Oncolytic Virotherapy for Breast Cancer Treatment

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Abstract: Breast cancer continues to be a leading cause of mortality among women. While at an early stage, localized breast cancer is easily treated; however, advanced stages of disease continue to carry a high mortality rate. The discrepancy in treatment success highlights that current treatments are insufficient to treat advanced-stage breast cancer. As new and improved treatments have been sought, one therapeutic approach has gained considerable attention. Oncolytic viruses are uniquely capable of targeting cancer cells through intrinsic or engineered means. They come in many forms, mainly from four major virus groups as defined by the Baltimore classification system. These vectors can target and kill cancer cells, and even stimulate immunotherapeutic effects in patients. This review discusses not only individual oncolytic viruses pursued in the context of breast cancer treatment but also the emergence of combination therapies with current or new therapies, which has become a particularly promising strategy for treatment of breast cancer. Overall, oncolytic virotherapy is a promising strategy for increased treatment efficacy for advanced breast cancer and consequently provides a unique platform for personalized treatments in patients.

Keywords: Breast cancer, Clinical trials, Oncolytic, Preclinical, Vectors, Virotherapy, Viruses.

1. INTRODUCTION: ADVANCING BREAST CANCER TREATMENT

Breast cancer continues to be the leading cause of death among women under 40 and the second leading cause of death in those over 40 [1]. In 2017, newly diagnosed breast cancer cases will make up nearly one-third of cancer diagnoses, with most of these cases being invasive [1, 2]. While early stage breast cancer is treated with high success, advanced breast cancer remains difficult to manage due to limitations of currently available treatments. Advanced breast cancer tends to develop resistance to standard therapies, thus leaving palliative care as the remaining option for these patients.

Current treatments for breast cancer fall under the cytotoxic, hormonal, and immunotherapeutic categories, all of which have demonstrated limited efficacy in advanced stages of breast cancer. With aggressive systemic therapies, patients often experience significant toxicity, while still only achieving a 50% or lower response rate [3]. These toxicities can persist as long-term ailments, affecting the cardiac and neurological systems and overall quality of life, as well as leading to the development of new primary cancers [4-9]. Combination therapies have been utilized to increase treatment efficiency, and are in extensive use today. However, tumors continue to develop resistance to these treatment combinations, leading to recurrences that become more challenging to treat. Thus, new therapies are in high demand for the systemic treatment of advanced breast cancer.

Research in oncolytic virotherapy has been ongoing for decades but only recently has the approach advanced to investigations at the clinical level. In recent years, oncolytic viruses have been moving towards clinical application at an accelerated pace. One example is T-VEC, an oncolytic herpes simplex virus (oHSV), which has been approved by the FDA for clinical use [10]. Oncolytic viruses are particularly attractive due to the myriad of targeting strategies they can utilize, thus extending them into the burgeoning field of personalized medicine. As a result, many oncolytic viruses have been identified as potential new therapeutic agents for the treatment of advanced breast cancer.

2. ONCOLYTIC VIRUSES

Oncolytic viruses are derived or engineered from naturally occurring viruses to target and specifically kill cancer cells. Currently, oncolytic viruses are derived from most groups of viruses, which are classified by their genome structure and modes of replication and transcription. These viruses have been subsequently engineered to utilize transcriptional and transductional targeting strategies that restrict replication of oncolytic vector constructs to cancer cells, thus sparing normal cells.
In breast cancer research, various viruses have already been extensively tested preclinically to assess their oncolytic efficacy. Of the seven groups in the Baltimore classification system, viruses from group I (double-stranded DNA viruses), group III (double-stranded RNA viruses), group IV (single-stranded RNA viruses – positive-sense), and group V (single-stranded RNA viruses – negative-sense), have been extensively investigated as candidates for breast cancer therapies, based on their previous use as vaccines or ease of handling and genetic manipulation. As with current treatments in the clinic, oncolytic viruses were initially being explored as single-agents and later in combination with existing therapies. Here we discuss oncolytic viruses produced within the last decade that are being widely studied for therapeutic application in breast cancer.

3. GROUP I VIRUSES

Group I viruses are double-stranded DNA (dsDNA) viruses, which have been explored for breast cancer therapy including oncolytic adenovirus (oAd), herpes simplex virus type 1 (HSV-1), and vaccinia virus (VV). Group I viruses require replication and transcription of their DNA within the host cell nucleus yet do not integrate into the host genome. Each virus utilized for oncolytic therapy has its unique cell entry and replication patterns, which can be exploited efficiently to deliver transgenes into the host cell nucleus.

3.1. Adenovirus

The human adenovirus (Ad) is the most studied oncolytic virus platform in breast cancer research as summarized in Table 1. Typically, Ad serotype 5 (Ad5) constructs target the cell through the human Coxsackievirus and adenovirus receptor (hCAR) receptor found on most cell surfaces. Subsequently, after binding to the receptor, the Ad undergoes endocytosis into the cell, after which the virus genome is transported into the host cell nucleus where it is transcribed and replicated for viral protein production and DNA packaging. Oncolytic Ads (oAds) have been engineered to take advantage of this lifecycle with modifications to the physical characteristics of the virion and the addition of targeting and therapeutic transgenes. One significant alteration found in many oncolytic Ads is the use of tumor-specific promoters, which restrict replication to cells expressing those genes. In addition, since breast tumors usually express low levels of the hCAR receptor the Ad5 vector uses [11], modifications to the Ad fiber protein involved in receptor binding have been shown to increase infectivity of cancer cells [12, 13].

In one study, tumor-specific promoters were utilized to increase breast cancer targeting [14]. A further modification was incorporated to display a chimeric Ad5 fiber protein that used the Ad serotype 3 (Ad3) knob domain. The Ad5/3 modification allowed higher infection rates of breast cancer stem cells in comparison to the wild-type Ad5 fiber [14]. In a subsequent clinical study, the same research team engineered an armed oAd using the Ad 5/3 platform. This virus, Ad5/3-D24-GM-CSF, was restricted to tumor cells containing defects in the p16-Rb pathway through a 24 base pair deletion of the E1A promoter gene [15]. Also, arming the virus with Granulocyte-Macrophage-Colony-Stimulating Factor (GM-CSF) allowed for the tumor-specific lymphocyte recruitment in human patients [15]. Another research team constructed an oAd armed with a CD40 Ligand (CD40L) transgene targeting breast cancer cells in vitro, in which early viral gene expression was regulated by an Estrogen Response Element (ERE) and a Hypoxia-Responsive Element (HRE) [16]. Expression of the CD40L was shown to directly inhibit cancer cell growth by binding to the surface receptor CD40. This oAd successfully inhibited breast cancer cell growth, reduced tumor volumes and displayed immune activation in vivo [16]. More recently, a study successfully targeted replication of Ad5-10miR145T to breast cancer cells through the insertion of 10 copies of the binding site for tumor suppressor microRNA (miRNA) miR145 downstream of Ad E1A gene [17]. This particular targeting technique is relatively new and was shown recently to suppress viral replication in cellular environments high in miR145 [17]. Due to decreased levels of miR145 in cancer, Ad5-10miR145T was able to replicate in breast cancer cells resulting in similar efficacy to the control virus [17].

Many studies have been conducted with a focus on systemic delivery with efficient viral targeting for the treatment of breast cancer metastasis. In a bone metastasis mouse model, oncolytic Ad.sTβRFc was shown to inhibit bone metastasis and reduce tumor burden [18]. This oAd was armed with a fusion protein, which targeted transforming growth factor beta (TGF-β) receptor 2 (TGFBR2). Expression of a soluble form of TGFBR2 fused with a human immunoglobulin Fc fragment inhibited the TGF-β signaling pathway associated with breast cancer bone metastasis [18]. Further investigation of this virus and a similar oAd, mhTERT-TAd.sTβRFc, which has its replication controlled by a modified human telomerase reverse transcriptase (hTERT) promoter, were conducted using a well-established bone metastasis mouse model [19]. In this model, both viruses resulted in low liver toxicity and were effective in inhibiting metastasis resulting in some cases of tumor-free mice [19]. Another oAd, Ad.dcn, was engineered to express the decorin (dcn) protein, and also inhibited bone metastasis and further prevented bone destruction by blocking the activity of TGF-β [20]. To further address the challenge of liver sequestering during systemic delivery, an oAd modified with a chimeric hexon protein containing the Ad serotype 48 (Ad48) hypervariable region was tested in the same bone metastasis model [21]. This oAd showed an improved safety profile in comparison to its unmodified counterpart with a reduction in liver uptake and damage [21].

