

Promising oncolytic agents for metastatic breast cancer treatment

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Abstract: New therapies for metastatic breast cancer patients are urgently needed. The long-term survival rates remain unacceptably low for patients with recurrent disease or disseminated metastases. In addition, existing therapies often cause a variety of debilitating side effects that severely impact quality of life. Oncolytic viruses constitute a developing therapeutic modality in which interest continues to build due to their ability to spare normal tissue while selectively destroying tumor cells. A number of different viruses have been used to develop oncolytic agents for breast cancer, including herpes simplex virus, adenovirus, vaccinia virus, measles virus, reovirus, and others. In general, clinical trials for several cancers have demonstrated excellent safety records and evidence of efficacy. However, the impressive tumor responses often observed in preclinical studies have yet to be realized in the clinic. In order for the promise of oncolytic virotherapy to be fully realized for breast cancer patients, effectiveness must be demonstrated in metastatic disease. This review provides a summary of oncolytic virotherapy strategies being developed to target metastatic breast cancer.

Keywords: oncolytic virus, virotherapy, breast cancer, metastasis

Introduction

Metastatic breast cancer is currently incurable. Advances in detection and treatment have improved the overall outlook for breast cancer patients in recent decades. Survival rates approach 100% when the disease is localized at diagnosis but are <25% for patients with distant metastases.¹ Despite continued improvements in patient care, survival rates have not changed appreciably in the past 20 years. It is therefore evident that metastasis to vital organs remains the key limitation in the clinical management of breast cancer. Currently used therapies can cause debilitating side effects and are limited in their effectiveness against metastases. This point is illustrated by two recent studies showing an increased long-term risk of heart disease in breast cancer patients treated with standard therapies.^{2,3} Additionally, patients aggressively treated with chemotherapy often suffer cognitive impairments sometimes referred to as “chemo brain”.^{4,5} Treatment of patients with metastatic disease is usually palliative, as aggressive tumors acquire resistance to established therapies. Therefore, there is a dire need for therapies that avoid risks of long-term toxicities and eliminate, or at least control, metastatic disease. Recently, it has been proposed that the current system of clinical trials for metastasis be revised to allow earlier interventions of combinatorial therapies.⁶ This view reflects the current prevailing notion that metastasis may be preventable if critical points along the metastatic cascade can be blocked. While prevention strategies are a worthy pursuit, the fact remains that these strategies do not offer benefits to

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patients with existing metastases. Tumor heterogeneity and the complexity of the tumor microenvironment mean that a “magic bullet” cure for breast cancer is unlikely. Rather, it is becoming increasingly clear that combinations of therapies will be necessary to yield therapeutic success.

Oncolytic viruses hold promise as potential therapeutics.⁷ A tumor-destroying (oncolytic) virus is naturally selective or genetically engineered to selectively replicate in and destroy tumor cells while sparing normal cells.⁸ During transformation, tumor cells acquire a variety of defects that make them particularly susceptible to oncolytic viruses, including overexpression of certain cell surface antigens, deficiencies in the interferon response, resistance to apoptosis, and increases in nucleotide synthesis, protein synthesis, and cell cycling pathways.⁹ Through multiple cycles of tumor cell infection, lysis, and spread to adjacent cells, an oncolytic virus has the potential to spread throughout and destroy an entire tumor. An oncolytic virus is perhaps an ideal therapeutic strategy for metastatic cancer due to a multimodal mechanism of action. Oncolytic viruses can directly destroy tumor cells, destroy tumor vasculature, and provoke antitumor immune responses. Moreover, oncolytic viruses can be “armed” with anticancer transgenes to enhance efficacy or can be combined with existing therapies such as small molecule inhibitors, chemotherapy, or radiation. Though the concept of treating cancer with viruses is decades old, it is only in recent years that advances in genetic engineering have allowed the field to truly mature.¹⁰ A diverse array of oncolytic viral platforms has been described in the literature, and this is an active area of investigation for breast cancer and other tumor types.^{7,8} Those most commonly used as oncolytic viruses include both DNA viruses (adenovirus, herpes simplex virus [HSV], and vaccinia virus) and RNA viruses (measles virus, reovirus, and Newcastle disease virus [NDV]). A literature search for “oncolytic virus” reveals that the annual number of publications in this field has approximately tripled over the past decade, highlighting the increasing interest in this potential treatment. In the context of this interest, it has been suggested that oncolytic virotherapy may come to represent a “fourth front” in the war against cancer, to complement the established treatment modalities of surgery, chemotherapy, and radiation.¹¹

