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REVIEW ARTICLE

Oncolytic viruses: A novel treatment strategy for breast cancer

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Abstract Breast cancer, an unceasingly occurring neoplasm, is one of the major determinants of mortality in women. Several ineffective attempts have been pursued using with conventional therapies against breast cancer. Resistance to existing therapies and their respective debilitating adverse effects have led research toward a new era of cancer treatment using viruses. Virotherapy constitutes a developing treatment modality with multiple mechanisms of

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Newcastle disease virus;
Reovirus;
Vaccinia;
Vesicular stomatitis virus;
Virotherapy

therapeutic activity in which the viruses can be directly oncolyticand can express transgenes or induce host immune response against tumor cells. Several different DNA- and RNA-containing viruses have been considered for virotherapy of breast cancer including adenovirus, herpes virus, vaccinia, reovirus, Newcastle Disease virus, measles virus and vesicular stomatitis virus. This review aims to summarize the viro-therapeutical agents against breast malignancies. Key Scientific Concepts of Review: In this review paper, we proposed a new strategy to virus's combinatorial treatments using several kinds of transgenes and drugs. These recombinant viruses have provided evidence of treatment efficacy against human breast cancer.

Background

Breast cancer is the most frequently diagnosed malignancy in women,[1–7] of whom it is one of the most prevalent cause of cancer related death worldwide.[4,5,8] Since traditional therapies have limited ability to treat breast tumors, especially when metastatic,[9,10] an alternative and more efficacious treatment approach is strongly recommended.[10]

The biological cycle of viruses involves cell infection, replication and subsequent cell death with release of a progeny of virions[11] (Fig. 1). This vital feature of viruses has initiated the oncolytic virotherapy era for cancer treatment.[12–14] This novel therapeutic strategy was first introduced in the early twentieth century[15] and administered to cancer patients in 1996 in the first clinical trial[16] resulting in cancer remission following viral infection.[17]

An oncolytic virus (OV) is a natural or modified replication-selective virus infecting target tumor cells yet relatively neglecting normal cells.[13,14,18–23] This serendipitous feature indicates the large therapeutic potential of OVs thanks to great amplification inside tumor cells and concurrent clearance from normal cells. No severe adverse effects and no deaths have been recorded with virotherapy so far.[14] In recent years the significant progress of molecular biology prompted the viral properties to be exploited in cancer therapy,[11] using a wide variety of DNA and RNA OVs.[21] Oncolytic virotherapy initially was not a successful method due to the inability to confine virulence exclusively

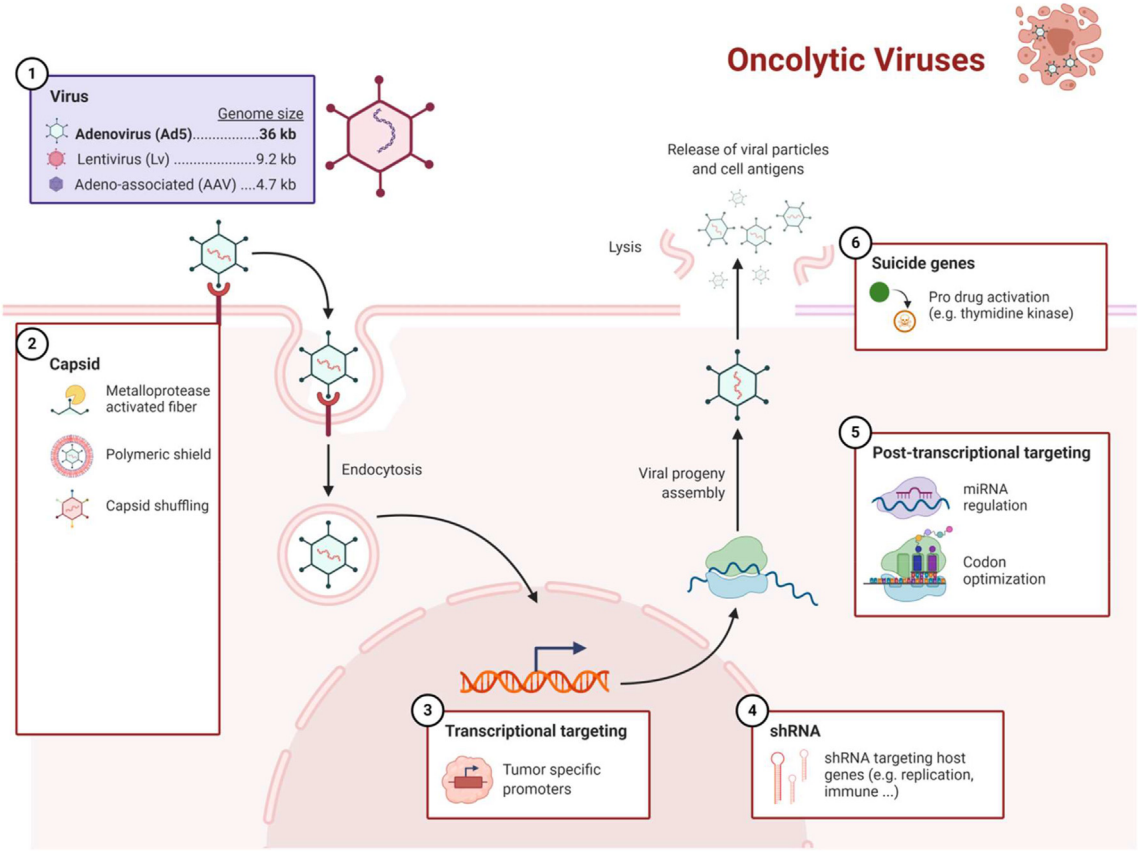

Figure 1 Schematic view of cancer cell infection and lysis by oncolytic virus (OV).

to cancer cells.[19] Characteristics of cancer cells including permanent division regardless of growth suppressors and DNA damage stress along with no cellular apoptosis and no proper response to host immune system have provided a suitable environment for OVs.[21] The Interferon-beta signal pathway is deemed a deteriorated protective mechanism of cancer cells against viral infection, thereby facilitating virus replication inside tumor cells (Fig. 2).[19]

OVs take advantage of the impaired cell signaling pathways to replicate properly in tumor cells.[22] Oncolytic virus therapy is rather favorable compared to conventional therapeutic modalities because it is capable of overcoming the resistance against traditional treatment methods[21]; hence, low toxicity[21,22] and a reasonable dose enhancement within tumor cells over time make OV therapy an appealing antineoplastic strategy.[21]

Oncolytic virus therapy has a dual mechanism of action[13,19–21,23]:

1) Oncotoxic activity of the virus; and
2) Systemic immunogenicity induced in host toward the OVs and tumor cells

The efficacy of OVs has proven both *in vitro* and *in vivo* by intravenous/systemic and local administration.[11] The intravenous (I.V.) has been recognized as the simplest treatment route to deliver OV [21,24] though the virus neutralization by a robust antibody or the complement cascade, is a concrete concrete limitation of this procedure. To overcome this obstacle, cell carriers or delivery vehicles can be used to prevent virus recognition by the immune system and facilitate its delivery to the tumor.[21,22] These vehicles include mesenchymal stem cells, monocytes, T cells and even autologous tumor cells.[22]

This paper examines the characteristics of viruses potential candidates for oncolytic therapy against breast cancer.