Low expression of the primary Ad receptor, hCAR, on breast cancer cells is often a limiting factor for efficacy of oAds. Due to the restricted expression of hCAR, infection is poor and alternative entry receptors have been explored to improve transductional targeting of Ads. For example, Ad-Luc(HRG-fiber) containing the Herelugin (HRG) ligand in the HI loop of the Ad knob domain successfully retargeted the virus to the receptor tyrosine-protein kinase erbB-3 (HER3) in breast cancer cells [22]. Another oAd, Ad5-pIX-RFP-FF/NK2, retargeted the oAd to the tyrosine kinase receptor Met (cMet), which was found to be overexpressed in a variety of cancers, including breast cancer [23, 24]. In addition, chimeric Ads using alternative serotypes, such as the
Table 1. Summary of oncolytic Ad (Group I) viruses used in the context of breast cancer therapy.

| Baltimore Classification System | Virus | Vector | Modifications | Aim/Target | Refs. |
|---------------------------------|-------|--------|---------------|------------|-------|
| Adenovirus (Ad) | | | E1 deletion; hTERT promoter insertion; Ad3 fiber knob | Increase breast cancer targeting | [14] |
| | Ad5/3-mdr-∆24; Ad5/3-hTERT-Agp; Ad5/3-cox2L-∆24 | | | | |
| | Ad5/3-D24-GM-CSF | Express GM-CSF; 24 bp deletion in E1A | p16-Rb pathway defects, tumor-specific immunotherapy | [15] |
| | AdEHCD401 | Insert HRE, ERE and E2F-1 promoters; delete Ad E3 19K/6.7K genes; arm with CD40L | Restrict to tumor cells over-expressing estrogen receptor and HIF-1α | [16] |
| | Ad5-10miR145T | Insert 10 copies of miR145 down stream of E1A | Restrict replication to cancer cells | [17] |
| | Ad.EHCD401; AdLuc2 | CMV promoter; Arm with sTGFβRIIFc gene | Target TGF-β | [18] |
| | mhTERTAd.sTβRFc | mhTERT promoter | Replication controlled | [19] |
| | Ad.dcn | Express Decorin protein | Produce functional decorin in vivo; target bone metastasis | [20] |
| | mHAd.luc2 | Ad48 hypervariable region in hexon gene | Reduce liver sequestration | [21] |
| | AdLuc(HRG-fiber) | HRG ligand in HI loop of Ad knob domain | Retarget to HER3 | [22] |
| | Ad5-pIX-RFP-FF-NK2 | NK2 ligand in HI loop of Ad 5 knob domain | Retarget to cMet | [23] |
| | Ad.KISS1 | Arm with KISS1; Ad5/3 chimeric fiber | Increase infection in breast cancer cells; tumor suppressive | [25] |
| | OAdmCherry | mCherry fused to pIX protein; Ad5/3 chimeric fiber | Oncolytic improvement with temozolomide | [26] |
| | CNHK600-IL24 | Arm with IL-24 | Induce apoptosis | [27] |
| | P55-HTERT-HRE-TRAIL | Arm with TRAIL | Target TNBC | [28] |
| | SG500-dNK | Arm with DmDNK | Increase cancer specificity; combination with BVDU or dFdC | [30] |
| | Ad5/3-∆24-tras | 24 bp deletion in E1A; Produce trastuzumab | Local antibody production at tumor sites | [31] |

Abbreviations: Ad: Adenovirus; BVDU: Bromovinyldeoxyuridine, CMV: Cytomegalovirus; dFdC: Difluorodeoxycytidine; GM-CSF: Granulocyte-macrophage colony stimulating factor; HER3: Receptor tyrosine-protein kinase erbB-3; HRG: Heregulin; IL-24: Interleukin-24; TGF-β: Transforming growth factor beta; TNBC: Triple negative breast cancer; TRAIL: TNF-related apoptosis-inducing ligand.

Ad3 fiber protein have been utilized to overcome reduced infection and immune surveillance. This chimeric fiber platform, as described above, utilizes the CD46 receptor, which is often upregulated in cancers. This platform was recently used in a study aimed at improving infection in a breast cancer brain metastases cell line using an oAd armed with the KiSS-1 metastasis suppressor protein (KiSS1) [25]. Ad-KiSS1 not only was able to infect the cell line, but it also resulted in increased cytotoxicity, suppression of invasive properties, and induction of apoptosis [25]. In another study using the Ad5/3 platform, triple negative breast cancer cells were targeted with OAdmCherry and the alkylating agent temozolomide [26]. This combination approach increased the efficacy of both treatments over mono-therapeutic controls.
by significantly increasing autophagy and oncolytic cell death [26]. These examples of oAds can provide new platforms for additional modifications such as liver detargeting strategies and therapeutic transgene expression for increased virus vector efficacy.

Further examples of modified oAds include those ‘armed’ with a therapeutically expressing transgene that is produced alongside the oncolytic effects of the replicating Ad. One research team created CNHK600-IL24, an oAd transcriptionally targeted by regulating Ad early gene expression with an hTERT promoter and a promoter containing Hypoxia-Response Elements (HRES). This construct was armed with an expression cassette in which the Cytomegalovirus (CMV) promoter regulated expression of the apoptosis-inducing cytokine IL-24 [27]. CNHK600-IL24 successfully inhibited breast cancer cell growth both in vitro and in vivo and reduced metastasis after systemic injection [27]. This research team also produced a similar oAd, P55-HERT-HRE-TRAIL, a virus armed with CMV-driven Tu-

mor Necrosis Factor-Related Apoptosis Inducing Ligand (TRAIL) [28], which has been shown to induce apoptosis in Triple-Negative Breast Cancer (TNBC) with a mesenchymal phenotype [29]. TNBC was successfully treated in both an orthotopic and a metastasis mouse model, resulting in tumor inhibition and significantly higher survival in the metastasis model when compared to a non-TRAIL expressing control vector [28]. Another transcriptionally targeted oAd, SG500-
dNK, armed with the suicide gene deoxyribonucleicase kinase (DmDNNK) from Drosophila melanogaster, also exhibited effective breast cancer targeting [30]. However, this oAd was observed to exhibit some off-target replication prompting the authors to recommend additional modification to further restrict replication to the targeted breast cancer cells [30]. Recently, another novel approach to engineering an oAd has been demonstrated with the addition of current monoclonal antibody immunotherapy trastuzumab. In a multiple targeting approach, Ad5/3-A24-tras was both transcriptionally and transcriptionally targeted, allowing for oAd-mediated breast cancer cell lysis and production of the immu-
notherapeutic anti-HER2-mAb trastuzumab [31]. The production of trastuzumab de novo in addition to the oAd-mediated oncolysis caused growth inhibition, tumor reduc-
tion and anti-tumor immune response [31].

As oAds approach the clinic, the question arises of whether the best therapeutic use of these oAds would be as a single therapy or as combination/adjuvant therapy. Given the lackluster results in single therapy treatments for breast cancer, a combination approach would be better suited to decrease the toxicity of treatments and increase their effectiveness. For example, the previously described oAd, SG500-
dNK, has also been paired with two common chemotherapeu-
tics to assess the effects that the oncolytic virus and che-
mothearpies have on each other [30]. The chemotherapeutic nucleoside analogs Bromovinyldeoxyuridine (BVDU) and Difluorodeoxyctydine (dFdC) were used after initial infection of TNBC cells in vitro. With both analogs, synergistic effects were observed with an increase in cell killing while normal cells were minimally affected [30]. In an in vivo xenograft model using the TNBC cell line MDA-MB-231, SG500-dNK in combination with dFdC resulted in significant reduction in tumor growth and increased survival when compared with the oAd alone [30]. To further illustrate combi-
nation approaches, the Ad 5/3-D24-GM-CSF previously described [15], was tested in vitro, in vivo, and in human patients using the chemotherapeutic drug Cyschophosphamide (CP) [32]. The MDA-MB-436 TNBC cell line was treated in vitro and in vivo, resulting in increased cell killing and anti-
tumor effects when Ad 5/3-D24-GM-CSF was used in com-
bination with CP [32]. In human patients, this combination was shown to be well tolerated without the occurrence of serious adverse events, and many patients exhibited decreases in blood tumor markers [32]. These studies highlight the potential of oAds in the clinic and suggest more focus on combined approaches may facilitate clinical development and application in the near future.

