protein, which will be produced during the viral replication process. The flagellin protein will interact with Toll-like receptor 5 (TLR 5), a portion of the innate immune system. When activated, TLR 5 causes inflammation and activation of the innate immune response.

For experiments of the efficacy for colorectal cancer, the colorectal cell line HCT 116 was selected due to the ability for the TPV recombinants to efficiently replicate within this cell line. HCT 116 tumors were induced in nude mice, which lack a functional adaptive immune system. These mice were deemed sufficient models for the TPV/Δ66R/Δ2L/fliC because the *fliC* protein activates the innate immune system, which is still fully functional in nude mice. Once the HCT 116 tumors grew to a specified size, the mice received intratumor injections of either the vehicle, or a TPV recombinant. The TPV/Δ66R, TPV/Δ2L, and TPV/Δ2L/Δ66R/fliC strains all displayed significant regression in tumor size over the study. However, only the TPV/Δ2L/Δ66R/fliC virus was able to show consistent significant reduction in tumor size, at multiple time points; this effect was not observed in the other two recombinant viruses. Therefore, this study suggests that the TPV/Δ2L/Δ66R/fliC strain was more effective, and able to reduce tumor size by two mechanisms.

The first mechanism is the innate cytolytic processes associated with viral replication, which caused cell cytolysis. The second is activation of the innate immune response by interactions of the viral flagellin protein with the host TLR 5.

TPV recombinants have also been effective in targeting the highly aggressive triple negative breast cancer (TBNC) cells. Effective treatment options for TBNC have been difficult to identify due to the absence of estrogen and progesterone receptors, which are prime targets for traditional breast cancer therapies. However, TPV recombinants have been shown to cause cancer cell death in MDA-MB231 cells, a TNBC cell line. In a study conducted by Suryawanashi et al. (2017), the TPV recombinants had the 66R gene ablated and had either an insertion of the m-CCL2 or m-IL2 transgenes into the viral genome. CCL2 is a chemotactic chemokine that will recruit immune cells, such as macrophages and dendritic cells, to the site of the tumor. However, apparent species differences are observed between the human and mouse strain of the CCL2 gene. The human strain of the CCL-2 chemokine has been observed to promote tumor growth and survival. This differs from the mouse *CCL-2* gene, which was incorporated into the TPV genome, and displayed no effect of prolonging the life span of *in vitro* MDA-MB231 cells. The second cytokine is IL-2, which possesses dual functionality. IL-2 acts as a T cell growth factor, initiating T cell maturation, in addition to activating tumor targeting macrophages. Based on these properties, both the TPV/Δ66R/m-CCL2 and the TPV/Δ66R/m-IL2 recombinants were produced to determine their efficiency in clearing the MDA-MB231 cells.

Both *in vitro* and *in vivo* studies were completed with these two TPV recombinants to determine the efficacy of TPV as a treatment for TNBC. First, the *in vitro* infection efficiency was

observed to determine which cell line would be utilized for the *in vivo* study as well as the role of m-CCL2 on tumor growth and survival. The MDA-MB231 cell line was selected due to the observation of efficient replication of TPV recombinants within these cells. Also, as stated earlier, the study determined that m-CCL2 does not promote cancer cell survival. MDA-MB231 tumors were induced in nude mice for the *in vivo* study to determine TPV recombinant functionality. The *in vivo* model is only capable of evaluating the viral cytolytic mechanism of cancerous cell death, because it lacks the functionality of the adaptive immune system. Both m-CCL2 and m-IL2 work by activating the adaptive immune system; therefore, the observed cell lysis must be attributed to the viral replication mechanism. As designed, both recombinant strains of the TPV, TPV/Δ66R/m-CCL2 and TPV/Δ66R/m-IL2, produced the desired effect to cause consistent and significant tumor regression in the induced MDA-MB231 tumors. To determine the role of the immunostimulatory transgenes, a follow up study will have to be conducted. This study will require the utilization of a model that will allow for tumor induction in mice, without rejection, while still having an intact adaptive immune system. Since both of these genes would increase the adaptive immune response, it can be hypothesized that an even larger increase in tumor regression would be observed in these new models.

However, not all recombinant viruses require the introduction of a transgene to increase the oncolytic activity. Sometimes removal of a viral gene can induce tumor disrupting activity. In a study conducted by Zhang et al. (2017) a, TPV recombinants with the ablated *15L* gene have been shown to significantly increase tumor regression in melanoma tumors induced in nude mice without the insertion of a transgene. The *15L* gene product mimics the neuregulin protein that

interacts with receptors in the ERB receptor kinase family. The activation of these receptors initiates a signal transduction pathway that results in the promotion of both DNA synthesis and cell division. The increase in cell division due to the 15L gene product, appears to promote increases in melanoma tumor size. However ablation of the 15L gene prevents the gene product from being produced during viral infection while also reducing the amount of cell division occurring during the infection. The ablation of the 15L gene is associated with increased interferon-ϒ1 (INF-ϒ1) production. Some types of INF have been observed to prevent proliferative pathways in cancerous cells. The ablation of the 15L gene not only reduces proliferation on its own, but the associated increase in INF-ϒ1 production further prevents cell division.