Discussion

DNA viruses

Adenovirus. Adenovirus is a small,[25] nonenveloped,[16,26–28] linear,[28] double-stranded DNA virus[16,26–29] with a genome size varying from 26 to 45 kb in size,[27] used in various studies as a safe OV[4,27–29] (Fig. 2). Approximately 50 serotypes of adenoviruses have been identified so far[11,26,27] with the serotype 2 and 5 tested most extensively in cancer trials.[28,30–32] Serotype 5 has been introduced as an efficacious vector for breast cancer therapy.[33] Adenovirus has low pathogenicity in humans,[12,27,28,32] causing mild infections in the respiratory tract,[27,28,32] gastro-intestinal (GI) tract and ophthalmic cavity.[27] Adenoviruses are grouped into two categories:

1) Replicative or oncolytic or replication-competent adenoviruses; and
2) Non-replicative adenoviruses, result of removing *E1-E3* genes.[34]

Adenovirus enters targets cells via receptor-mediated endocytosis[35] by primary attachment with Coxsackie–adenovirus receptor (CAR). The entrance of the virus is

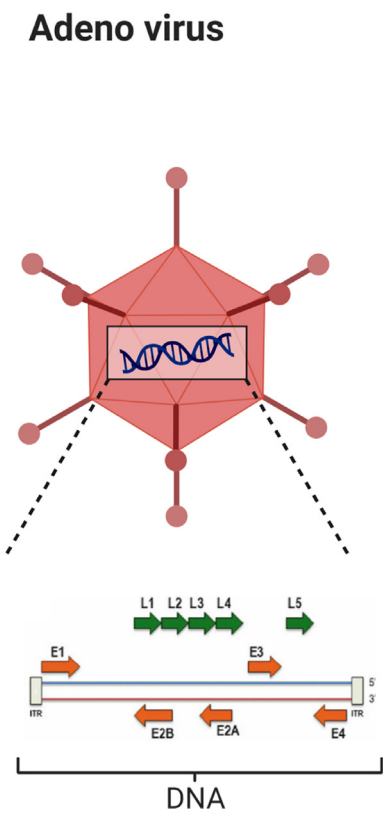

Figure 2 Schematic view of adenovirus.

mediated by the capsid arginine–glycine–aspartic acid motif interaction with αV integrins.[27,29,30,32,35,36] The higher CAR expression, the higher number of target cells are infected with adenovirus. Moreover, there is an inverse correlation between tumor stage and aggressivity with CAR expression,[31] which is low in various tumors including breast malignancies.[4] CAR is down-regulated by excessive activity of the RAS-MAPK pathway in various types of cancers[29]; In contrast, histone deacetylase inhibitors (HDIs) known as a type of antineoplastic agents, upregulate CAR expression.[22] Therefore, various signals can control CAR expression.[33] Although transduction of tumor with viruses is almost dependent on CAR expression, the extracellular matrix (ECM) plays a crucial role in the infectivity of cancer cells[31] (Fig. 3).

Oncolytic adenoviruses have been grouped into four specific classes so far.[37] Oncorine or H101[19] or ONYX-015 (dl1520) enrolled as the first OV with genetic modification in humans.[11,16] ONYX-015 is a replicative adenovirus with deletion in the E1-b region,[11,16,19,27,32,37] resulting in the suppression of E1B-55kd expression.[28] E1B-55kd is a p53-inhibitory protein and is selective for tumors with abolished p53 function[11,37] since interfering the function of p53 is a condition for virus replication.[16] The gene of interest is engineered into the deleted region[27] (Table 1).

Despite promising advances in viral therapy, some limitations feature adenoviruses as anti-cancer treatment, including[38]:

1) Inefficacy of virus to reach metastatic lesions;
2) Poor distribution in tumor lesions;
3) Narrow target cancer cell spectrum;
4) Difficulties in monitoring the transgenes being carried.

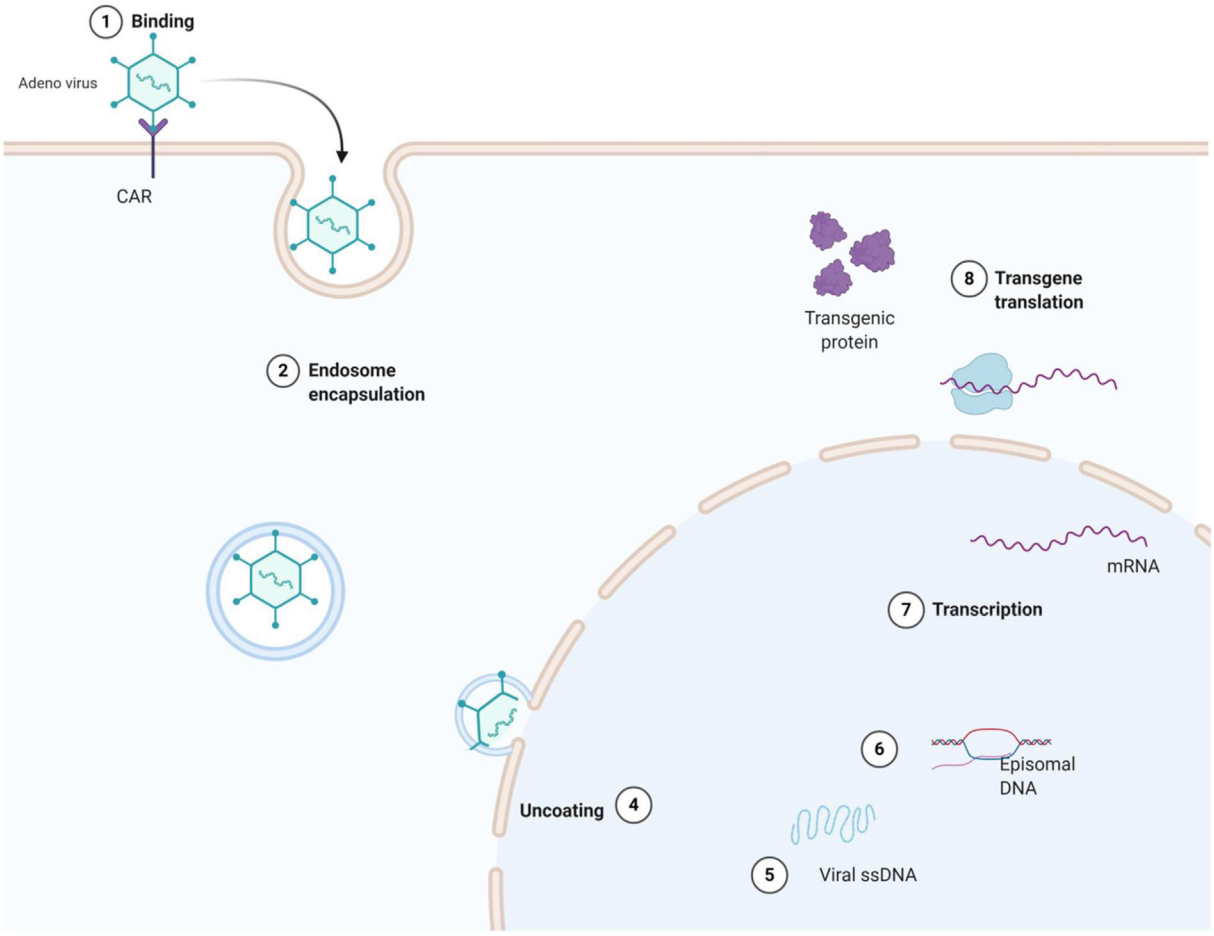

Figure 3 Schematic representation of adenovirus binding and internalization via CAR and α_V integrins, respectively.

Although OVs do not necessarily need transgenes to perform as anti-cancer agents - unlike non_oncolytic viruses -, arming them with functional immunogenic transgenes including cytokines or prodrug-activating enzymes has the potential to strengthen their tumoricidal activity.[16] Granulocyte macrophage-colony stimulating factor (GM-CSF) is a therapeutic transgene that has been introduced into the genome of several OVs.[19] The antineoplastic activity of GM-CSF is attributed to T cell activation.[16] Adenovirus vectors are the most frequently utilized viruses in gene therapy against malignant tumors,[33] as for instance Ad5—Δ24—GMCSF, an oncolytic adenovirus armed with GM-CSF. The integrin retargeted form of this recombinant virus was tested on 7 patients with breast and colorectal cancer, 3 of whom showing disease stabilization.[39] Furthermore, in a study by Yang et al[40] an adenovirus encoding for *decorin* gene yielded promising antitumor results in a metastatic breast cancer model originating from MDA-MB-231 cells. In another study human interferon consensus gene was inserted into the genome of an oncolytic adenovirus

Table 1 Summary of modified adenoviruses applied on breast malignancies.