### 3.2. Herpes Simplex Virus

Herpes simplex virus type 1 (HSV-1) is a large, en-
veloped, dsDNA virus that fuses its envelope to the host cells subsequently releasing its naked virion into the cell. Many of the oncolytic HSV vectors incorporate mutations in viral genes or introduce additional therapeutic or targeting ap-
proaches (Table 2). To restrict HSV-1 replication to cancer cells, the γ34.5 gene was deleted, resulting in a transcription-
tally targeted vector unable to replicate in neurons [33]. Additional modifications to the entry mediator glycoprotein gD found on the HSV-1 envelope allowed for retargeting to specific overexpressed receptors in breast cancer, such as the Human Epidermal Growth Factor Receptor 2 (HER-2). This approach was utilized in the oHSV construct R-LM249, which contained the anti-HER-2 single chain antibody trast-
uzumab in the gD domain [34]. This oHSV was successfully retargeted to the HER-2 receptor in breast cancer cells [35], a receptor commonly overexpressed in some breast cancer subtypes [36]. In addition, treatment with R-LM249 in mice displayed no signs of toxicity, inhibited HER-2 positive tu-

cor cells growth and even resulted in tumor-free mice [35].

A separate oHSV, G47Δ, contained several gene muta-
tions to restrict replication to breast cancer cells further. The additional mutations in the ICP6 and α47 genes restricted replication to dividing cells [37] and enhanced immune stimulation [38]. In a study of pulmonary breast cancer me-
tastasis treatment with G47Δ, the virus significantly reduced the number of tumors compared to the control [39]. In addi-
tion, G47Δ was successfully tested in a breast cancer stem cells both in vitro and in vivo to assess its ability to target stem cells contributing to tumor growth [40]. In tamoxifen-
resistant breast cancer cells and tumors, G47Δ was able to target, replicate in and reduce tumor growth, demonstrating its potential as adjuvant therapy in the clinic [41]. In an at-
tempt to negate the decreased virulence associated with the deletion of γ34.5 a recent study introduced the C-terminus of murine protein phosphatase 1 regulatory subunit 15A (MyD116) to the N-terminus of the γ34.5 gene in a G47Δ recombinant (GD116) [42]. This insertion enhanced the rep-
lication and cytotoxicity of GD116 in breast cancer cells in vitro, thus introducing a new possible platform to develop oHSV with higher efficiency [42].

Some oHSV vectors have been armed with cancer-combating proteins, enzymes, or drugs to achieve a greater therapeutic effect. In breast cancer treatment, the oHSV-based OSVP virus...
Table 2. Summary of oncolytic HSV and VV (Group I) viruses used in the context of breast cancer therapy.

| Baltimore Classification System | Virus | Modifications | Aim/Target | Refs. |
|---------------------------------|-------|---------------|------------|-------|
| **Herpes simplex virus (HSV)**  | R-LM249 | γ34.5 gene deletion; Trastuzumab scFv in gD domain | Retarget to HER2 receptors | [34] |
|                                 | G47A  | γ34.5 gene deletion; ICP6 gene mutation; α47 gene mutation | Restriction to breast cancer cells; immune reaction enhancement | [37–41] |
|                                 | GD116 | C-terminus of MyD116 inserted in place of the C-terminus of γ34.5 | Enhance replication and cytotoxicity | [42] |
|                                 | OSVP  | 15-hydroxy prostaglandin dehydrogenase gene | Break down tumor promoting prostaglandin E2 | [43] |
|                                 | HF10  | Naturally mutated strain | Cellular effects; Combination effects with Bevacizumab targeting VEGF | [44, 45] |
|                                 | MGH2  | Transcriptionally targeted; Express GFP | Combination with apoptosis-inducing compounds; Tumor penetration improvement | [46] |
|                                 | M002  | Express IL-12 | Viral replication in combination with HDAC inhibitors | [47] |
|                                 | HSV1-hGM-CSF | Transcriptionally targeted; Produce GM-CSF | Combination with doxorubicin; Target cancer stem cells and chemoresistant cancer cells | [48] |
|                                 | rQNestin34.5 | ICP34.5 mutation under control of the Nestin promoter | Combination with CAR NK cells expressing anti-EGFR; Target breast cancer brain metastasis | [50] |
| **Vaccinia virus (VV)**         | GLV-1h68 | Natural tropism to cancer cells; RUC-GFP gene; β-galactosidase gene; β-glucuronidase gene insertions | Target mammary tumors; Replication in cancer cells; Combination approach using prodrugs | [53, 54, 56] |
|                                 | GLV-1h164 | Armed with GLAF-2 antibody | Target VEGF | [55] |
|                                 | Vvdd  | Deletions in TK and VGF genes or Serpin-1 and Serpin-2 genes | Replication restricted to tumor cells; enhanced cytotoxicity | [57, 58] |

Abbreviations: EGFR: Epidermal growth factor receptor; GFP: Green fluorescent protein; GM-CSF: Granulocyte-macrophage colony-stimulating factor; HDAC: Histone deacetylase; HER2: Human epidermal growth factor receptor 2; IL-12: Interleukin-12; NK: Natural killer; TK: Thymidine kinase; VEGF: Vascular endothelial growth factor; VGF: Vaccinia growth factor.

incorporated a 15-hydroxyprostaglandin dehydrogenase gene encoding an enzyme that breaks down tumor promoting prostaglandin E2 [43]. In mouse models of orthotopic and metastatic breast cancer, this oHSV inhibited tumor growth, metastasis, and even contributed to immune stimulation after treatment [43].

In recent years, the oHSV HF10 virus, a naturally mutated strain, was evaluated in human breast cancer patients. In one study, breast cancer patients who had recurrences were treated with single or repeated doses of HF10 injected into single tumor nodules [44]. Interestingly, these patients demonstrated tumor size reductions and CD8-positive T cell infiltration that was suggestive of an antitumor response [44]. Another phase I dose escalation clinical trial was completed using HF10, in which six with recurrent breast cancer of seventeen patients with advanced cancers were included [45]. While HF10 injections were safe and well-tolerated, a follow-up clinical trial enrolling a larger cohort of breast cancer patients would likely yield more relevant data to assess its efficacy as a therapy in this disease setting.

Several studies have evaluated oHSV efficacy in combination with other treatments such as chemotherapies, immunotherapies, and targeted therapies. MGH2, a transcriptionally targeted oHSV was assessed in conjunction with doxycycline-induced caspase 8 expression, recombinant TRAIL, and/or chemotherapy paclitaxel [46]. Treatment in vivo with doxycycline to induce caspase 8 expression resulted in apoptosis which increased MGH2 infection by facilitating virus
spread and therefore increased cell death intratumorally [46]. Pretreatment with a paclitaxel-TRAIL combination also increased MGH2 spread within tumors and contributed to higher cell death and necrosis [46]. Similarly, an Interleukin-12 (IL-12) expressing oHSV, M002 also exhibited increased replication in breast cancer cells including in HSV-resistant cells when paired with select histone deacetylase inhibitors [47]. Another oHSV, HSV1-hGM-CSF, has been constructed to transcriptionally target breast cancer cells and produce human granulocyte-macrophage colony-stimulating factor upon replication [48]. HSV1-hGM-CSF treatment given as an adjuvant therapy with chemotherapeutic agent doxorubicin was able to significantly reduce tumor volume in a breast cancer mouse model when compared to either treatment alone [48]. Similar effects were shown with HF10 when combined with the monoclonal antibody bevacizumab in treatment against a xenograft mouse model [49].

Recently, a unique study was carried out combining the glioma-specific oHSV, rQnestin34.5 with Chimeric Antigen Receptor (CAR) modified Natural Killer (NK) cells expressing an Epidermal Growth Factor Receptor (EGFR) antibody fusion (EGFR-CAR-NK-92) [50]. This approach can target both EGFR expressing cancer cells and EGFR-negative cancer cells within the tumor. Herein, Breast Cancer Brain Metastases were initially treated intratumorally with the EGFR-CAR-NK-92 cells and subsequently treated with the oHSV [50]. Similar to the studies previously described, the combination here reduced tumor growth more than the single therapy controls and resulted in significantly increased survival in the mice [50]. Overall, the trend observed with oAd experiments was replicated using oHSVs, suggesting that combination approaches are superior to single therapy approaches. Due to the recent FDA approval of Imlygic (talimogene laherparepvec or TVEC) as an oncolytic virus, one of the most promising agents for immunotherapy, VV is expected to advance from clinical trials in the near future.