Armed oncolytic viruses

A variety of viral types have shown an ability to replicate in breast cancer cells and suppress tumor growth *in vivo* by viral replication alone. However, many investigators have sought to enhance the efficacy of an oncolytic viral platform

by “arming” the virus with anticancer transgenes targeting directly to cancer cells or the tumor microenvironment. To date, these efforts have largely involved the application of transgenes with broad anticancer activity rather than being specific to breast cancer. Factors chosen to target the tumor microenvironment have been shown to be advantageous in several settings. Gholami et al¹² recently described a vaccinia virus that expresses a single chain antibody against vascular endothelial growth factor (VEGF). Due to the resultant antiangiogenic ability, this virus was shown to exhibit higher antitumor activity than a control virus in a murine model of triple-negative breast cancer. The oncolytic adenovirus AdEHE2F expresses two inhibitors of angiogenesis: soluble Flt-1 to inhibit VEGF receptor activity and a soluble extracellular domain of Delta-like 4 to inhibit Notch signaling and thus limit endothelial maturation. This virus more effectively suppressed tumor growth in two flank tumor models and extended survival versus a control virus, and this effect was attributed to both oncolysis and disruption of tumor vasculature.¹³ Gil et al¹⁴ describe an oncolytic vaccinia virus that also inhibits angiogenesis by targeting CXCR4. The microenvironment can also be targeted in a site-specific manner. In a series of studies, it has been shown that targeting transforming growth factor-beta signaling enhances the ability of oncolytic adenovirus to limit the progression of bone metastases.^{15–18} Similarly, an adenovirus expressing soluble osteoprotegerin fused to the Fc portion of immunoglobulin G (IgG) is also effective at limiting progression of bone metastases.¹⁹

Another strategy is to enhance viral efficacy by arming the oncolytic platform with immune-stimulating factors. An oncolytic HSV (oHSV) armed with interleukin-12 (IL-12) more effectively inhibited the growth of breast cancer brain metastases than an unarmed control oHSV.²⁰ Another oHSV, armed with 15-prostaglandin dehydrogenase, reduced tumor growth and suppressed metastasis development by promoting antitumor immunity in an immunocompetent model.²¹ Enhanced tumor control has also been described for oncolytic adenoviruses armed with IL-24 and with CD40 ligand.^{22,23} Other immune-stimulating factors used to arm oncolytic viruses include IL-2 in an oncolytic NDV²⁴ and an oncolytic measles virus armed with *Helicobacter pylori* neutrophil-activating protein.²⁵

Several investigators have sought to enhance oncolytic virus potency by arming viruses with factors intended to either increase viral replication or enhance killing of infected cells. An oHSV armed with inhibitor of growth 4 exhibited enhanced replication in breast cancer cells *in vivo*.²⁶ An oncolytic adenovirus armed with tumor necrosis

factor-related apoptosis-inducing ligand (TRAIL) was shown to be effective against triple-negative breast cancer cells both in vitro and in vivo.²⁷ Other oncolytic adenoviruses have been armed with factors for modulating or targeting cellular stress responses, such as p53,²⁸ heat shock transcription factor 1,²⁹ and mortalin.³⁰ Finally, genes for prodrug-converting enzymes have been used to arm several oncolytic viruses used in breast cancer studies, including vesicular stomatitis virus (VSV),³¹ vaccinia virus,³² and adenovirus.³³ Overall, these studies demonstrate that the efficacy of an oncolytic platform can be made more potent by the inclusion of an anticancer transgene.