Applied virus	Design features	Benefit and limitation of the therapy	References
Ad5—Δ24—GMCSF	Featured by GMCSF gene and retargeted towards integrin	Disease stabilization in 3 of 7 patients with breast and colorectal cancer	39
Ad.dcn	Expressing human decorin gene	Oncolysis of metastatic breast cancer	40
ad5/IFN	Armed with human IFN gene	Oncolysis of human breast cancer xenografts	28
Ad5/3-Δ24 and Ad5.pk7-Δ24	Capsid modification of Ad	Oncolysis of $CD44^{+}CD24^{-}$/low breast cancer cells *in vitro* and *in vivo*	41—43
Ad5ERE2	Inserting a pS2 promoter section into the E1a and E4 promoters of Ad5	Oncolysis of breast epithelial cells	37
Ad.DF3-E1	Regulation of the E1A gene expression with MUC1 promoter	Oncolysis of breast cancer cells *in vitro*	44
CNHK600-IL24	Armed with IL-24 gene	Oncolysis of breast tumor cells *in vitro* and *in vivo*	45

providing encouraging results in inducing regression of human breast cancer xenografts.[28] Cancer stem cells (CSC) are one of the key factors in cancer initiation, relapse, metastasis formation and resistance to treatment.[3] Two types of modified OVs Ad5/3-Δ24 and Ad5.pk7-Δ24 showed promising results against breast cancer stem cells both *in vitro* and *in vivo*.[41–43] Tissue- or tumor cell-specific promoters proved promising in developing potential therapeutic viruses, providing additional tissue selectivity.[16,37] A conditionally replicative adenovirus named Ad5ERE2 was developed to target epithelial breast cells by inserting a pS2 promoter section with two estrogen response elements into the *E1a* and *E4* promoters of parental Ad5 with the aim to regulate *E1a* and *E4* genes expression.[37] Since approximately 70% breast malignancies are considered to be estrogen-receptor positive,[7,33,37] estrogen can activate this promoter thereby stimulating virus replication and enhancement in cancer therapy.[37] On the other hand, an adenovirus was developed by regulating *E1A* gene expression with MUC1 promoter. MUC1 is an antigen being expressed aberrantly in approximately 80% of primary breast neoplasms. MUC1 promoter showed efficient gene transduction and expression of the viral gene in MUC1-expressing breast cancer cells.[44] The antitumor effect of IL-24 has also been eye-catching since it causes cell cycle arrest when transduced with adenovirus. An Ad5 armed with *IL-24* gene named CNHK600-IL24 provided potential effects on apoptosis and immune response stimulation, resulting in considerable disruption of breast tumor cells both *in vitro* and *in vivo*[45] (Table 1).

HSV. Herpes simplex virus (HSV) is an enveloped, double-stranded DNA virus[11,34,35] with a large linear genome of about 152 Kb,[11,35] used in cancer therapy as a delivery vector for oncolysis.[34] HSV-1 is more advantageous over other OVs due to[46]:

1) A wider spectrum of target cells;
2) Native oncotropism of the virus;
3) Large genome with high capacity for transgenes;
4) Availability of antiviral medicines in case of unfavorable replications;
5) Avoidance of unwanted mutations

HF10 is a spontaneously attenuated form of HSV-1 that was tested in a pre-clinical study on breast cancer-bearing mice, showing prolonged survival rate in treated mice.[1,34,47] These encouraging findings led to a clinical trial testing HF10 on six breast cancer patients, achieving a 30–40% tumor regression.[47] Another pilot clinical trial investigating the effect of HF10 on tumor micro-environment of six patients with recurrent breast cancer showed potent antitumor immunity.[1]

Talimogene laherparepvec (T-VEC), also known as or IMLYGIC[48] or OncoVEX GM-CSF[49] - a cancer therapeutic drug approved in the USA, Europe and Australia-was the first OV tested in humans.[13,20] T-VEC is a live[20] modified intralesional[49] oncolytic HSV-1 with replacement of the herpes simplex virus *ICP34.5* and *ICP47* genes with two copies of the *GM-CSF* gene.[13,47] The removal of the *ICP34.5* gene limits the neuro-pathogenicity of HSV-1,[13,20] enhancing the tumor-induced immunity with a long-term immunological memory thanks to GM-CSF factor.[13,19,34] T-VEC was clinically tested in a phase 1 clinical trial in 2006 on 30 patients with various types of metastatic tumors, including patients with head and neck malignancies, colorectal cancer, refractory breast cancer and melanoma. There was no relation between antibody titers, severity of adverse effects and therapeutic responses in the latter clinical trial. Tumors injected with T-VEC showed size reduction and an inflammatory response was observed in 14 out of 19 biopsies. There was no evidence of necrosis in non-cancerous cells in support of tumor-specificity of the virus.[49,50] In another phase 1 clinical trial on 14 breast cancer patients treated with OncoVEX GM-CSF, a reasonable efficacy and safety was detected in injected and metastatic lesions.[51] G47Δ is a further oncolytic HSV-1 which proved as effective treatment against breast cancer patients[19,46] (Fig. 4).

As mentioned above, arming viruses with immunogenic factors is a strategy to enhance their viro-therapeutical potential.[51] In this regard, a γ134.5-deleted HSV-1 expressing IL-12 displayed more effective oncolytic activity against breast cancer with brain metastases compared to an oHSV without inserted genes.[2] Furthermore, the insertion of an inhibitor of growth 4 (Ing4) (HSV1716Ing4) into an oHSV yielded high replication of virus in breast cancer cells *in vivo*. Combinational therapies have shown beneficial results in improving onco-selectivity and oncotoxicity of OVs.[51] A synergic effect of oHSV HF10 and bevacizumab was observed in a pre-clinical study of human breast cancer xenografts.[52] Furthermore, a potent antitumor effect and increased apoptosis of breast cancer cells both *in vitro* and *in vivo* studies was detected when paclitaxel and oHSV G47Δ were applied together[53] (Table 2).

Another chemo-therapeutic drug, doxorubicin, was reported to elicit enhanced antitumor activity when combined with a Type-2 oHSV in a subcutaneous syngeneic model. Moreover, combining an oHSV with the chemotherapeutic drug mitoxantrone increased neutrophils and $CD8^+$ T cell infiltration, leading to enhanced immunity response and survival rate in an immuno-competent model.[51] Moreover, the cytolytic effect of EGFR-CAR NK-92 cells was synergized in co-therapy with oHSV-1 both *in vitro* and in a mouse model bearing intracranial breast tumor[54] (Table 2). Since 10–20% of breast cancers develop brain metastases, intra-carotid injection of oHSV G47Δ after blood brain barrier disruption followed by mannitol administration was undertaken on nude mice bearing intracerebral human breast tumors, significantly increasing their survival.[24] Breast cancer is the most common cause of metastasis in meninges - affecting 5–8% of breast cancer patients -, with devastating impact on the respective survival. oHSV-1 showed promising therapeutic effect against meningeal metastatic from breast tumors.[55] Furthermore, metastatic breast malignancies reduced by more than half with (I.V.) delivery of fully HER2-retargeted oHSV using mesenchymal stromal cells (MSCs) as a carrier in NSG mice.[56]

Several agents have been used to increase the virotherapy effect against breast cancer.[51] For instance, HDIs proved to increase oHSV replication in breast cancer cell