Two independent experiments were conducted to determine the effect of the 15L ablation during a TPV infection of the SK-MEL-3 strain of melanoma, as well as the role of INF-ϒ1 on cell proliferation. To examine the effects of the 15L gene ablation, an *in vitro* experiment was conducted to determine in which cell line the TPV best replicated. The SK-MEL-3 melanoma cell line was determined to be best suited for efficient replication and therefore, these cells were utilized to induce tumors in nude mice. The SK-MEL-3 tumors were then treated with wild type TPV, TPV/Δ66R, TPV/Δ15L, or TPV/Δ15L/Δ66R. Both viral strains were able to show significant regressions in tumor size, the Δ66R and Δ15L single knockout viruses. However, only the Δ15L virus was able to show a consistent significant regression at multiple time points. The TPV/Δ15L/Δ66R recombinant did not show any ability to significantly reduce the size of the tumor. It is hypothesized that the ineffectiveness of the double knockout can be attributed to

the increased replication time that was observed during the *in vitro* portion of this experiment. The increase in replication time allows for fewer viral particles to be produced due to the slower replication rate.

A second study was conducted by Zhang et al (2017) b, to determine the influence of secondary infection components, such as INF-ϒ1, on the proliferation of melanoma cells. The experiment analyzed the role of INF-ϒ1 and compared its effects to type one INFs, INF-α and INF-β. INFs act as a mechanism of antiviral immune defense and will be produced by a virally infected cell to act as a warning mechanism of viral presence. As stated earlier, the TPV/Δ15L infection increases the amount of INF-ϒ1 that is produced, which is less common and not as well understood as the type one INFs. Type one interferons, α and β, have shown to decrease the proliferation of melanoma cells *in vitro*. To determine the antiproliferative effects of INF-ϒ1, an *in vitro* experiment was conducted where SK-MEL-3 cells were grown in culture with either INF-ϒ1, INF-α, or INF-β. Observations from the study indicated that INF-ϒ1 was more effective at preventing SK-MEL-3 cell division than either INF-α or INF-β. A follow up study was conducted to determine whether INF-ϒ1 was the factor responsible for the reduction in cell proliferation. SK-MEL-3 cultures were infected with the TPV/Δ15L virus, so that INF-ϒ1 would be produced by the cells. After the allotted amount of time, anti-INF-ϒ1 antibody was introduced to neutralize the INF to determine its effects on cell growth. After the addition of anti-INF-ϒ1 antibody, there was an increased rate of cell survival and proliferation that was not observed during the initial TPV/Δ15L infection. This experiment confirms the role of INF-ϒ1 in limiting tumor cell division.

Tanapox Altering the TME

OVs not only influence intracellular pathways, as has been noted thus far, but OVs also are capable of interacting and altering the TME (Martin & Bell, 2018). Matrix metalloproteinase-9 (MMP-9) is one component of the extracellular matrix found in the TME. The presence of MMP-9 in melanoma indicates a poor prognosis, because the protein is associated with increased tumor growth and metastasis. MMP-9 has the ability to inhibiting the viral infection, as observed during TPV infections; however, the exact mechanism has yet to be determined. To determine the impact that MMP-9 has on a TPV infection, an *in vitro* study was conducted. SK-MEL-3 cells were infected with TPV with the fluorescent protein GFP (TPV/egfp), and grown in medium with varying concentrations of the MMP-9 protein. It was shown that the presence of the MMP-9 protein is capable of dramatically reducing the amount of viral replication within these melanoma cells by reducing the amount of fluorescence associated with the infection.

A follow-up study was conducted by Zhang and Essani (2017), to confirm the role of MMP-9 in inhibition of viral replication. The study was repeated exactly as before, however an anti-MMP-9 antibody was introduced at varying concentrations. The addition of anti-MMP-9 antibody neutralized the MMP-9 protein and prevented its native effects on the viral infection. As expected, the addition of the antibody allowed for a significant increase in viral replication, which was observed as a correlated increase in GFP fluorescence. Once again, this confirms the role of MMP-9 in inhibiting viral replication.