Combination therapies

Oncolytic viruses have been used in a number of combination therapeutic strategies to increase their effectiveness against breast cancer. Of particular interest are studies in which virotherapy has been combined with agents that have already been used in breast cancer patients. The microtubule-targeting chemotherapeutic agent paclitaxel was shown to increase viral uptake and cytotoxicity of an IL-24-expressing adenovirus, without altering viral replication.²² Similarly, paclitaxel in combination with the oHSV G47Δ led to increased tumor cell apoptosis without changes in viral replication, and this yielded a synergistic inhibition of tumor growth in vivo.³⁴ In another study, paclitaxel was used in a regimen to induce tumor cell senescence and was combined with oncolytic measles virus; this combination more effectively mediated growth inhibition of breast cancer cells than either treatment alone.³⁵ Another chemotherapeutic agent, doxorubicin, was used in combination with a Type-2 oHSV to yield enhanced tumor growth suppression in a subcutaneous syngeneic model³⁶ and in combination with coxsackievirus A21.³⁷ Bevacizumab, a monoclonal antibody targeted against VEGF, has been used in breast cancer patients with mixed results. However, a combination therapy of the oHSV HF10 with bevacizumab yielded synergistic antitumor activity in a preclinical model.³⁸

Several agents that have been used in clinical trials for breast cancer have also been demonstrated to enhance oncolytic virotherapy. Inhibitors of histone deacetylase (HDAC) enzymes can have multiple antitumor effects and are currently being investigated in clinical trials for breast cancer and other tumor types.³⁹ HDAC inhibitors have been shown to suppress the interferon-mediated antiviral response⁴⁰ and thus have attracted attention as a potential combination for virotherapy with oHSV.^{41–43} In accordance with these earlier studies, it has been shown that HDAC inhibitors increase oHSV replication in a panel of breast cancer cell lines but

do not alter replication in normal breast epithelial cells, an effect that was attributed to inhibition of Class I HDACs in particular.⁴⁴ Inhibitors of heat shock proteins (HSPs) are also in clinical trials as cancer therapeutics.⁴⁵ HSP inhibition has been shown to enhance the cytopathic effect of an oncolytic measles virus in breast cancer cells without altering toxicity in normal cells.⁴⁶ Combination of an oHSV with the chemotherapeutic drug mitoxantrone yielded enhanced survival in an immunocompetent model by enhancing the immunogenicity of the dying tumor cells and increasing the infiltration of neutrophils and CD8+ T cells into treated tumors.⁴⁷ Sunitinib is a receptor tyrosine kinase inhibitor that targets multiple intracellular pathways. In a study by Jha et al,⁴⁸ the combination of sunitinib and oncolytic VSV led to the complete elimination of flank tumors in a syngeneic immunocompetent model.⁴⁸ The results of this study further suggested that the enhanced effect was a result of the suppression of innate immune pathways by sunitinib. Thalidomide, which has been investigated for anticancer properties as a monotherapy,⁴⁹ was used in combination with a fusogenic oHSV to enhance suppression of tumor growth and metastasis to the lungs in an immunocompetent model.⁵⁰

A number of other agents in clinical use for other disease processes have nonetheless proved useful for improving the potency of oncolytic viruses against breast cancer. The vaccinia virus GLV-1h153 expresses a transgene for the human sodium iodide symporter. In an orthotopic model of triple-negative breast cancer, a combination therapy of the radionuclide ¹³¹I and GLV-1h153 was shown to increase tumor regression six fold versus the virus-only treatment group.⁵¹ In addition to this therapeutic approach, the same virus was demonstrated via positron emission tomography to be useful in combination with ¹²⁴I in identifying and controlling residual tumor at surgical margins, in an orthotopic tumor model.⁵² The prodrug 5-fluorocytosine used in combination with an oncolytic VSV expressing a cytosine deaminase/uracil phosphoribosyltransferase “suicide” transgene has been shown to enhance antitumor activity and improve survival of tumor-bearing mice.³¹ Finally, the hypertension drug losartan has been shown to inhibit collagen I synthesis from mammary carcinoma-associated fibroblasts in vitro and improve intratumoral spread of oHSV by reducing tumor fibrosis (results obtained in non-breast cancer models but may imply effectiveness in a breast cancer setting as well).⁵³