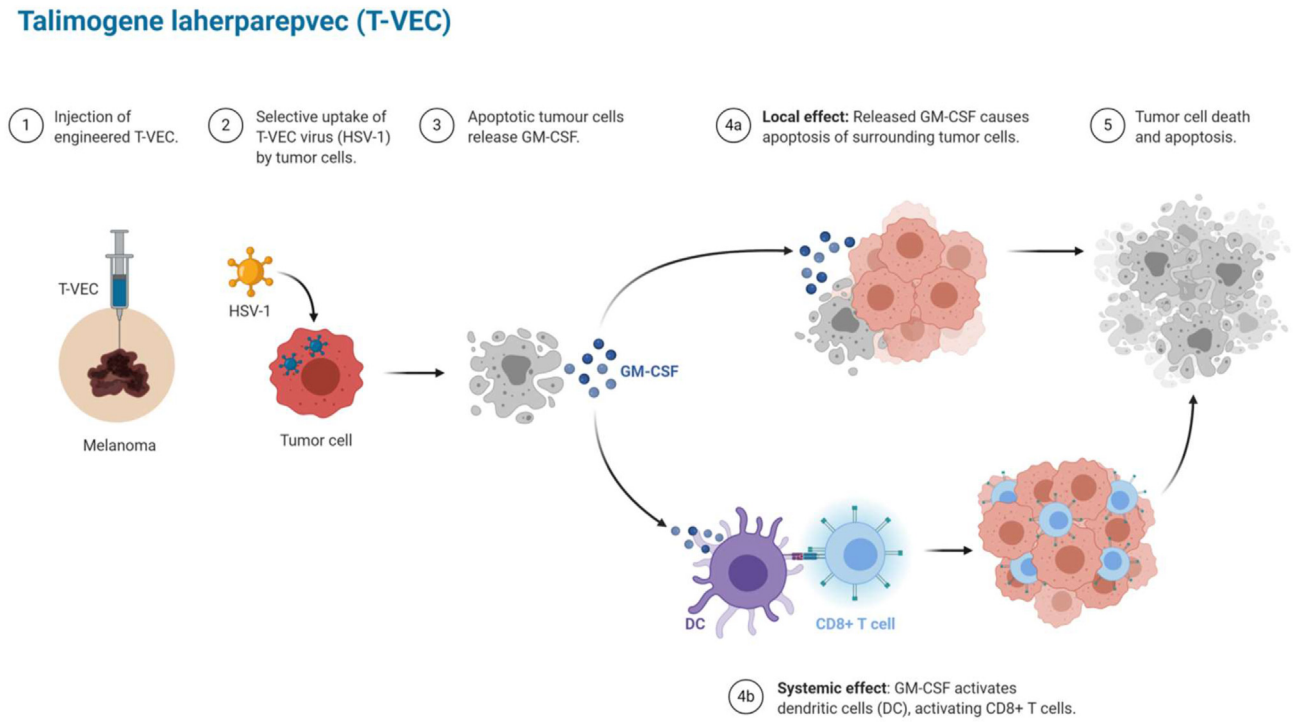

Figure 4 Schematic view of T-VEC and oncolytic HSV-1 efficacy in tumor cell.

lines than in normal breast cells.[57] Some agents used to treat other diseases could also be used as combination therapy approach for breast cancer. For instance, losartan drug showed to increase oHSV dissemination within tumors by inhibiting collagen I synthesis from mammary carcinoma-associated fibroblasts *in vitro*. Though metastatic lesions are still a challenge in breast cancer treatment, I.V. delivery as well as local injection of some viruses provided promising perspectives.[51] An oHSV expressing 15-prostaglandin dehydrogenase hampered the tumor growth and the development and accumulation of tumor cells in the lungs of mice treated by intra-lesional administration.[58] In some cases, cancer therapy yielded long-lasting anti-neoplastic immune response.[51] The HSV-1 1716 mutant, which is another version of ICP34.5-deleted HSV,[11] was used to target primary tumors of an immuno-competent mouse model, showing moderate reduction of tumor growth rate, prolonged survival and a decreased rate of lung metastases.[59] In an immunocompetent animal model, the injection of an ICP0 mutant HSV-1 virus designated oHSV KM100 into flank tumors yielded long-term tumor regression in 80% cases, averting tumor relapse and enhancing the survival rate.[60]

Two families of oHSV, the G207 and NV1020 series, were tested as breast cancer therapies in a mouse model, showing that third generation oHSV vector G47Δ, a G207, with deletions of ICP47 and US11 promoters, is efficacious against both primary and metastatic tumors of the brain, with vector NV1042 containing IL-12 being highly effective in the periphery.[61] The efficacy of I.V. adminstration of oHSV G47Δ was also assessed on secondary lung tumors originating from breast cancer in nude mice, showing a 9-fold decrease rate of lung metastasis than in control mice.[62] Oncolytic HSV G47Δ are seemingly effective in treating breast malignancies both *in vitro* and *in vivo*.[3] Likewise, oHSV G47Δ was successfully directed towards CSCs *in vitro*, diminishing their propensity of self-renewal and inducing regression of BALB/c mice tumor xenografts. Furthermore, a synergic effect of paclitaxel against NCSCs and CSCs was achieved.[63] OVs can take advantage of activated signaling pathways in cancer cells.[64] Triple-negative breast cancer (TNBC) is a sub-division of breast neoplasms with considerably high relapse rate accompanied by poor health outcomes of the respective patients.Triple negative breast cancer (TNBC) accounts for approximately 15% of breast cancers[65,66] and over-expressed levels of MEK/MAPK - a protein chain signaling from a surface cell receptor to the DNA in the nucleus of the cell - have been identified in TNBC. The effect of a genetically engineered HSV-1 (NV1066) on TNBC cell lines targeted the MEK/MAPK pathway and effectively treated TNBC[64] (Table 2).

Since hypoxia affects 40% of breast cancers, whose cells become unresponsive to chemotherapy, oHSV vectors may be useful to treat hypoxic cancer cells. The concentration of HSV-1-derived OV was estimated to be nine-fold higher in hypoxic breast cancer cells when compared with normoxic counterparts.[67]

Vaccinia. Vaccinia virus is an enveloped,[11,16,28,34] linear,[16,68] double-stranded DNA virus[11,16,28,34,68] with a large genome size of approximately 200 Kb[11,28,35] belonging to the genus orthopoxvirus and the poxvirus family.[68] Vaccinia virus is a genetically modified,[69] cytoplasmically replicating virus,[11,16,34,35,48,68,70] being appealing for oncolytic virotherapy[71] in the past 20 years.[68] The excision of two genes, including vaccinia growth factor (VGF) and

Table 2 Summary of herpes simplex viruses applied on breast malignancies.

Applied virus	Design features	Benefit and limitation of the therapy	References
HF10	Naturally mutated HSV-1	Prolonged survival rate in breast cancer-bearing mice	47
		30–100% regression of tumor in breast cancer patients	
		Oncolysis in 6 patients with recurrent breast tumor	1
HF10 + bevacizumab	Combination therapy	Oncolysis in human breast carcinoma xenografts	52
T-*VEC* or IMLYGIC or OncoVEXGM-CSF	Deletion of the herpes simplex virus ICP34.5 and ICP47 genes, armed with two copies of GM-CSF gene replaced in ICP34.5 gene region	Oncolysis in 30 patients with various types of metastatic tumors, including breast cancer	49,50
		Reduction in tumor size	
		Oncolysis in regional and distant lesions in 14 breast cancer patients	51
M002	*γ134.5-deleted HSV-1 expressing* IL-12	Oncolysis against breast cancer with brain metastases	2
HSV1716Ing4	Insertion of an inhibitor of growth 4 into an oHSV	Oncolysis in breast cancer cells *in vivo*	51
oHSV + Doxorubicin	Combination therapy	Oncolysis in a subcutaneous syngeneic model	51
oHSV + Mitoxantrone	Combination therapy	Increasing immune response and oncolysis in an immunocompetent model	51
HER2-retargeted oncolytic HSV	Generated by modification of single-chain antibody (scFv) to HER-2 in gD	>50% reduction in metastatic breast cancer in NSG mice	56
oHSV + Losartan	Combination therapy	Oncolysis by increasing oHSV dissemination within tumors	51
HSV-15PGDH	oHSV-1 expressing 15-prostaglandin dehydrogenase	Oncolysis and prevention of lung metastasis in breast cancer-bearing mice	58
HSV-1 1716	Deletion of ICP34.5	Oncolysis of primary breast tumors in a mouse model and reduction of lung metastasis	59
oHSV KM100	ICP0 mutation of HSV-1	Long term tumor regression in a breast cancer model with increased survival rate	60
G47Δ	A G207 with ICP47 and the US11 promoter deletions	Oncolysis of both primary and secondary breast tumors in brain	61
		9-fold reduction in lung metastasis of breast cancer models	62
		oncolysis of breast cancer stem cells *in vitro* and *in vivo*	3
G47Δ + paclitaxel	A G207 with ICP47 and the US11 promoter deletions accompanied by paclitaxel	Increased apoptosis in breast cancer cells both *in vitro* and *in vivo*	53
		Increased oncolysis against NCSCs and CSCs	63
G47Δ + mannitol	Combination therapy	Oncolysis and increased survival rate in nude mice bearing intracerebral human breast tumors	24
NV1042	Insertion of a murine interleukin (IL)-12 in NV1023	Oncolysis of peripheral breast tumors	61
NV1066	Removing single-copy of ICP-4, ICP-0, and g134.5 on HSV-1	Oncolysis of TNBC cell lines by targeting the MEK/MAPK pathway	64

thymidine kinase (TK) genes renders vaccinia virus exclusively selective for tumor.[69,72] The unique features of vaccinia to fight tumors are:

1) Possibility to insert large transgenes[14,28,34,73];
2) Hematogenous dissemination with a reasonable stability and efficiency,[14,34] differently from adenovirus and HSV[38];
3) High safety[14,34,73];
4) High immunogenic property[28];
5) Rapid dissemination within tumor[14,34];
6) Elevated tumor tropism.[34]

Furthermore, vaccinia enters tumor cells via a unique way called apoptotic bleb mimicry a receptor-independent entry considered an advantage of vaccinia against other OVs.[14] In a study with four breast cancer patients, the administration of 3×10^7 PFU of vaccinia within tumors was well tolerated and showed selective oncolytic features.[69] JX-594 (also Pexa-Vec) is a vaccinia virus used as the first

engineered oncolytic vaccinia virus for oncolytic therapy of cancer cells.[19,68] JX-594 has a large transgene capacity[14] and is a thymidine kinase gene deleted mutant armed with a human *GM-CSF* gene and a *Lac-Z* gene[19,38] under the control of a synthetic early/late promoter and the p7.5 promoter, respectively.[14] Although vaccinia is a virus with innate tumor cell specificity,[38] the removal of the *TK* gene and the insertion of the above mentioned genes effectively limits virus replication in cancer cells.[14,39] Low doses of JX-594 (<1 PFU/cell) proved beneficial in killing tumor cells, including breast cancer cells.[14] The potency of vaccinia virus GLV-1h68 strain was assessed in breast cancer stem-like cells (CSC) and xenografts.[74] GLV-1h68 was injected *I.V.* into human breast GI-101A cancer xenografts in nude mice to evaluate its oncolytic feature and replication capacity, showing complete regression and suppression of tumors, thanks to a reasonable tumor targeting, entry and amplification capacity.[71] For several years, recombinant vaccinia viruses have been enrolled in gene delivery as well as cancer vaccines. Furthermore, there is evidence that vaccination against small-pox decreases the risk of some malignancies as melanoma and breast cancer due to cross-immunity against *HERV-K* oncogenes.[75,76] Targeting vascular endothelial growth factor proved an advantageous therapeutic approach for breast cancer patients. With this view, a type of vaccinia virus was developed by arming it with a gene against vascular endothelial growth factor (VEGF). The anti-vasculature property of the virus yielded high anti-tumor activity in Triple negative breast cancer (TNBC) xenografts in an orthotopic murine model.[65] An oncolytic vaccinia virus with CXCR4 antagonizing property was also designed against tumor vasculature. CXCR4 antagonizing resulted in anti-angiogenesis and tumor regression in TNBC syngenic mice with primary tumors. This oncolytic vaccinia virus also effectively prevented the metastasis risk and increased overall survival of mice.[34,75] Since TNBC is an invasive breast cancer, complete margin-free surgical resection is recommended to enhance patients' survival.[77] A recombinant vaccinia virus carrying the human sodium iodide symporter (hNIS) designated GLV_1h153 targeted positive surgical margins after resection of TNBC in a murine model.[77] To augment the oncolysis effect of OVs, several other therapeutical agents involved in treatment of other diseases are also being employed against breast cancer.[51] An oncolytic vaccinia virus named GLV-1h153 expressing the *hNIS* gene and combined with radionuclide I targeted orthotopic xenografts of MDA-M-231 cells in TNBC. Tumor regression was six-fold higher in the treatment group as compared to controls treated with parental virus.[78] Furthermore, vaccinia virus VG9-IL-24 with the insertion of IL-24 was targeted against human breast cancer cells and xenografts, showing cell lysis and increased apoptosis.[70] A rather promising double recombinant vaccinia virus was also created by inserting a combination of lactopin (human milk protein with anti-tumor properties) and *GM-CSF* genes. The VV-GMCSF-Lact exhibited remarkable suppression activity of tumor growth in breast cancer cells and xenografts.[48] The efficacy and safety of a vaccinia virus recombined with tumor-associated antigen *MUC1* and *IL-2* genes was tested in breast cancer patients, showing no adverse effects and long-term T cells in tumor biopsies.[34] Furthermore, a prime-boost immunization with a vector carrying tumor-associated antigen and co-stimulatory factors elicited significant anti-tumoral immune response. Taking into account all this evidence, PANVAC-VF, a cancer vaccine therapy delivered by two combining viral vectors encoding MUC1 and CEA antigens with human T-cell co-stimulatory molecules was developed against breast and ovarian cancers, yielding significant efficacy in limiting the tumor burden.[39,79] Moreover, the median progression-free survival was 7.9 months in metastatic breast cancer patients treated with co-therapy of PANVAC and docetaxel and 3.9 months in the docetaxel-only treated group, respectively[34,68] (Table 3).

RNA viruses

RNA viruses, featured by double-stranded RNA, are alternative prominent agents for viro-therapy against a wide range of cancers.[80] Protein Kinase R (PKR), an interferon-inducible double stranded RNA-activated enzyme, plays a key role in protection against viral infection by promoting apoptosis, hence defects of PKR and/or interferon signaling pathway lead to selective replication of tumor cells and consequent viral propagation.[16]

Reovirus

REOvirus (Respiratory Enteric Orphan virus)[81] is a non-enveloped, double-stranded, RNA-containing virus[16,28,81–83] belonging to genus Orthoreoviridae[16] and Reoviridae family.[83] REOvirus is considered a benign virus[16,28,81–84] causing mild enteric and respiratory tract infections in humans.[81,82,84] Reovirus is considered a naturally occurring OV[28,43] and experimental investigations showed its ability to infect several cancer cell types including brain,[43,84] breast,[28,43,80,82,84] bladder,[43] colon,[28,43,80,82,84] glioma,[80,82] ovaries,[28,43,80,82,84] lymphoid,[28,43,82] pancreatic,[82,84] prostatic[84] and spinal[43] cancer cells. A reovirus like HSV-1 84 has an inherent propensity to infect cells with activated RAS pathway,[11,16,47] featuring 30% of human malignancies.[16,84] Whilst in normal cells PKR is phosphorylated and viral gene activation is not facilitated, in cancer cells the activation of RAS-pathway leads to a lytic infection.[16] RAS pathways can be frequently activated by factors other than mutations of RAS itself.[83,84] Although RAS mutations are infrequently observed in breast cancers,[82,85] overexpression of pathways downstream of RAS leading to aberrant RAS activation is considered to be associated with breast tumor growth.[82] Other mutations may occur in malignancies, including the activation of *c-erbB* gene, which is responsible for encoding EGF-R. C-erbB-2/HER2/neu is overexpressed in ovarian[83,86] and breast cancers.[85,86] Studies have shown that cells with an activated neu can be infected by reovirus.[83] On the other hand, reovirus was introduced as a promising therapeutic agent for breast cancer with high RAS activity regardless of HER2 overexpression. No correlation was observed between sensitivity to reovirus and HER2 expression.[82] Src is a family of non-receptor tyrosine kinases also implicated in breast cancer by activating the RAS pathway and inducing cell infection by reovirus.[83] Additionally, reovirus takes advantage of the immune system stimulation by activating dendritic cells to combat cancer.[17,81] By presenting the antigen,

Table 3 Summary of modified vaccinia viruses applied on breast malignancies.