### 3.3. Vaccinia Virus

The vaccinia virus (VV) is unique among dsDNA viruses in that its replication occurs entirely in the cytoplasm, and not the nucleus of the cell [51]. This feature is touted as an additional safety benefit for an oncolytic virus due to the genome integration risk being eliminated. In addition, VV has a naturally tropism to tumors, making it an ideal candidate as an oncolytic virus [52]. In breast cancer, an oncolytic VV (oVV) has been shown to have high tumor cell infectivity, replicate well and cause tumor regression (Table 2). One strain, GLV-1h168, an oVV containing three gene modifications for successful visual and immunohistochemical tracking, was able to successfully replicate in and kill canine mammary tumor cells both in vitro and in vivo within a nude mouse model [53]. In human breast cancer stem cells demonstrating increased resistance to chemotherapy and irradiation, GLV-1h168 was able to replicate more efficiently when compared to the non-stem cell type counterparts [54]. When assessed in a xenograft mouse model using breast cancer stem cells, GLV-1h168 was also able to significantly inhibit tumor growth, making this a potential oncolytic virus to target hard to kill cancer stem cell populations [54]. In another study, an oVV named GLV-1h164, armed to express the single-chain antibody GLAF-2 against Vascular Endothelial Growth Factor (VEGF) was tested in triple negative breast cancer [55]. GLV-1h164 significantly regressed xenografts of triple negative breast cancer tumors when compared to the non-GLAF-2 expressing parent virus [55]. In addition, VEGF was successfully targeted, as was seen by the decrease in vascular flow and the inhibition of tumor vasculature post-treatment [55].

A few studies in recent years have combined oVV with anti-cancer agents to increase vector potency in treating breast cancer. One study combined GLV-1h68 with (1S)-secob-DMAI-β-D-galactoside 1, a produg activated by β-galactosidase, which is expressed in the virus [56]. This study was the first attempt using this type of produg in vivo in a tumor-bearing model. Herein, a human metastatic breast cancer cell line, GI-101A, was used to form xenograft tumors in nude mice that were treated first with the oVV GLV-1h68 and subsequently with the produg [56]. As a result, tumors were significantly reduced in volume compared with the controls, leading the research team to surmise the potential of produg combinations with oVV [56]. In another study, also using a transcriptionally targeted strain, Vvdd, tested the virus in combination with a 4-1BB (CD137) receptor antagonist [57]. Vvdd contains additional deletions that further restrict replication and cytolytic activity to tumor cells, enhancing this oVV tumor targeting and cytotoxicity [58]. The combination of the 4-1BB antagonist and Vvdd was able to inhibit tumor growth and increase survival in an immunocompetent mouse model, as well as impact metastatic tumors at other sites of the body [57]. Also, antitumor effects were seen as a reduction of breast cancer metastasis and tumor infiltration by CD8+ T cells, NK cells, and myeloid cells, highlighting the potential use of VV in immunotherapy [57]. As this VV research in breast cancer treatment expands, more drug combinations and immunotherapeutic applications should be addressed. With more studies, oVV may advance to clinical trials in the near future.

### 4. GROUP III VIRUSES

The double-stranded RNA (dsRNA) class of viruses represents a diverse group of pathogens that infect a broad range of host species, from bacteria and fungi to animals and plants. Most of these viruses haveicosahedral capsid structures, and contain from one to a dozen different RNA molecules, each coding for one or more viral proteins. Upon infection, the genomic dsRNA is transcribed into mRNAs that will serve for both translation and replication.

#### 4.1. Reovirus

Reovirus is a dsRNA virus whose exact lifecycle mechanism is still not fully understood. However, reovirus has been accepted as generally nonpathogenic in humans [59], and consequently have been exploited as oncolytic viruses (Table 3). Interestingly, Type 3 Dearing reovirus strain is naturally oncolytic and preferentially infects tumor cells. The oncolytic effect of this strain in breast cancer cells has been explored in several studies. One study tested a panel of breast cancer cell lines and found that all were susceptible to reovirus infection regardless of hormone receptor status, whereas normal breast epithelial cells were not [60]. This broad infection capacity has been attributed to activated Ras pathway or mutated Ras protein in cancer cells [61].
tested in mice using core biopsies of a human breast cancer tumor, reovirus treatment successfully caused tumor regression [62]. This study also found that reovirus is a sufficient vector to target breast cancer stem cells, as they also exhibit aberrant Ras activity [62]. Because of encouraging studies indicating the reovirus type 3 Dearing strain would make an ideal oncolytic virus for the clinic, it quickly rose to clinical testing. In 2013, a dose-escalation phase I trial was published reporting on reovirus (Reolysin) used as a local injection at the tumor site [63]. While patients ranged in cancer type, three were metastatic breast cancer patients. Of these three, one was determined to have stable disease after treatment, and the study concluded that treatment with reovirus proved to be safe in various advanced stage cancers [63].

Reovirus has also been tested in combination with docetaxel and gemcitabine to study possible enhancement of its oncolytic activity. In the phase I clinical trial, 25 oncology patients were treated with docetaxel in combination with reovirus. Of these patients, one presented with metastatic breast cancer which was considered to have undergone a complete response to the treatment [64]. A phase I trial combining gemcitabine with reovirus showed some positive effects in cancer patients including one breast cancer patient. However, the results of this study were less definitive, prompting a suggestion for further exploration on this particular combination [65].

Given that these combination studies had only two breast cancer patients enrolled, further exploration in a breast cancer cohort would be more enlightening on the potential of reovirus in combination with commonly used breast cancer treatments. Recently, a preclinical study combined reovirus with an anti-PD-1 inhibitor to target breast cancer cells both in vitro and in vivo with an immunocompetent mouse model [66]. The authors demonstrated that reovirus was capable of inducing an immune response and when combined with anti-PD-1 therapy, tumor reduction, and immune response was so marked that 70% of mouse cohort was cured [66]. Remarkably, this combination enabled a systemic protective anti-tumor response that inhibited tumor growth during a tumor re-challenge, thus providing further evidence in support for using of reovirus in clinical trials [66]. However, while reovirus has quickly risen to clinical trials, further exploration with a breast cancer cohort of patients should be conducted to determine its potential as a breast cancer treatment.

The remainder of this review will touch on two additional groups of viruses that have been advanced in breast cancer research. These more recent studies involve virus platforms that could be utilized in breast cancer therapy. While some of these examples have been used in treating other tumor types, breast cancer could be similarly targeted.

### 5. GROUP IV VIRUSES

The positive-sense single-stranded RNA (+ssRNA) class of virus is unique in that the genome can immediately produce proteins as positive sense ssRNA that function as mRNA within the cytoplasm. Those explored for use in breast cancer treatment are picornaviruses within the genus Enterovirus, also known as intestinal viruses. Here we discuss the coxsackievirus and polioviruses that have been examined in breast cancer research (Table 3).

#### 5.1. Coxsackievirus

The naturally occurring Coxsackievirus A21 (CVA21) strain, which is mildly pathogenic to humans, enters the cell through receptor-mediated infection, particularly using a complex of the intercellular adhesion molecule 1 (ICAM-1) and the Decay-Accelerating Factor (DAF). This receptor complex is found to be overexpressed in many cancers including breast cancer [67]. One study using CVA21 successfully destroyed breast cancer cells in single monolayers and spheroid cultures, as well as in vivo SCID mouse models of xenograft and orthotopic metastatic breast cancer [68]. Recently, the coxsackievirus B3 strain has been genetically modified to increase safety by inserting transcriptionally regulated miRNA sequences [69]. Here, triple negative breast cancer was treated in vitro and in vivo with results indicating an increase in safety as tumor growth was suppressed [69]. The encouraging results from these studies introduce coxsackievirus strains to oncolytic virotherapy for breast cancer and pave the way for further safety studies as a single agent as well as in combination drug approaches.
5.2. Poliovirus

Recently, a study using poliovirus has explored the treatment of breast cancer xenograft models. While poliovirus is associated with neurological pathogenesis resulting in the debilitating polio disease, this study utilizes the live-attenuated polio vaccine with an additional rhinovirus gene insert to further prevent replication in neural cells [70]. In addition, the poliovirus uses the CD155 receptor for entry, which is found in nearly all cancers, making it an ideal candidate for oncolytic therapy. Here, PVSRIPO was tested on breast cancer cells in vitro and in vivo xenografts resulting in cell lysis and delayed tumor growth [71]. Most interestingly, treatment with PVSRIPO resulted in robust immune activation and neutrophil infiltration in tumors, highlighting its potential as an immunotherapeutic vector [71].