Other investigators have demonstrated proof of principle that agents with potential clinical application may be of use in future combination strategies with oncolytic virotherapy. In particular, combination therapies that target

components of the cytoskeleton can enhance the efficacy of certain oncolytic viruses. A small molecule inhibitor (Y27632) of Rho signaling enhanced measles virus replication in breast cancer cells both *in vitro* and *in vivo*, in a flank xenograft model.⁵⁴ Irwin et al⁵⁵ demonstrated that cell-to-cell spread of myxoma virus can be similarly enhanced by disrupting the cortical actin cytoskeleton, as shown both by chemical inhibition and by the generation of a mutant myxoma virus expressing the vaccinia virus F11 protein. Accordingly, the mutant virus exerted greater tumor growth inhibition in an orthotopic model versus the unarmed control virus, in both injected and uninjected tumors. Other investigators have shown that replication of oHSV can be enhanced *in vitro* by inhibitors of MEK⁵⁶ or by inhibitors of caspases.⁵⁷

In total, the combination strategies summarized here illustrate the point that the antitumor efficacy of oncolytic viruses can be enhanced by a variety of other agents. However, caution must be taken to not interpret these findings too broadly. Whether or not a particular drug will enhance virotherapy for breast cancer must be carefully tested on a case-by-case basis. Studies of combination strategies in other cancers indicate that the effectiveness of a combination depends upon the cell line, the virus, the relative timing of each agent, and the mechanism of action of the compound. Comprehensive reviews of oncolytic viruses in combination therapies for cancer in general are given elsewhere,^{58–62} with a few examples of these complex interactions provided here. For instance, the combination of temozolomide and oHSV was synergistic in certain glioma cell lines but antagonistic in others.⁶³ In thyroid cancer cells, paclitaxel enhanced oHSV cell killing, while doxorubicin mediated either additive or antagonistic effects.⁶⁴ Moreover, whether or not a compound enhances viral replication *in vitro* does not always reflect whether or not antitumor efficacy *in vivo* is enhanced. In a pancreatic cancer model, gemcitabine inhibited the replication of two different oHSVs *in vitro* but nonetheless increased cytotoxicity overall. However, *in vivo*, the combination enhanced antitumor activity of one virus but decreased that of the other.⁶⁵ In pancreatic cancer cells, 5-fluorouracil inhibited oncolysis mediated by wild-type HSV but enhanced oncolysis of the oHSV NV1066.^{66,67} For breast cancer, it may be that the most effective compounds for enhancing virotherapy are yet to be identified. A high-throughput screen of compounds in the murine mammary carcinoma line 4T1 identified a novel compound that enhanced VSV replication in this otherwise-VSV-resistant cell line.⁶⁸ A similar approach might be taken to identify compounds that enhance the effectiveness of other oncolytic viruses as well.

Systemic efficacy

The key challenge in the treatment of breast cancer is the targeted destruction of disseminated metastases. Initial preclinical virotherapy studies have generally involved local or regional delivery of oncolytic agents to individual tumor sites, an approach that would obviously not be ideal for situations in which patients presented with multiple or inaccessible lesions. Despite the challenges associated with systemic delivery of oncolytic viruses (liver uptake, binding to and inactivation by serum factors, etc), the potential utility of this approach has been demonstrated for several viral platforms, both in terms of systemic delivery and the ability of locally delivered viruses to mediate systemic antitumor effects. Clinical application of this approach has shown it to be generally well tolerated in patients with multiple tumor types.⁶⁹