Applied virus	Design features	Benefit and the limitation of the therapy	References
JX-594 (Pexa-Vec)	Deletion of a thymidine kinase gene plus arming with a human GM-CSF gene and a Lac-Z gene	Oncolysis of tumor cells including breast cancer cells	14
GLV-1h68	—	Oncolysis of breast cancer stem-like cells (CSC) in cell line and xenografts	74
		Oncolysis of human breast GI-101A cancer xenografts in nude mice	71
GLV-1h164	Armed with a gene against VEGF	Oncolysis in TNBC xenografts in an orthotopic murine model	65
OVV-CXCR4-A	Armed with CXCR4 antagonist gene	Oncolysis in TNBC syngeneic mice with primary tumors	34,75
GLV_1h153	Armed with the human sodium iodide symporter (hNIS) gene	Oncolysis of positive surgical margins after resection in TNBC murine model	76
GLV_1h153 + radionuclide I	Combination therapy	Six fold higher oncolysis in an orthotopic xenografts of MDA-M-231 cells in TNBC	78
VG9-IL-24	Vaccinia virus strain Guang9 armed with IL-24 gene	Oncolysis of human breast cancer cells and xenografts	70
VV-GMCSF-Lact	Armed with lactopin and GM-CSF genes	Oncolysis in breast cancer cells and xenografts	48
VV-MUC1-IL-2 (*TG-1031*)	Armed with the tumor-associated antigen MUC1 and IL-2 genes	Potent oncolysis in advanced inoperable breast cancer recurrences	34
PANVAC-VF	Combined with human T-cell costimulatory molecules	Oncolysis of breast and ovarian cancers in limited tumor burden	39,79
PANVAC + docetaxel	Vaccinia prime, fowlpox boost	Combination therapy: Increased median progression-free survival in metastatic breast cancer patients	34,68

dendritic cells collaborate with reovirus to initiate innate and adaptive immune system responses against the virus regulating also their magnitude.[17] Hence, reovirus proved capable of inducing a systemic effect even by intralesional injection.[51] TRAIL (TNF-related apoptosis-inducing ligand) is a potential therapeutic ligand implicated in apoptosis. Research on HEK293 cell lines, lung cell lines (A157/H549) and breast malignancies (MDA231/ZR75-1) have shown that reovirus-infected cells are more susceptible to death by exogenous TRAIL.[17] By contrast, immuno-suppression promoted reoviral-induced oncolysis in immune-competent animal models.[84]

Since cancer-initiating cells are resistant to commonly used cancer treatment, CSC may be targeted, as pointed out by a study on human breast cancer xenografts using an oncolytic reovirus, showing significant tumor regression and reduction of the CSC population equalizing the reduction of non-CSC tumor cells.[43] To increase the treatment efficacy of reovirus, a study by Mostafa et al was done.

A study on murine breast cancer cells was undertaken to increase the treatment efficacy of reovirus by synergizing its oncolytic effect with the immune checkpoint blockade feature of PD-L1, showing promising results in terms of tumor regression and improved survival rate.[87] Several studies have confirmed the oncolytic property of reovirus. In a study on six breast cancer cell lines, Hs578Bst normal mammary gland epithelial cell line (the control) showed no infection, upholding the concept of normal cells spared from reovirus infection differently from tumorous cells.[17] Moreover, the oncolytic capacity of reovirus was tested on 8 severe combined immunodeficient (SCID) mice models whose hind flank was implanted with v-erbB—transformed NIH-3T3 fibroblasts, undergoing tumor regression in 6 of them 12 months following intra-lesion injection of the virus in tumor xenografts.[83] The same outcome was achieved also against xenografts of other human cancer cell lines (breast, colorectal and prostate malignancies).[83] Similar to other OVs, the efficacy of reovirus may be weakened by circulating antibodies, as confirmed by a phase I study on immunocompetent mice, whose tumors relapsed three weeks after an initial growth inhibition following systemic administration of reovirus, with the concomitant rise of serum anti-reoviral antibodies. In the latter study the maximum anti-reoviral antibody concentration was reached on day 7 for 36% mice and day 14 in 61% of them, suggesting that the systemic administration of reovirus should be fast, frequent and at high doses during the first week of treatment, before the rise of antiretroviral antibodies[19] (Table 4).

A combination of topoisomerase inhibitors as DNA-damaging agents with reovirus yielded promising therapeutic effects in breast cancer therapy.[66]

Pelareorep[69] (Reolysin®) is a wild form of reovirus[34] administered for the first time in the USA to 2 patients affected by breast cancer and 16 other solid tumor-bearing patients to evaluate the maximum dose tolerated. A low toxicity resolving over time was observed with Reolysin, along with a partial response in one breast cancer, whose size shrank by 34%.[47] Furthermore, is administration of

Table 4 Summary of reoviruses applied on breast malignancies.

Applied virus	Design features	Benefit and the limitation of the therapy	References
Reovirus	—	Oncolysis of human breast cancer xenografts by targeting cancer stem cells (CSCs)	[43]
		Oncolysis of six breast cancer cell lines and sparing the control Hs578Bst normal mammary gland epithelial cell line	[17]
		Oncolysis of breast cancer cell lines as tumor xenografts on SCID mice models	[83]
Reovirus	Combined with PD-1 blockade	Oncolysis of murine breast cancer cells with increasing survival rate	[86]
Reovirus + topoisomerase inhibitors	Combination therapy	Potent oncolysis in triple-negative breast cancer patients	[66]
Pelareorep (Reolysin®)	Wild form of reovirus	Applied on 2 breast cancer patients that resulted in partial oncolysis with 34% tumor shrinkage in one of them	[47]
		Increased median overall survival (OS) in 74 advanced breast cancer patients	[69]
REO-010	Combination therapy of Reolysin + Docetaxel	Reasonable oncolysis of advanced tumors including breast cancer patients	[34]
REO-009	Combination therapy of Reolysin + Gemcitabine	Partial oncolysis of advanced tumors including breast cancer patients	[34]

Reolysin with docetaxel was also performed in a phase 1 clinical trial (REO-010) on patients affected by advanced tumors including breast cancer, exhibiting safe as well as reasonably effective antitumor response. A partial anti-tumor response was also observed in another phase I trial (REO-009) on co-therapy with Reolysin and gemcitabine in patients affected with advanced tumors.[34] Finally, the synergistic effect of pelareorep combined with paclitaxel was evaluated in a phase II trial on 74 advanced breast cancer patients, showing a significantly improved median overall survival in the group undergoing combinatory therapy.[69]

Measles

Measles virus (MV) is an enveloped[9,15,88] single-stranded RNA virus[15] belonging to the genus Morbillivirus[9,15] and the family of Paramyxoviruses,[9,15,88] featured by a long non-segmented genome[15,88] of 16 kb size[15] (Table 5).

The anti-cancer property of MV was first discovered in 1949 by regression of Hodgkin's lymphoma following infection by wild type MV.[89] The oncolytic MV is an attenuated vaccine strain derived from the Edmonston-B (MV-Edm) vaccine lineage,[89] proving reasonable safety and potency as anti-cancer treatment both *in vitro* and *in vivo* preclinical studies.[90] MV replicates totally in the

Table 5 Summary of measles viruses applied on breast malignancies.

Applied virus	Design features	Benefit and the limitation of the therapy	References
Measles	—	Managing pleural effusions in breast cancer patients by direct intrapleural and IV administration	[51,88]
MV-s-NAP and MV-lambda-NAP	Armed with helicobacter-pylori neutrophil-activating gene	Oncolysis in breast cancer metastases	[15,93]
rMV-SLAMblind	Engineered toward SLAM blindness	Oncolysis of PVRL4-positive breast cancer cells	[9]
rMV-BNiP3	Armed with BNiP3 gene	Oncolysis in breast cancer cells *in vitro*	[8]
Measles + Aurora A kinase inhibitor alisertib	Combination therapy	Oncolysis in aggressive breast cancer cell lines and xenografts	[89,93]
Measles + small molecule inhibitor of the Rho family	Combination therapy	Oncolysis of breast cancer cells *in vitro* and *in vivo*	[51]
MV-m-uPA and MV-h-uPA	Designed as stromal-selective	Oncolysis of breast cancer in both syngeneic and xenotransplant models	[90]

cytoplasm,[9] using CD46, CD150 and nectin-4 receptors to enter the cell. CD150 or signal lymphocyte-activation molecule (SLAM) is merely used by wild-type MVs 15 but CD150 also is found both in wild type and vaccine strains,[9] hence SLAM-positive cells are the main targets of MVs.[9] CD46, represented only in attenuated types of MVs,[9,15] is a highly-expressed receptor in variety of cancers including breast malignancies[91] and its density is a good determinant of targeted selectivity for attenuated MV.[22,91] Nectin-4 receptor or poliovirus-receptor-like-4 (PVRL-4) is a transmembrane glycoprotein on epithelial cells featuring both wild types and attenuated MVs.[9,15,89] Nectin-4 receptor, a reliable indicator of viral spread into human airways,[9,15,89] is an up-regulated biomarker of breast tumor,[9,89,92] ovarian and lung cancer.[89,92] Cell to cell fusion of infected cells forming syncytia is an exclusive characteristics of the infection by MV leading to apoptosis.[15]