6. GROUP V VIRUSES

There has been additional breast cancer research conducted with viruses from the negative-sense single-stranded RNA (-ssRNA) group. The -ssRNA group encompasses viruses that have frequently been used to treat a variety of different cancers. However, the application in breast cancer has only been recently explored both in vitro and in vivo as shown in Table 4 and includes Vesicular Stomatitis Virus (VSV), Measles Virus (MV), Maraba virus, and Newcastle Disease Virus (NDV).

6.1. Vesicular Stomatitis Virus

Vesicular Stomatitis Virus (VSV) is a relatively new virus in breast cancer virotherapy, and initial studies reveal its oncolytic potential as well as challenges that will require more engineering and testing. VSV is unable to replicate in normal human cells yet can replicate in oncogenic human cells through the cellular mutations accumulated in cancer cells, possibly through antiviral pathways. This unique characteristic, in addition to its low pathogenicity in humans, provides a unique safety profile sought after in virotherapy. However, treatment approaches have struggled to increase its efficacy to rival that of more commonly used oncolytic viruses. For example, a study using the oncolytic VSV (oVSV) mutant rM51R-M was unable to completely inhibit progression of tumor growth in an in vivo breast cancer model, even in combination with IL-12 [72]. Recently, a study using the mutant VSVΔ51 tested the vector in combination with Microtubule-Destabilizing Agents (MDAs) to increase the efficacy of the oVSV vector [73]. Herein, VSVΔ51-resistant 4T1 breast cancer cells were treated in vitro with MDAs, followed by the virus resulting in synergistic effects on the viral spread and cell death, including VSVΔ51-resistant breast cancer cells [73]. In vivo, the vector in combination with MDAs was able to delay tumor progression and increase survival, as well as trigger antitumor activity [73].

Interestingly, a study that examined a combination of VSV and VV in various established cancer cell lines showed that the VV significantly enhanced VSV replication [74]. Administering the viral combination in an aggressive 4T1 breast cancer model, corroborated the in vitro data while simultaneously establishing the safety of the combination [74]. This result was further supported by using a more extensive panel of cancers using ex vivo tumor tissue slices, also finding significantly enhanced viral replication when compared to singularly infected cultures [74]. While breast cancer specimens were not included in this study, the virus combination approach can be utilized with other established oncolytic viruses in breast cancer research, as seen in Table 4. Recently, a study using an oVSV armed with a reovirus Fusion-Associated Small Transmembrane protein (FAST) demonstrated successful decreases in tumor growth and increased survival in a syngeneic murine breast cancer model [75]. This study highlighted the ability of the FAST protein (p14) to increase virus transmission and dissemination within the model as well as the induction of an anti-tumor immune response [75]. Overall, VSV is just beginning to enter breast cancer research; its natural oncolytic activity makes it a candidate for breast cancer research, particularly in an immunotherapeutic capacity.

6.2. Measles Virus

Oncolytic measles virus (oMV) derived from the attenuated Edmonston-B (MV-Edm) vaccine strain have been tested in clinical trials for various cancers, and in recent years, the exploration into breast cancer applications has begun. MV utilize the following receptors: CD46 [76] ubiquitously expressed on all nucleated cells, SLAM (signaling lymphocytic activation molecule) [77] often overexpressed in cancer cells, and the Poliovirus Receptor-related protein 4 (PVRL4) [78]. Attenuated oMV have been utilized to specifically target cancer cells, by limiting their replication to oncogenic cells. In a study using both MV-GFP virions and MV-GFP-infected dendritic cells, breast cancer cells were successfully infected by both modes and virus was able to eradicate the cancer cells [79]. This result illustrated an important approach of oncolytic virotherapy in the context of preexisting immunity. The data from this study suggest that carrier cells (such as dendritic cells used in these experiments) are efficient in bypassing MV-neutralizing antibodies and successful in delivering the vector to breast cancer cells [79]. These results were further supported by a pleural effusion xenograft model where MV-GFP rapidly infected and spread amongst tumors including distant metastasis using either free-virions or carrier dendritic cells [79]. In another study a CD150 (SLAM) blind strain was created, rMV-SLAMblind, resulting in infection of breast cancer cells via the Nectin cell adhesion molecule 4 Nectin-4 or PVRL4 receptor, which coincidently is also overexpressed in breast cancer cells [80]. This virus improved upon the vaccine derivative, MV-Edm, in enhancing oncolytic activity both in vitro and in vivo in breast cancer cells [80]. Furthermore, safety testing in Rhesus monkeys concluded that rMV-SLAMblind did not demonstrate symptoms typically seen in a measles infection [80].

A separate research team sought to retarget MV to the urokinase-type Plasminogen Activator Receptor (uPAR) which is primarily expressed in cancer and is associated with tumor progression and metastasis [81]. This study utilized both syngeneic and xenograft breast cancer mouse models to test species-specific versions of the uPAR-targeting oMV vectors (MV-m-uPA and MV-h-uPA). Both viruses were cancerspecific, and were shown to delay tumor progression in both models and significantly increased survival in a human xenograft model [81]. A subsequent study with these viruses
utilized uPAR overexpression in tumor stroma fibroblasts and determined that the tumor stroma could be utilized to transfer infection to tumor cells, induce apoptosis and significantly delay tumor progression [82]. Further modification of an oMV to dual target murine and human cells in a xenograft breast cancer mouse model resulted in increased survival and decreases of tumor-associated fibroblasts and endothelial cells [83]. These studies illustrate a unique approach to breast cancer treatment by targeting both the tumor stroma and tumor cells that can provide additional avenues for successful clinical treatment.

As with many oncolytic viruses being explored in clinical trials, combination therapies with MV are of particular interest due to the evidence that these approaches can increase the efficacy of viral therapies. For example, several MVs (e.g., MV-GFP, MV-lambda, MV-s-NAP, and MV-lambda-NAP) have recently been tested with alisertib. Alisertib (MLN8237) is an Aurora A kinase inhibitor whose activity is synergistic with viral replication. The combination of these oMV vectors with alisertib significantly improved breast cancer cell eradication compared to virus-only treatment, and in some cases resulted in complete eradication in vitro [84]. When this combination was repeated in vivo using MV-lambda-NAP, survival of a xenograft metastasis mouse model of breast cancer was significantly improved, and in some cases resulted in complete regression [84]. In the pleural effusion model previously described, a combination of alisertib and MV-s-NAP also increased survival significantly compared to single-agent therapy [84]. With the combination of drugs such as alisertib, the efficacy of MVs can be increased and utilized in clinical trials, leading to better outcomes and possibly the advancement of the oMV vector to clinical use.

Table 4. Summary of oncolytic Group V viruses used in the context of breast cancer therapy.

| Baltimore Classification System | Virus | Vector | Modifications | Aim/Target | Refs. |
|--------------------------------|-------|--------|---------------|------------|-------|
| Vesicular Stomatitis Virus (VSV) | rM51R-M | Naturally oncolytic | Breast cancer cell infection and cell death in combination with IL-12 | [72] |
| VSVΔ51 | Naturally oncolytic; Deletion in matrix protein | Efficacy in combination with MDAs | [73] |
| VSVΔ51; VVD-eGFP; VVΔB18R-eGFP | Vaccinia virus B18R gene deletion | B18R gene product contribute to viral replication; synergistic effect of viral co-infection | [74] |
| VSV-p14 | Armed with FAST protein | Increase virus infection and spread | [75] |
| MV-GFP | Green fluorescent protein expression | Modes of infection using dendritic cell carriers or MV alone in cancer cells | [79] |
| rMV-SLAMblind | Mutated to be incapable of binding CD150 receptor | Infection via PVRL4 receptor | [80] |
| MV-m-uPA; MV-h-uPA | Retarget to uPAR | Increased infection and targeting through tumor stroma | [81, 82] |
| MV-un-muPA | Modified for murine and human targeting; Targeted to human CD46 and murine uPAR | Effects on tumor stroma and tumor infection by oncolytic MV | [83] |
| MV-lambda; MV-s-NAP; MV-lambda-NAP | Express human lambda Ig chain (and/or) neutrophil-activating protein | Effects of combination treatment with alisertib | [84] |
| MV-lambda-NAP | Express human lambda Ig chain (and/or) neutrophil-activating protein | Effects of combination treatment with alisertib | [84] |
| Maraba Virus | MG1 | G protein mutation (Q242R); M protein mutation (L123W) | Increase virus oncolysis; Attenuate replication in normal cells | [85-88] |
| Newcastle Disease Virus (NDV) | Lentogenic LaSota strain | None | Tumor selectivity | [89] |
| Oncolytic strain MTH-68 | None | Combination radiofrequency hyperthermia treatment of a breast cancer patient | [90] |