Intravenous (IV) injection has been used to deliver several types of oncolytic viruses in breast cancer-targeted studies, including both viruses with native tropism and those modified for the purposes of targeting. Proof of principle validating IV delivery as a viable option for metastasis therapy has been shown in studies in which viruses are systemically delivered to primary breast tumors. IV delivery to orthotopic xenografts has been shown with both native and capsid-modified oncolytic adenoviruses.^{70,71} Jing et al⁷² described a measles virus targeted to the urokinase-type plasminogen activator receptor (uPAR). IV delivery of this virus mediated tumor growth suppression and extended survival in an orthotopic xenograft model. Oncolytic vaccinia virus can also be delivered IV to suppress tumor growth, as shown using the virus GLV-1h68 in a flank tumor model.⁷³ In subsequent studies, the same virus eradicated orthotopic xenograft tumors when delivered IV⁷⁴ or by retro-orbital injection to orthotopic tumors derived from breast cancer stem-like cells.⁷⁵ Another vaccinia virus, GLV-1h153, eliminated primary tumors when delivered intratumorally or IV, and prevented the development of systemic metastases in a model of triple-negative breast cancer.⁷⁶ As impressive as these results are, still more encouraging are studies in which oncolytic viruses have reduced the growth of metastases⁷⁷ or have been delivered to established systemic metastases. Iankov et al⁷⁸ report the delivery of a nontargeted oncolytic measles virus to pleural metastases by both direct intrapleural administration and IV administration, improving survival in treated mice. The uPAR-targeted measles virus mentioned above has also been used against systemic metastases, as survival of tumor-bearing mice was extended by the IV delivery of this virus in both syngeneic immunocompetent and xenograft models of lung metastasis.⁷⁹

Several other oncolytic viruses have demonstrated systemic efficacy against lung metastases. IV delivery of a TRAIL-armed adenovirus more effectively suppressed the growth of established lung metastases and led to extended survival in a xenograft model versus a control virus that lacked TRAIL,²⁷ and similar results have been observed with IV delivery of an IL-24-expressing adenovirus.⁸⁰ Fernandez et al⁸¹ demonstrated efficacy against established lung metastases, including improved survival, with both an IL-4-expressing and a thymidine kinase-expressing mutant VSV following IV administration to tumor-bearing immunocompetent mice. Similarly, IV administration of a mutant interferon-inducing VSV inhibited the growth of established lung metastases, extending survival of mice versus mock treatment in an immunocompetent model.⁸² An oncolytic vaccinia virus expressing a CXCR4 antagonist was designed to target tumor vasculature.¹⁴ This virus not only mediated greater growth inhibition and tumor vasculature disruption in primary tumors than a control virus when given IV, but also more effectively limited the growth of lung metastases and enhanced survival, when given either preoperatively or postoperatively in an experiment involving surgical removal of primary tumors. Moreover, mice that remained tumor free following treatment showed a significant resistance to tumor rechallenge, suggesting that therapy had stimulated antitumor immune activity.¹⁴ Oncolytic HSV has also been delivered IV, as demonstrated in a study by Wang et al,⁸³ in which mice given G47Δ oHSV had nine fold fewer metastatic lung lesions than mock-treated mice. IV administration of coxsackievirus A21 has demonstrated activity against primary tumors and has been reported to eliminate metastases.⁸⁴ Finally, IV administration of oncolytic viruses is not limited to delivery to the lung. In a series of studies, oncolytic adenoviruses expressing a soluble transforming growth factor-beta receptor II fused to the human IgG1 Fc fragment have been delivered to established bone metastases by IV injection. In each case, viral therapy limited the progression of bone lesions and the associated tumor-mediated bone destruction in both immunodeficient xenograft^{16,17} and syngeneic immunocompetent models.¹⁸ In a similar study, an unarmed chimeric adenovirus mutated to avoid liver uptake achieved comparable results with reduced liver toxicity versus an unmodified control virus.⁸⁵