Attenuated MV strains look promising for oncolysis of human breast cancer xenografts.[88,93] Tumor cell-specificity of MV is merely related to high expression of CD46 on cancer cells compared to non-cancerous normal cells.[88] Expression of transgenes such as helicobacter-pylori neutrophil-activating protein by MVs showed effective antitumor immunity in breast cancer metastases.[15,93]

Direct intra-pleural and *I.V.* administration of MV proved effective as an oncolytic approach to manage pleural effusions in breast cancer.[51,88] An engineered MV named rMV-SLAMblind, with mutations leading blindness to SLAM, was designed to infect breast cancer cells while sparing SLAM-positive lymphoid cells. This recombinant MV, which is both SLAM-negative and CD46-negative, was recognized to have a relevant effect on PVRL4-positive breast cancer cells[9] (Table 5).

The oncolytic property of MV was also enhanced by insertional modifications, although a pro-apoptotic gene named BNiP3 was inserted into the MV genome (rMV-BNiP3) to make an armed MV proving effective to kill and induce apoptosis of breast cancer cells.[8] Since combinational strategies maximize the oncolytic activity of OVs, the MV-mediated cytolysis has been increased by co-treatment with heat shock protein 90 (HSP90) inhibitors.[51,89] Co-therapy of MV with geldanamycin (GA) as a HSP90 inhibitor enhanced the virus activity in several tumor cell lines.[22] Moreover, the combination of Aurora A kinase inhibitor alisertib with MV, boosted the therapeutic effects of MV in aggressive breast cancer cell lines and xenografts.[89,93] Additionally, the combinatorial effect of paclitaxel with oncolytic MV showed significant tumor growth inhibition.[51] These satisfactory results pushed to investigate combinatorial strategies to maximize the effect of MVs against cancers,[93] for instance by targeting cytoskeleton components. A small molecule inhibitor of the Rho family proved effective to improve the oncolytic effect of MV against breast cancer cells both *in vitro* and *in vivo*.[51] Tumor microenvironment (TME) components are one of the key factors in viro-therapy efficiency. Tumor-associated macrophages (TAMs) are one of these components potentiating the antitumor property of OVs.[94] The biological effect of OVs on TME plays a crucial role on tumor progression[90] and factors targeting the tumor micro-environment are more effective.[51] A stromal-selective MV, directed towards breast cancer cells in both syngeneic and xenotransplant models, mediated prevention of tumor progression and extended survival when compared with the control group[90] (Table 5).

Newcastle disease virus (NDV)

Newcastle disease is a viral avian disease caused by a virus named Newcastle Disease Virus (NDV), firstly reported in Indonesia and England[95] (Table 6). NDV is an enveloped,[28,96] non-segmented, single-stranded RNA virus with a genome size of 15.9 kb[95] belonging to the genus Rubulavirus and the family Paramyxoviridae.[95,96] Oncolysis by NDV, an appealing anti-cancer agent with minimal side effects,[97] was pioneered by Cassel et al in the 1960s[98–101] with NDV 73-T,[98] a lytic strain[98] replicating in cancerous cells and inducing syncytia formation and apoptosis.[80,98] It is reported that the apoptotic mechanism of NDV 73-T strain uses the extrinsic death pathway based upon enhanced secretion of INF-alpha and TNF-alpha by peripheral blood mononuclear cells (PBMCs).[98] Many studies on NDV 73-T strain against human tumor xenografts of neuroblastoma, fibrosarcoma, epidermoid, colon, lung, breast, prostate and subcutaneous IMR-32 neuroblastoma have shown significant tumor regression.[28,80] A purified NDV AF2240 strain elicited apoptotis in 50% of cancer cells MDA-MB-231 *in vitro*, inhibiting also their proliferation, while it had no detrimental effect on non-cancerous human umbilical endothelial cells HUVECs and human epithelial breast cells line Hs578Bst. NDV-AF2240 replicates more efficiently in MDA-MB-231 breast cancer cells than in MCF-7 cells.[102]

Several pre-clinical studies have been conducted with native and recombinant NDV.[103] In a phase I pre-clinical study, the systemic administration of a naturally attenuated strain of NDV (PV701) to 79 patients with advanced solid tumors achieved complete tumor regression in several types of cancers with different origins, including epithelial derived cancers (breast, colon, lung, and prostate) and cancers with neuro-ectodermal and mesenchymal origins, with minimal toxicity even at high doses of NDV-PV701[98] (Table 6). Therefore, NDV can be administered at high doses, and when the infusion time is extended from 1 to 3 h, the NDV toxicity progressively reduces.[16] The immunogenicity of NDV can be amplified by adding IL-2 and GM-CSF recombinant genes to the viral coding sequence.[22] *In vitro* studies with recombinant NDV, especially rNDV/IL2, have shown robust anti-cancer activity against several human cell lines including human mammary carcinoma cell line MCF-7, human colon adenocarcinoma cell line HT29, and human Jurkat cell line.[98] Treating mice bearing tumor xenografts with NDV/IL2 also showed T-cell infiltration and tumor regression. Furthermore, the insertion of GM-CSF as an incorporated therapeutic gene activates the innate immunity amplifying the production of interferon and increasing the anti-neoplastic properties of NDV.[104]

Various clinical trials were performed on autologous tumor cell vaccine NDV (ATV-NDV) following inactivation of patient-derived tumor cells by irradiation,[103,105] increasing the production of INF a/b and IL-2 thereby stimulating host immunogenicity. Considerable anti-cancer outcomes and increased survival were obtained by using ATV-NDV on various types of cancers, including breast, colorectal, ovarian, renal cell, head and neck and glial tumors.[100,103]

In end-stage breast and ovarian cancer patients and especially in early-stage breast cancer, two or more

Table 6 Summary of Newcastle Disease Viruses applied on breast malignancies.

Applied virus	Design features	Benefit and the limitation of the therapy	References
NDV AF2240	—	Oncolysis in 50% of human breast cancer cell line MDA-MB-231 *in vitro* with no effect on noncancerous cells (HUVECs and Hs578Bst)	97
		Oncolysis of MDA-MB-231 breast cancer cells compared to MCF-7 cells	102
NDV-PV701	Naturally attenuated strain	Oncolysis and complete tumor regression in seventy-nine patients with advanced solid tumors including breast cancer	98
rNDV/IL2	Armed with IL-2	Oncolysis of several human cell lines including human mammary carcinoma cell line MCF-7, the human colon adenocarcinoma cell line HT29, and human Jurkat cell line	98
ATV-NDV	An autologous NDV-modified tumor vaccine	Increase host immunogenicity and oncolysis of various types of cancers including breast cancer	100,103
		Increased survival rate, especially in early-stage breast cancer patients	103
NDV + radiofrequency hyperthermia (RHT) + dendritic cell (DC) vaccination	Combination therapy	Increased survival rate and quality of life in patients with secondary malignancy of breast in the liver	106
NDV + glycolysis inhibitor 2-Deoxyglucose (2-DG)	Combination therapy	Oncolysis of *in vitro* mouse and human breast cancer cell cultures and xenografts	107

administrations of ATV-NDV vaccination significantly increased the survival rate of cancer patients.[103] Schirrmacher indicated that the efficacy of ATV-NDV seems to vary by quality of the vaccine, showing about 36% higher five-year survival rate in high-grade breast cancer patients administered with high-quality vaccine than low-quality one.[105]