Abbreviations: FAST: Fusion-associated small transmembrane protein; IL-12: Interleukin-12; MDAs: Microtubule-destabilizing agents; MV: Measles virus; PVRL4: Poliovirus receptor-related protein 4 Nectin-4; uPAR: Urokinase-type plasminogen activator receptor.
6.3. Maraba Virus

The Maraba virus, another relatively new member of oncolytic virotherapy vectors, has made its way into breast cancer research. In a study exploring the virus as a VSV-related rhabdovirus with potent oncolytic activity, a recombinant Maraba, MG1 was engineered to increase its oncolytic potential while attenuating its ability to replicate in normal cells [85]. Maraba MG1 was safely administered intravenously, and repeated doses in a syngeneic colon cancer model resulted in complete regression of tumors [85]. In a subsequent study investigating the 4T1 mouse breast cancer metastasis model, administration of MG1 or a UV-inactivated version in a preoperative treatment scheme dramatically reduced lung metastasis [86]. Assessment of MG1 in combination with paclitaxel treatment further enhanced breast cancer cell killing by enhancing viral replication both in vitro and in vivo [87]. Even more remarkable, a recent study examined long-term immune response effects to intratumorally injected MG1 when combined with surgical resection post-treatment, showed that the virus was able to slow metastases and even resulted in complete responses in a subset of in vivo breast cancer models examined [88]. Through re-challenge mouse models and gene expression analysis, the authors concluded that immune activation was crucial to the overall response in vivo [88]. Further illustrating this point, mouse cohorts that were first treated in vivo with MG1, followed by surgical resection and anti-PD-1 therapy, demonstrated 60-90% complete responses after tumor re-challenge [88]. These recent results further support previously described data points in other studies that show an increase in treatment efficacy when oncolytic viruses are used in conjunction with other anti-cancer therapeutics.

6.4. Newcastle Disease Virus

The final vector of the ssRNA group explored in breast cancer research is Newcastle Disease Virus (NDV). While NDV has been examined in the past as an oncolytic vector, only in recent years has it been tested in breast cancer. A recent study, assessing the pro-inflammatory response to NDV in a number of tumor lines, including breast cancer, found that NDV is a potent activator of type I and II interferon responses in addition to Interleukin 6 (IL-6) expression [89]. The authors of this study concluded that immune activation was crucial to the overall response in vivo [88]. Further illustrating this point, mouse cohorts that were first treated in vivo with MG1, followed by surgical resection and anti-PD-1 therapy, demonstrated 60-90% complete responses after tumor re-challenge [88]. These recent results further support previously described data points in other studies that show an increase in treatment efficacy when oncolytic viruses are used in conjunction with other anti-cancer therapeutics.

CONCLUSION: A MULTI-COMBINATION APPROACH

Throughout the development of oncolytic virotherapy, a reoccurring theme that has been gaining traction in the field, particularly in breast cancer, has been combination approaches. Monotherapeutic approaches have been crucial to the understanding the mechanisms involved in virus-specific contributions to therapeutic response and optimizing oncolytic activity. However, the inadequate efficacy and lack of complete responses at the clinical level are driving new combination approaches. Anti-cancer drugs can often result in synergistic effects when combined with oncolytic virotherapy, presenting a platform for personalized therapies. These combinations have enhanced both the drug and viral vector efficacy in vitro and in vivo in most cases provided a greater therapeutic effect. Importantly, the combinations discussed in this review have shown to improve and support anti-cancer immune responses.

An innovative approach to oncolytic virotherapy would be the combination of different oncolytic viruses to target the same disease in distinct ways. The first example of this strategy was published in 2010 by a research team in Canada, combining VSV and VV. This study examined the combination in the context of various established cancer cell lines, which resulted in the finding that the VV significantly enhanced VSV replication [74]. As described earlier, VSV is new to breast cancer virotherapy and is still met with challenges affecting its overall efficacy. However, this study introduced a new method that could enhance VSV replication dramatically [74]. Administering the treatment in the context of an aggressive 4T1 mouse tumor model in vivo, corroborated the in vitro data as well as established its safety [74]. Further infection of ex vivo tumor tissue slices from a range of cancers supported the in vitro and in vivo data as well, showing significant enhancement of viral replication when compared to singularly infected cultures [74]. While breast cancer specimens were not included in this particular study, the virus combination approach opens the door to exploit those more established oncolytic viruses in breast cancer research.

Although oncolytic viruses for use in breast cancer treatments are taking great strides towards the clinic, many hurdles still remain. For example, Ad vectors have faced challenges in clinical trials because of limited efficacy observed in patients to date. However recent studies suggest that therapeutic benefits can be improved whenAds are used in combination with therapeutic drugs [26, 30-32] or immune checkpoint inhibitors [91]. This approach is a particularly promising avenue as Ads have already been studied extensively for breast cancer treatment and have an established safety profile in clinical trials. Likewise, HSV-based therapeutic vectors such as T-VEC may be insensitive to treating all cancer cell types due to deletion of the γ34.5 gene, which also compromises the replication of the virus [42]. Novel improvements to the HSV platform could be utilized to enhance the anti-tumor effects on breast cancer [42]. VV vec-
titors have a number of advantages including selective and robust cancer cell killing using in vitro and in vivo preclinical models of breast cancer [53-58]. However, despite extensive safety experience as a live vaccine, clinical trials using oVV vectors have not included breast cancer patients to date. Recently, newer vectors such as reovirus [66], MV [82, 83], and Maraba virus [88] have shown promising preclinical results in the treatment of breast cancer. It is too soon to determine the clinical impact of these virus platforms in a clinical setting, since they will likely need further vector improvements and extensive preclinical testing.

As discussed throughout this review, combination approaches using current therapeutic drugs promise an increase in therapeutic efficacy, and highlight how quickly the oncolytic virotherapy field is developing in cancer research. Currently, there are a number of Phase I and II clinical trials using oncolytic viruses that are completed or ongoing for treating breast cancer patients, as shown in Table 5. The majority of these are utilizing a combination approach to treat advanced-stage cancers. However, while the combination approach appears promising, further challenges lie in identifying and developing successful and safe combinations. Proper combinations will likely rely on patient disease progression, prior chemotherapy and resistance, the milieu of gene mutations in oncogenes and tumor suppressor genes, virus receptor expression, and immune status. This approach will likely present a challenge in clinical trials as it suggests a degree of personalization that may not be easily replicated among individuals. Nevertheless, with safety profiles established for many of the vector platforms, oncolytic virotherapy represent a new era of breast cancer therapy in which potentially effective and well-tolerated regimens may also further improve quality of life post-treatment.