Local and/or regional delivery of oncolytic viruses to various metastatic sites has also been reported. An oHSV targeted to human epidermal growth factor receptor-2 (HER-2) given intraperitoneally reduced the growth of peritoneal metastases. Interestingly, treated mice also exhibited reduced development of brain metastases.⁸⁶ Intratibial delivery of an

oncolytic adenovirus armed with a transgene consisting of osteoprotegerin fused to the Fc portion of IgG reduced the progression of established bone metastases and limited tumor-mediated bone destruction.¹⁹ Regional delivery methods have been used to target brain metastases, including direct injection of an IL-12-expressing oHSV into metastatic lesions^{20,87} or both intracranial and intrathecal delivery of oncolytic poliovirus.⁸⁸ Additionally, brain metastases have been targeted by intracarotid administration of the oHSV G47Δ.⁸⁹ In that study, disruption of the blood-brain barrier by the prior administration of mannitol was used to improve delivery of the oHSV and to yield a survival benefit in treated mice.

In addition to IV delivery, some oncolytic viruses have shown an ability to exert a systemic effect even when injected intratumorally, as described above for oncolytic vaccinia virus.⁷⁶ In a bilateral orthotopic xenograft model, oncolytic reovirus mediated regressions in both injected and uninjected tumors.⁹⁰ An oHSV expressing 15-prostaglandin dehydrogenase, when administered to primary tumors, inhibited both tumor growth and the formation of lung metastases in an immunocompetent model.²¹ In some cases, tumor treatment led to lasting antitumor immunity. The oHSV 1716 was used to treat primary tumors in an immunocompetent model. While tumor growth suppression was moderate, there was nonetheless an extension of survival and a reduction in the number of lung metastases, and treated mice resisted tumor rechallenge.⁹¹ Injection of the oHSV KM100 into flank tumors led to complete tumor regressions in the majority of treated animals and conferred a resistance to tumor rechallenge, in an immunocompetent model.⁹² Israyelyan et al^{93,94} constructed oHSV with either one or two mutations intended to induce syncytia formation among infected breast cancer cells. Both viruses suppressed tumor growth when injected intratumorally and reduced the incidence of metastases in multiple internal organs.⁹⁴ An oncolytic adenovirus expressing beta-defensin-2 mediated intratumoral infiltration and activation of dendritic cells; treatment of primary tumors with this virus suppressed tumor growth and inhibited the formation of lung and liver metastases. Furthermore, treated mice were resistant to tumor rechallenge.⁹⁵

Clinical studies

Several oncolytic agents have advanced to clinical trials, for a variety of cancers.⁹⁶ Although a clinical trial specific for breast cancer patients has not yet been conducted, breast cancer patients have been included in trials involving solid tumors (Table 1). In a Phase I trial, patients with cutaneous or subcutaneous metastatic tumor deposits (including 14 breast cancer

Table 1 Clinical trials of oncolytic viruses involving breast cancer patients

| Virus designation | Virus type | Phase, indication* | Status | Reference [#] |
|---|--------------------|--------------------|------------|------------------------------|
| OncoVexGM-CSF (Talimogene laherparepvec) | HSV | I, solid tumors | Completed | PMID 17121894 ⁹⁷ |
| Ad3-hTERT-E1A | Adenovirus | I, solid tumors | Completed | PMID 22871667 ⁹⁸ |
| HF10 | HSV | Pilot, breast | Completed | PMID 16865590 ⁹⁹ |
| HF10 | HSV | Pilot, breast | Completed | PMID 22193629 ¹⁰⁰ |
| Onyx-015 | Adenovirus | I, solid tumors | Completed | PMID 17704755 ¹³⁴ |
| Telomelysin | Adenovirus | I, solid tumors | Completed | PMID 19935775 ¹³⁵ |
| PV701 | NDV | I, solid tumors | Completed | PMID 16638865 ¹³⁶ |
| PV701 | NDV | I, solid tumors | Completed | PMID 11980996 ¹³⁷ |
| CAVATAK | Coxsackievirus A21 | I, solid tumors** | Completed | NCT00636558 |
| wDD-CDSR | Vaccinia | I, solid tumors** | Ongoing | NCT00574977 |
| HF10 | HSV | I, solid tumors** | Ongoing | NCT01017185 |
| ColoAd1 | Adenovirus | I/II, solid tumors | Recruiting | NCT02028442 |
| VCN-01 | Adenovirus | I, solid tumors | Recruiting | NCT02045602 |
| Reolysin | Reovirus | II, breast | Recruiting | NCT01656538 |
| GL-ONC1 (GLV-1h68) | Vaccinia | I, solid tumors | Recruiting | NCT00794131 |