Combination of radiofrequency hyperthermia (RHT) with virotherapy by systemic oncolytic NDV and dendritic cell (DC) vaccination led to expansion of tumor-reactive memory T-cells resulting in longer survival (>66 months) in comparison to counterparts receiving conventional treatment. Furthermore, stable disease and a high quality of life were elicited.[106]

Since glucose uptake is higher in cancer cells compared to normal counterparts, targeting glucose metabolism is considered a rational strategy to reduce tumor growth. The combination of NDV and a glycolysis inhibitor 2-Deoxyglucose (2-DG) can have a synergic effect *in vitro* in mouse and human breast cancer cell cultures and xenografts.[107]

Vesicular stomatitis virus (VSV)

Vesicular stomatitis virus (VSV) is an enveloped,[108–111] negative, single-stranded RNA genome[81,111,112] of 11 Kb size[113] belonging to the genus Vesiculovirus of the Rhabdoviridae family.[80,112] The five proteins encoded by VSV include the envelope glycoprotein (G), matrix (M) protein, nucleocapsid (N) protein, large polymerase (L) and phosphoprotein (P).[111–113] Glycoprotein G cooperates with the infectious property of VSV in most mammalian cells.[113]

VSV is a another promising candidate for viro-therapy against malignant tumors[108–110] (Table 7). Since it is highly sensitive to Interferon, VSV is safe for normal human cells but has oncolytic effect on cancer cells, featured by a malfunctioning antiviral immune response with diminished interferon signaling.[22,81,114,115]

The rapid replication kinetics of VSV (8–12 h) ensures an oncolytic activity before the production of neutralizing antibodies by the host 114. The combination of VSV with HDIs decreases the interferon-mediated antiviral effect, thereby enhancing the anticancer activity of VSV against several kinds of tumors.[22]

The combinational therapy of prodrug 5-fluorocytosine (5FC) with recombinant VSV encoding for cytosine deaminase/uracil phosphoribosyl-transferase suicide gene increased the anti-tumor activity of VSV and the bystander killing of uninfected cells in tumor bearing mice.[116] Furthermore, in a virus/cell-based assay virus-sensitizer molecules were used to enhance VSV replication in VSV-resistant breast cancer cell lines.[117]

Immunotherapy can have a synergic effect on oncolysis induced by OVs. In this regard, recombinant VSV with the insertion of *IL-4* gene (rVSV-IL4) functioned as a potent anti-tumor agent against breast and melanoma tumor xenografts.[112]

Several studies have been conducted restricting the replication of VSVs exclusively to tumor cells[113] Generating viruses targeting receptors over-expressed in cancer cells is

Table 7 Summary of vesicular stomatitis viruses applied on breast malignancies.

Applied virus	Design features	Benefit and the limitation of the therapy	References
VSV-CD:UPRT + 5FC	Armed with the cytosine deaminase/ uracil phosphoribosyl-transferase suicide gene and combined with prodrug 5-fluorocytosine	Increased oncolysis of non-infected bystander cells *in vitro* including breast MCF7 cells on tumor-bearing mice	[116]
VSV + VSe	Combination therapy of VSV with virus-sensitizer molecules	Enhanced VSV replication in VSV-resistant breast cancer cell lines	[117]
rVSV-IL4	Armed with IL-4 gene	Oncolysis of breast and melanoma tumor xenografts	[112]
VSV-S-GP	The entire G gene on VSV was altered to a modified glycoprotein of Sindbis virus	High affinity towards human epidermal growth factor receptors overexpressed on breast cancer cells on mice and oncolysis of tumor	[113]
[A pseudotyped VSV]	The G gene of VSV was pseudotyped with the glycoprotein of a chimeric Sindbis virus containing the Fc region of a synthetic immunoglobulin G (IgG) targeting *Staphylococcus aureus* protein A	Targeted human epidermal growth factor receptor 2 (Her2/neu) in breast cancer	[112]
rrVSV-GMCSF	Armed with a mouse GM-CSF	Increased T cell response and faster tumor elimination by targeting her2 receptor-positive breast cancer cells	[112]
VSV-TK + ganciclovir	Combination therapy of an armed VSV with the prodrug ganciclovir	Oncolysis of melanoma or breast malignancy in immunocompetent mice	[113]
VSV (M51R)-LacZ	Containing the M51R mutation in the matrix protein gene	Oncolysis of metastatic lesions of experimental breast cancer-bearing mice	[114]

a promising means to enhance the their respective oncolytic function.[51] In a mice study, the pseudotyped virus (VSV—S-GP) obtained by replacing the entire *G* gene of VSV with a modified glycoprotein of Sindbis virus, showed a high affinity towards human epidermal growth factor receptors overexpressed on breast cancer cells, reducing tumor bulk.[113] Likewise, another pseudotyped virus replacing the *G* gene of VSV with the glycoprotein of a chimeric Sindbis virus containing the Fc region of a synthetic immunoglobulin G (IgG) targeting *Staphylococcus aureus* protein A showed high affinity against human epidermal growth factor receptor 2 (Her2/neu) of breast cancer cells.[112] HER2 receptor-positive breast cancer cells were also targeted by another recombinant VSV with an insertion of mouse GM-CSF, showing increased T cell response and faster tumor elimination.[112]

To increase the bystander effect of VSV therapy, a VSV armed with the herpes thymidine kinase (TK) gene accompanied by the prodrug ganciclovir was administered to melanoma or breast malignancies in immuno-competent mice, significantly increasing the oncolytic and immunogenic activity of VSV.[113]

However, the virus activity can also be diminished by various factors and type I Interferon immunity was reportedly stimulated by macrophages surrounding various tumor cell lines (including breast tumors), giving these cells a VSV resistant status[118] (Table 7).

Tamoxifen, an anti-estrogen receptor drug being used in estrogen receptor-positive breast cancers proved a stimulatory effect on macrophage activation and type I interferon-mediated immunity response. Consequently, pre-treatment with tamoxifen showed suppressive effects on VSV replication both *in vitro* and *in vivo*.[119] Aside from *in situ* onco-toxicity of VSV, distant metastasis of breast adenocarcinoma are also responsive to VSV infection.[120] In this regard, a matrix protein mutant VSV was administered systemically to experimental breast cancer-bearing mice, selectively replicating within metastatic lesions and manifesting effective oncolytic outcomes.[114] There is evidence that $CD4^+$ T-cells are crucial elements against tumor cells and OV immunotherapy by recombinant VSV generates a long-lasting immune memory in T cells compared to other conventional therapies[121] (Table 7).

Conclusion

Viro-therapy is relatively a novel promising treatment for a wide range of human diseases, including cancer. Oncolytic viruses have led to integrated advances allowing more efficient and translatable therapies with very favorable risk—benefit ratio. Oncolytic viruses (e.g., Ad, NDV, HSV, reovirus, etc.) or alternative viruses (e.g., measles, poliovirus, VSV, vaccinia, etc.) can be tested against human cancers.

Non-engineered wild-type virus strains and innovative recombinant selectivity-enhanced viruses have shown limited success over the past 60 years in clinical trials as the first and second generation of oncolytic viruses, respectively. The use of third generation transgene-delivering armed oncolytic viruses has gained prominent appeal in

clinical settings since their potency against tumor treatment is increased by engineering additional factors to viruses.

A growing body of evidence indicates that the development of new class of drugs (i.e., viral-based therapy) in combination with other therapies or approaches (e.g. checkpoint blockade immunotherapy [Opdivo, Keytruda, etc.]) can be applied to deal with the heterogeneous nature of the tumors. Combinatorial treatments of oncolytic viruses with several kinds of transgenes and drugs can achieve highly potent viro-therapeutical effect, especially against human breast cancer. Combinatorial therapies in preclinical trials can also combine chemotherapeutical and oncolytic viruses as well as oncolytic viruses and immunomodulating agents. Oncolytic virus immunotherapy will offer new class of drugs to clinicians to optimize cancer immunotherapy.

Author contributions

M. J., S.T., L.C., N.G.H., M.K., H.N., M.M., M.M., S.H.Z., M.K.H., M.I., S.F., and R.A.S., were responsible for drafting the manuscript, reading, writing and editing supervision and contributed equally to the development of the manuscript and its revision. All authors read and approved the final manuscript.

Conflict of interests

Authors declare no conflict of interests.

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