**LIST OF ABBREVIATIONS**

- ssRNA = Negative-Sense Single-Stranded RNA
- +ssRNA = Positive-Sense Single-Stranded RNA
- Ad3 = Ad Serotype 3
- Ad5 = Ad Serotype 5
- Ad48 = Ad Serotype 48
- Ad = Adenovirus
- BVDU = Bromovinyldeoxyuridine

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Table 5. Clinical trials for breast cancer treatment using oncolytic virotherapy approaches.

| Phase | Virus | Additional Therapy | Disease | Status | ID |
|-------|-------|-------------------|---------|--------|----|
| I     | vvDD-CDSR (VV) | None | Melanoma, HNSCC, Breast, Liver, colorectal, and Pancreatic cancers | Completed | NCT00574977 |
| I     | CVA21 (Coxsackievirus) | None | Solid tumor cancers | Completed | NCT00636558 |
| I     | HF10 (HSV) | None | Refractory HNSCC, Skin SCC, Breast carcinoma, Melanoma | Completed | NCT01017185 |
| II    | Reolysin (reovirus) | Paclitaxel | Metastatic breast cancer | Completed | NCT01656538 |
| I     | MV-NIS (MV) | None | Metastatic breast cancer and HNSCC | Active, not recruiting | NCT01846091 |
| I     | VCN-01 (Ad) | Gemcitabine Abraxane | Advanced/metastatic tumors pancreatic adenocarcinoma | Recruiting | NCT02045602 |
| I/II | MG1MA3 (oncolytic Maraba) and AdMA3 (Ad vaccine) | None | Advanced/metastatic solid tumors | Recruiting | NCT02285816 |
| I     | Toca 511 (retroviral replicating vector) | Toca FC (5-fluorocytosine formulation) | Solid tumors, Lymphoma | Recruiting | NCT02576665 |
| I/II | JX-594 (VV) | Metronomic CP | Advanced breast cancer, soft-tissue sarcomas | Recruiting | NCT02630368 |
| I/II | Talamogene Laherparepvec (HSV) | Paclitaxel | TNBC | Recruiting | NCT02779855 |
| I     | Pexa-Vcc (VV) | Ipilimumab | Metastatic/Advanced tumors | Recruiting | NCT02977156 |
| II    | ADV/HSV-tk (Ad) | Valacyclovir, Pem-brolizumab, and stereotactic XRT | TNBC and NSCLC | Recruiting | NCT03004183 |
| I     | PVSRIPO (oncolytic poliovirus) | None | Stage II-IV TNBC | Not yet recruiting | NCT03564782 |

Abbreviations: Ad: Adenovirus; CP: Cyclophosphamide; HNSCC: Squamous cell carcinoma of the head and neck; HSV: Herpes simplex virus; MV: Measles virus; NSCLC: Non-small cell lung cancer; SCC: Squamous cell carcinoma; TNBC: Triple negative breast cancer; VV: Vaccinia virus; XRT: Radiation therapy.
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**REFERENCES**

1. Siegel RL, Miller KD, Jemal A. Cancer statistic, 2017. Cancer J Clin 2017; 67: 7-30.
2. American cancer society. Breast cancer facts & figures 2017-2018. [online] 2018 [cited 2017] Available at: https://www.cancer.org/content/dam/cancer-org/research/cancer-facts-and-statistics/breast-cancer-facts-and-figures/breast-cancer-facts-and-figures-2017-2018.pdf
3. Gonzalez-Angulo A, Morales-Vasquez F, Hortobagyi GN. Advances in experimental medicine and biology. Adv Exp Med Biol 2007; 608: 1-22.
4. Azim HA, de Azambuja E, Colozza M, Bines J, Piccart MJ. Long-term toxic effects of adjuvant chemotherapy in breast cancer. Ann Oncol 2011; 22(9): 1939-47.
5. Anderson RA, Themmen APN, Qahtani AA, Groome NP, Cameron DA. The effects of chemotherapy and long-term gonadotrophin suppression on the ovarian reserve in premenopausal women with breast cancer. Human Reprod 2006; 21(10): 2583-92.
6. Mols F, Beijers T, Vreugdenhil G, van de Poll-Franse L. Chemotherapy-induced peripheral neuropathy and its association with quality of life: a systematic review. Support Care Cancer 2014; 22(8): 2261-9.
7. Taylor CW, McIsaac P, Darby SC. Cardiac risks of breast-cancer radiotherapy: a contemporary view. Clin Oncol 2006; 18(3): 236-46.
8. Bird B, Swain SM. Cardiac toxicity in breast cancer survivors: review of potential cardiac problems. Clin Cancer Res 2008; 14(1): 14-24.
9. Mols F, Vingerhoets A, Coebergh J, van de Poll-Franse LV. Quality of life among long-term breast cancer survivors: A systematic review. Eur J Cancer 2005; 41(17): 2613-19.
10. Amgen. FDA approves IMLYGIC™ (Talimogene Laherparepvec) as first oncolytic viral therapy in the US. [online] 2018 [cited Oct 27, 2015]. Available at: https://www.amgen.com/media/news-releases/2015/10/fda-approves-imlygic-talimogene-laherparepvec-as-first-oncolytic-viral-therapy-in-the-us/
11. Kim M, Zinn KR, Barnett BG, et al. The therapeutic efficacy of adenoviral vectors for cancer gene therapy is limited by a low level of primary adenovirus receptors on tumour cells. Eur J Cancer 2002; 38(14): 1917-26.
12. Dimitriev I, Krasnykh V, Miller C, et al. An adenovirus vector with genetically modified fibers demonstrates expanded tropism via utilization of a coxsackievirus and adenovirus receptor-independent cell entry mechanism. J Virol 1998; 72(12): 9706-13.
13. Glasgow JN, Curiel DT. Transductional targeting of adenovirus vectors for gene therapy. Cancer Gene Ther 2006; 13(9): 830-44.
target solely HER-2-positive cells. Proc Natl AcadSci USA 2009; 106(22): 9039-44.

Cobleigh MA, Vogel CL, Tripathy D, et al. Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. J Clin Oncol 1999; 17(9): 2639-39.

Carroll NM, Chioccia E, Takahashi K, Tanabe KK. Enhancement of gene therapy specificity for diffuse colon carcinoma liver metastases with recombinant herpes simplex virus. Ann Surg 1996; 224(3): 323-30.

York IA, Roop C, Andrews DW, et al. A cytolic herpes simplex virus protein inhibits antigen presentation to CD8 T lymphocytes. Cell 1994; 77(4): 525-35.

Wang J, Hu P, Rabkin SD, Liu R. Oncolytic herpes simplex virus treatment of metastatic breast cancer. Int J Onco 2012; 40: 757-63.

Li J, Zeng W, Huang Y, et al. Treatment of breast cancer stem cells with oncolytic herpes simple virus. Cancer Gene Ther 2012; 19(10): 707-14.

Fan J, Jiang H, Cheng L, Liu R. The oncolytic herpes simplex virus vector, G47A, effectively targets tamoxifen-resistant breast cancer cells. Oncol Rep 2016; 35(1): 1741-49.

Cheng L, Jiang H, Fan J, et al. A novel oncolytic herpes simplex virus armed with the carboxyl-terminus of murine MyD116 has enhanced anti-tumour efficacy against human breast cancer cells. Oncol Lett 2018; 15(5): 7046-52.

Walker JD, Sehgal I, Kousoulas KG. Oncolytic Herpes Simplex Virus I encoding 15-Prostaglandin Dehydrogenase mitigates immune suppression and reduces ectopic primary and metastatic breast cancer in mice. J Virol 2011; 85(14): 7363-71.

Sahin T, Kasuya H, Nomura N, et al. Impact of novel oncolytic virus HF10 on cellular components of the tumour microenvironment in patients with recurrent breast cancer. Cancer Gene Ther 2011; 19(4): 229-37.

Kasuya H, Koderia Y, Nakao A, et al. Phase I dose-escalation clinical trial of HF10 oncolytic herpes virus in 17 Japanese patients with advanced cancer. Hepato-gastroenterology 2014; 61: 599-605.

Nagano S, Perentes J, Jain RK, Boucher Y. Cancer cell death enhances the penetration and efficacy of oncolytic Herpes Simplex Virus in tumors. Cancer Res 2008; 68(10): 3795-802.

Cody JJ, Markert JM, Hurst DR. Histone deacetylase inhibitors improve the replication of oncolytic herpes simplex virus in breast cancer cells. PLoS One 2014; 9(3): e92919.

Zhuang X, Zhang W, Chen Y, et al. Doxorubicin-enriched, ALDH1+ breast cancer stem cells are treatable to oncolytic herpes simplex virus type 1. BMC Cancer 2012; 12(1): 1-16.

Tan G, Kasuya H, Sahin T, et al. Combination therapy of oncolytic herpes simplex virus HF10 and bevacizumab against experimental model of human breast carcinoma xenograft. Int J Cancer 2015; 137(1): 171-82.

Potent antitumor effects of targeted promoter-driven oncolytic adenovirus armed with Dm-dNK for breast cancer in vitro and in vivo. Cancer Lett 2013; 328(1): 95-103.

The effect of the carboxyl-terminal portion of HER2-positive cancer. Mol Cancer Ther 2016; 15(9): 2259-69.

Bramante S, Koski A, Liikanen I, et al. Oncolytic virotherapy for treatment of breast cancer, including triple-negative breast cancer. OncolImmunology 2015; 5(2): e107805.

Chou J, Kern ER, Whitley RJ, Roizman B. Mapping of herpes simplex virus 1 neurovirulence to (gamma)134.5, a gene nonessential for growth in culture. Science 1990; 250: 1262-6.