There are several proposed anti-viral mechanisms for MMP-9. These include the potential of the protein to cleave proteins necessary for the virus to enter the cell, prevention of virus attachment to the cell, or alteration in the signaling process that occurs during TPV

replication. The decrease in the TPV replication in the presence of MMP-9 is positively correlated with a decrease in the amount of INF secreted by these cells. It is hypothesized that the upregulation of MMP-9 inactivates the production of the INF cytokines, and therefore acts as a protective factor for melanoma cells by allowing for uninhibited tumor cell replication. These examples illustrate that the interaction of the OV and the TME is crucial in determining the therapeutic effectiveness of an OV.

Conclusion

TPV has been associated with a regression in tumor size for a variety of different cancerous cell lines through a different mechanisms. The amount of variance in treatment strategies with TPV indicate strong potential as a future OV. This variance is associated with the fact that the TPV genome is quite large, allowing for transgene insertion, and creating multiple different potential mechanisms for tumor clearance. In tandem with the large portion of the world's population that is immunologically naïve, the therapeutic potential of TPV warrants further investigation. Due to the difficulty in treating cancer and the limited potential of a single OV as a treatment, TPV could be a great addition to the collection of OVs that are being utilized to combat cancer. Future experiments will utilize the TPV recombinant viruses for treatment of tumors induced in immunocompetent models to observe the effects of the immunostimulatory transgenes.

References

Chaurasiya, S., Chen, N.G.,Warner, S.G. (2018). Oncolytic virotherapy versus cancer stem cells: a review of approaches and mechanisms. *Cancers 10,* (124). doi:10.3390/cancers10040124

Conrad, S.J., El-Aswad, M., Kurban, E., Jeng, D., Tripp, B.C., Nutting, C., Eversole, R., Mackenzie, C., Essani, K. (2015). Oncolytic tanapox virus expressing FliC causes regression of human colorectal cancer xenografts in nude mice. *Journal of Experimental & Clinical Cancer Research*, 34 (19). DOI 10.1186/s13046-015-0131-z

Dhar, D.A., Werchniak, A.E., Li, Y., Brennick, J.B., Goldsmith, C.S., Kline, R., Damon, I., Klaus, S.N. (2004). Tanapox infection in a college student. *The New England Journal of Medicine*, 350 (4) 361-366.

Manson-Bahr, P.E.C., Downie, A.W. (1973). Persistence of Tanapox in Tana River Valley. *British Medical Journal 2,* 151-153.

Martin, N.T., Bell, J.C. (2018). Oncolytic virus combination therapy: killing one bird with two stones. *Molecular Therapy 26,* (6). https://doi.org/10.1016/j.ymthe.2018.04.001

Mundi, N., Um, S., Yoo, J., Rizzo, G., Black, M., Pinto, N., Palma, D.A., Fung, K., MacNeil, D., Mymryk, J.S., Barrett, J.W., Nichols, A.C. (2014). The control of anaplastic thyroid carcinoma cell lines by oncolytic poxviruses. *Virus Research 190,* 53-59. http://dx.doi.org/10.1016/j.virusres.2014.07.009

Nazarian, S.H., Barrett, J.W., Stanford, M.M., Johnston, J.B., Essani, K., McFadden, G. (2007). Tropism of *Tanapox virus* infection in primary human cells. *Virology 368,* 32-40. doi:10.1016/j.virol.2007.06.019

Suryawanashi, Y.R., Zhang, T., Woyczesczyk, H.M., Christie, J., Byers, E., Kohler, S., Eversole, R., Mackenzie, C., Essani, K. (2017). T-independent response mediated by oncolytic tanapox virus recombinants expressing interleukin-2 and monocyte chemoattractant protein-1 suppresses human triple negative breast tumors. *Med Oncology 34,* (112). DOI 10.1007/s12032-017-0973-7

Twumasi-Boateng, K., Pettigrew, J.L., Kwok, Y.Y.E., Bell, J.C., Nelson, B.H. (2018). Oncolytic viruses as engineering platforms for combination immunotherapy. *Nat Rev Cancer.* doi: 10.1038/s41568-018-0009-4.

Zhang,T., Essani, K. (2017). Tanapox virus lacking the 15L gene inhibits melanoma cell growth in vitro by inducing interferon-λ1 release. *Virus Genes 53,* 477-482. DOI 10.1007/s11262-017-1434-2 201

Zhang, T., Suryawanshi, Y.R., Kordish, D.H., Woyczesczyk, H.M., Jeng, D., Essani, K. (2017) (a). Tanapox virus lacking a neuregulin-like gene regresses human melanoma tumors in nude mice. *Virus Genes 53,* (1) 52-62. doi:10.1007/s11262-016-1402-2.

Zhang, T., Suryawanshi, Y.R., Szymczyna, B.R., Essani, K. (2017) (b). Neutralization of matrix metalloproteinase-9 potentially enhances oncolytic efficacy of tanapox virus for melanoma therapy. *Med Oncology 34,* (129). DOI 10.1007/s12032-017-0988-0