Notes: *Solid tumors included breast cancer patients; **these studies specifically recruited breast cancer patients among a variety of other tumor types; [#]NCT number is the identifier number on ClinicalTrials.gov.

Abbreviations: HSV, herpes simplex virus; hTERT, human telomerase reverse transcriptase; NDV, Newcastle disease virus; PMID, PubMed unique identifier number.

patients) were given the oHSV OncoVEX GM-CSF.⁹⁷ Overall, safety and evidence of viral efficacy (transgene expression and viral-associated tumor necrosis) were demonstrated. Some breast cancer patients showed stable disease in the injected lesions and/or shrinkage of both injected and adjacent uninjected lesions. Hemminki et al⁹⁸ reported safety and potential efficacy in a trial for chemotherapy-refractory tumors, including five breast cancer patients given a human telomerase reverse transcriptase (hTERT)-selective type 3 oncolytic adenovirus, and this effect was enhanced by concomitant trastuzumab administration. For two of these patients, the complete viral dose had been given through the IV route (the others received partial IV doses). The oHSV HF10 has been used in a pilot study involving direct intratumoral injection in six patients with recurrent breast cancer.⁹⁹ A follow-up study showed histological evidence of viral replication and CD8+ lymphocyte infiltration in injected tumors.¹⁰⁰ Perhaps lending support to additional clinical trials, evidence of activity of several oncolytic viruses has also been demonstrated in studies involving primary tumor tissue cultured *ex vivo*, including reovirus,⁹⁰ adenovirus,^{101–105} and parvovirus H-1.¹⁰⁶ These studies may provide a basis for bridging the gap in efficacy often observed between preclinical and clinical studies for oncolytic viruses.

In addition to direct administration to patients, there is evidence that oncolytic viruses may have an additional clinical application based on their ability to eliminate contaminating tumor cells from bone marrow samples in patients given autologous grafts.¹⁰⁷ Proof of principle for potential application to breast cancer has been shown using both reovirus

(in a study¹⁰⁸ in which breast cancer cell lines were admixed with patient apheresis products) and an oHSV.¹⁰⁹

Overcoming challenges

Many of the challenges for successful oncolytic virotherapy of metastatic breast cancer are the same as those for virotherapy in general and have been reviewed in detail elsewhere.^{7,8,110} Accordingly, the strategies devised to overcome those challenges for other tumor types will likely be directly applicable or readily adaptable to the metastatic breast cancer setting.¹¹¹ These strategies can be organized into three broad categories: enhancing systemic delivery, promoting efficient intratumoral spread (overcoming matrix barriers, diffusion gradients, and poor viral replication), and limiting the antiviral immune response. For breast cancer in particular, advances have been made on all these fronts. Despite the progress already attained with current oncolytic agents described in this review, vascular delivery remains an obstacle to successful virotherapy, as the bulk of a systemically delivered viral dose is sequestered by the liver and spleen. Efforts to modify the infectivity of adenovirus have perhaps been at the forefront of oncolytic virus applications. In particular, the generation of chimeric adenoviruses with composite fiber proteins derived from multiple serotypes can improve systemic delivery, as shown with an Ad5/35 chimeric virus.²⁸ Delivery of a chimeric Ad5/3, with reduced liver uptake, to orthotopic tumors was further improved by pharmacological pretreatment of mice to ablate coagulation factors, Kupffer cells, and thrombocytes.¹¹² Another chimeric virus, Ad5/48, displayed reduced liver uptake and

greater delivery to bone metastases when given IV, leading to inhibition of tumor progression.⁸⁵ Tumor uptake and penetration of oncolytic adenovirus can also be improved by combining polymer coating of the virus to reduce hepatic sequestration and improve circulation half-life with focused ultrasonography to enhance tumor extravasation. This combination led to enhanced suppression of tumor growth and increased survival of tumor-bearing mice.¹¹³ Finally, the delivery of oncolytic adenovirus using mesenchymal stem cells has also been described.¹¹⁴