Menotti L, Cerretani A, Hengel H, Campadelli-Fiume G. Construction of a fully retrograded herpes simplex virus 1 recombinant capable of entering cells solely via human epidermal growth factor Receptor 2. J Virol 2008; 82(20): 10153-61.

Menotti L, Nicoletti G, Gatta V, et al. Inhibition of human tumor growth in mice by an oncolytic herpes simplex virus designed to...
Oncolytic Virotherapy for Breast Cancer Treatment

[57] John LB, Howland LJ, Flynn JK, et al. Oncolytic virus and anti-4-1BB combination therapy elicits strong antitumor immunity against established cancer. Cancer Res 2012; 72(7): 1651-60.

[58] McCart J, Ward JM, Lee J, et al. Systemic cancer therapy with a tumor-selective vaccinia virus mutant lacking thymidine kinase and vaccinia growth factor genes. Cancer Res 2001; 61: 8751-57.

[59] Sabin AB. Reoviruses. A new group of respiratory and enteric viruses formerly classified as ECHO type 10 is described. Science 1959; 130(3386): 1387-9.

[60] Hata Y, Etoh T, Inomata M, et al. Efficacy of oncolytic reovirus against human breast cancer cells. Oncol Rep 2008; 19: 1395-8.

[61] Strong JE, Coffey MC, Tang D, Sabinin P, Lee PWK. The molecular basis of viral oncolysis: usurpation of the Ras signaling pathway by reovirus. EMBO J 1998; 17(12): 3351-62.

[62] Marcato P, Dean CA, Giacomantonio CA, Lee PWK. Oncolytic reovirus effectively targets breast cancer stem cells. Molecular Therapy 2009; 17(6): 972-9.

[63] Morris DG, Feng X, DiFrancesco LM, et al. REO-001: A phase I trial of percutaneous intraskeletal administration of reovirus type 3 dearing (Reo lysin®) in patients with advanced solid tumors. Invest New Drugs 2013; 31(3): 696-706.

[64] Comins C, Spicer J, Protheroe A, et al. REO-10: A phase I study of intravenous reovirus and docetaxel in patients with advanced cancer. Clin Cancer Res 2010; 16(22): 5364-72.

[65] Loikema MP, Arkenau H-T, Harrington K, et al. A Phase I study of the combination of intravenous Reovirus Type 3 Dearing and gemcitabine in patients with advanced cancer. Clin Cancer Res 2011; 17(3): 581-8.

[66] Mostafa AA, Meyers DE, Thirukkanmaran CM, et al. Oncolytic reovirus and immune checkpoint inhibition as a novel immunotherapeutic strategy for breast cancer. Cancers 2018; 10(6): 205.

[67] Regidor P, Callies R, Regidor M, Schindler A. Expression of the cell adhesion molecules ICAM-1 and VCAM-1 in the cytosol of breast cancer tissue, benign breast tissue and corresponding sera. Eur J Gynaecol Oncol 1998; 19: 377-83.

[68] Skelding KA, Barry RD, Shafren DR. Systemic targeting of metastatic human breast tumor xenografts by Coxsvackievirus A21. Breast Cancer Res Treat 2009; 113(1): 21-30.

[69] Sagara M, Takishima Y, Miyamoto S, et al. 409. Novel recombinant Coxsvackievirus B3 infection elicits robust oncolytic activity against human non-small lung cancer and triple-negative breast cancer. Mol Ther 2016; 24: S162.

[70] Gromeier M, Alexander L, Wimmer E. Internal ribosomal entry site substitution eliminates neurovirulence in intergeneric poliovirus recombinants. Proc Natl Acad Sci USA 1996; 93(6): 2370-5.

[71] Holl EK, Brown MC, Boczkowski D, et al. Recombinant oncolytic poliovirus, PVSRIPO, has potent cytotoxic and innate inflammatory effects, mediating therapy in human breast and prostate cancer xenograft models. Oncotarget 2016; 7(48): 79828-41.

[72] Ahmed M, Puckett S, Lyles DS. Susceptibility of breast cancer cells to an oncolytic matrix (M) protein mutant of vesicular stomatitis virus. Cancer Gene Ther 2010; 17(12): 883-92.

[73] Arulananandam R, Batenchuk C, Varette O, et al. Microtubule disruption synergizes with oncolytic virotherapy by inhibiting interferon translation and potentiating bystander killing. Nat Commun 2015; 6: 6410.

[74] Le Boeuf F, Diallo J-S, McCart AJ, et al. Synergistic interaction between oncolytic viruses augments tumor killing. Mol Ther 2010; 18(5): 888-95.

[75] Le Boeuf F, Gebremeskel S, McMullen N, et al. Reovirus FAST protein enhances vesicular stomatitis virus oncolytic virotherapy in primary and metastatic tumor models. Mol Ther - Oncolytics 2017; 6: 80-9.

[76] Dörig RE, Marcil A, Chopra A, Richardson CD. The human CD46 molecule is a receptor for measles virus (Edmonston strain). Cell 1993; 75(2): 295-305.

[77] Tatsuo H, Ono N, Tanaka K, Yanagi Y. SLAM (CDw150) is a cellular receptor for measles virus. Nature 2000; 406(6798): 893-7.

[78] Mühlbach MD, Mateo M, Sinn PL, et al. Adherens junction protein nectin-4 is the epithelial receptor for measles virus. Nature 2011; 480(7378): 530-3.

[79] Iankov ID, Msaouel P, Allen C, et al. Demonstration of anti-tumor activity of oncolytic measles virus strains in a malignant pleural effusion breast cancer model. Breast Cancer Res Treat 2010; 122(3): 745-54.

[80] Sugiyama T, Yoneda M, Kuraishi T, et al. Measles virus selectively blind to signaling lymphocyte activation molecule as a novel oncolytic virus for breast cancer treatment. Gene Ther 2012; 20(3): 338-47.

[81] Jing Y, Bejarano M, Ziais J, Merchant JR. In vivo anti-metastatic effects of uPAR retargeted measles virus in syngeneic and xenograft models of mammary cancer. Breast Cancer Res Treat 2015; 149(1): 99-108.

[82] Jing Y, Chavez V, Ban Y, et al. Molecular effects of stromal-selective targeting by uPAR-retargeted oncolytic virus in breast cancer. Mol Cancer Res 2017; 15(10): 1410-20.

[83] Jing Y, Chavez V, Karishma N, Merchant J. Antitumor efficacy of a dual stromal and tumor targeted oncolytic measles virus in breast and colon cancer models. Cancer Res 2018; 78(13 supplement): 5920-20.

[84] Iankov ID, Kurokawa CB, D’Assoro AB, et al. Inhibition of the Aurora A kinase augments the anti-tumor efficacy of oncolytic measles virotherapy. Cancer Gene Ther 2015; 22(9): 438-44.

[85] Brun J, McManus D, Lefebvre C, et al. Identification of genetically modified Maraba virus as an oncolytic rhabdovirus. Mol Ther 2010; 18(8): 1440-9.

[86] Zhang J, Tai L-H, Ilkow CS, et al. Maraba MG1 virus enhances natural killer cell function via conventional dendritic cells to reduce postoperative metastatic disease. Mol Ther 2014; 22(7): 1320-32.

[87] Bourgeois-Daigneault M-C, St-Germain L, Roy D, et al. Combination of Paclitaxel and MG1 oncolytic virus as a successful strategy for breast cancer treatment. Breast Cancer Res 2016; 18(1): 83.

[88] Bourgeois-Daigneault M-C, Roy D, Aitken A, et al. Neoadjuvant oncolytic virotherapy before surgery sensitizes triple-negative breast cancer to immune checkpoint therapy. Sci Transl Med 2018; 10(422): eaao1641.

[89] Ginting T, Suryatenggara J, Christian S, Mathew G. Proinflammatory response induced by Newcastle disease virus in tumor and normal cells. Oncolytic Virother 2017; 6: 21-30.

[90] Schirmacher V, Sticker W, Lulei M, Bihari A-S, Sprenger T. Long-term survival of a breast cancer patient with extensive liver metastases upon immune and virotherapy: a case report. Immunother 2015; 7(8): 855-60.

[91] Feola S, Capasso C, Fuciliero M, et al. Oncolytic vaccines increase the response to PD-L1 blockade in immunogenic and poorly immunogenic tumors. Oncolimmunology 2018; 7(8): e1457596.