Other oncolytic viruses have been targeted directly to receptors often overexpressed in breast cancer. Both oHSV and VSV mutants have been generated to target HER-2.^{86,115,116} Efforts are also under way to improve the replication and spread of oncolytic viruses within tumors. Efficient replication has been shown to be critical for late-phase antitumor responses mediated by vaccinia virus.¹¹⁷ It has also been shown that breast cancer cells in three-dimensional culture can be more resistant to HSV-1 replication than those in traditional monolayer culture, and this was attributed to interference of the extracellular matrix with viral spread.¹¹⁸ In some cases, tumor cells of a certain phenotype may be resistant to oncolytic virus replication or can acquire resistance over time. A study by Bazan-Peregrino et al,¹¹⁹ in which multiple adenoviral mutants were compared for effectiveness in breast cancer, may provide a template for identifying more effective oncolytic agents. Similarly, a study by Song et al,¹²⁰ in colon and hepatic carcinoma cells, identified gene signatures associated with tumor cell resistance to oHSV that may provide a guide for developing countermeasures that can enhance virotherapy of breast cancer.

Finally, antiviral immunity within the tumor microenvironment has been shown to be a key limitation of the effectiveness of some oncolytic viruses; a study of VSV in several tumor cell lines, including breast cancer, demonstrated that macrophages present in the microenvironment stimulate a Type I interferon response that renders otherwise-susceptible tumor cells resistant to viral replication.¹²¹ However, this antiviral state could be reversed by pharmacological inhibition of JAK signaling. The interaction of oncolytic viruses with the immune system is complex, however. Whereas defects in innate immunity can promote viral replication,¹²² an efficient adaptive immune response can be an important component of the antitumor effect mediated by oncolytic viruses.¹²³

Future directions

As the field of oncolytic virotherapy continues to mature, new oncolytic viruses are constantly being identified.¹²⁴

As an example, most studies with oHSV have thus far been based on an HSV-1 platform, but HSV-2 can also be engineered into an oncolytic virus.^{36,125} Furthermore, an oncolytic bovine herpes virus type 1 with activity against a range of breast cancer subtypes has also been described.¹²⁶ Likewise, while most oncolytic adenovirus constructs have been based upon serotype 5, a number of other serotypes exist as possible oncolytic platforms for breast cancer.^{127,128} Recently, a method has been described in which experimental evolution was used to develop an oncolytic VSV highly selective for p53-deficient tumor cells. This selective process was shown to yield a virus more potent than the parental strain in a syngeneic model.¹²⁹ Similarly, reverse genetics was used to guide the engineering of a novel measles virus that more effectively infects breast cancer cells.¹³⁰ Novel oncolytic vaccinia viruses with activity against breast cancer are also being developed.¹³¹ Aside from the identification of new oncolytic viruses, modifications to existing viruses will also continue to improve their utility against breast cancer. In particular, it is likely that many future oncolytic viruses will include transgenes allowing the real-time monitoring of efficacy by noninvasive imaging.¹³² Eisenberg et al,¹³³ in a proof-of-principle study, demonstrated the utility of using a green fluorescent protein-expressing oHSV to assess surgical efficacy, as intratumoral administration of the oHSV enabled the detection of axillary lymph node metastases.

Conclusion

Oncolytic virotherapy, as a field, has rapidly advanced in a relatively short period of time. Early efforts to destroy tumors by viral oncolysis alone have graduated into efforts in which the potency of the viral platform is enhanced by the inclusion of therapeutic transgenes or in combination with other agents. Utilizing improved viral constructs that can be delivered systemically will lead to effective treatments for metastatic breast cancer patients.

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