Oncolytic Viruses PANVAC and Pelareorep as Treatment for Metastatic Breast Cancer

Willie Mieke Iwema

Rijksuniversiteit Groningen

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BSc Thesis Life science & Technology
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Department of Medical Microbiology: Molecular Virology
D. Bhatt PhD candidate
Prof. dr. C.A.H.H. Daemen

university of groningen

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Abstract

Breast cancer is the most prevalent diagnosed malignancy and the leading cause of cancer death in women. Metastatic breast cancer is a serious problem, since the 5-year survival rate drops to 27% when metastasis has occurred in different parts of the body. There is a need for new treatments for metastatic breast cancer since there is no curative treatment available yet. The oncolytic viruses PANVAC and pelareorep have shown promising anticancer activity in pre-clinical and clinical studies in a broad spectrum of malignancies, but it remains unclear whether they are suitable as anticancer treatments for patients with metastatic breast cancer.

The poxviral-based cancer vaccine PANVAC (CEA-MUC-1-TRICOM) induces antigen-specific CD4 and CD 8 T cells and antitumor activity in the host. Since the majority of the breast tumors express the antigens CEA and MUC-1, and the PANVAC vaccine consists of the vectors encoding for the transgenes of CEA and MUC-1, PANVAC can target these tumors. Therefore, PANVAC is an important vaccine against breast cancer. Several clinical trials with advanced-stage carcinomas showed that PANVAC is well-tolerated, safe to use, and clinically beneficial.

The reovirus-based cancer vaccine pelareorep (Reolysin®) induces oncolysis in different ways, using mutations in RAS-signaling pathways. As a variety of human tumors have these RAS mutations, making pelareorep an interesting anticancer agent. Clinical trials with pelareorep as mono- or combination treatment concluded that pelareorep is well-tolerated, safe to use, and induces antitumor immune responses. The clinical trial in patients with metastatic breast cancer showed no benefit of the addition of pelareorep to paclitaxel.

Even though PANVAC in combination with docetaxel showed improvement of progression-free survival in patients with metastatic breast cancer, both PANVAC and pelareorep are not suitable as a treatment for metastatic breast cancer. Further exploration on these oncolytic virus therapies in metastatic breast cancer may be of interest to design a good combinatorial therapy.

1. Introduction

Breast cancer is the most prevalent diagnosed malignancy and the leading cause of cancer death in women. Moreover, it is the second most common cause of cancer-related death worldwide[1]. The 5-year survival rate of women suffering from breast cancer is 99%, which worsens to 85% if cancer spreads to the lymph nodes. The survival rate drops even more (27%) when metastasis has occurred in different parts of the body, making metastatic breast cancer a serious problem.[2] After metastasis, there is no curative treatment available. The only option left is palliative care, to improve or maintain a good quality of life.

In the last couple of years, new treatments have become available. Next, to conventional therapies like surgery, chemotherapy, and radiotherapy, targeted therapy is upcoming. The need for new treatments is rising due to therapy resistance, which means that tumors do no longer respond to therapy. Therapy resistance occurs as a result of several genetic and epigenetic changes in the cancer cell or the microenvironment around the tumor[3,4]. For patients in which therapy resistance occurs, targeted therapy may be of interest, like oncolytic virus (OV) therapy.

Interest in OV therapy has increased since 2015 after the approval of talimogene laherparepvec (T-VEC, Imlygic®) by the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for melanoma treatment. In the past 25 years, a lot of clinical studies on the use of OV therapy have been carried out, which has brought us more knowledge on these anticancer agents[5]. A lot of different OVs have been tested as monotherapy or in combination with other anticancer treatments, for several different tumors. However, due to the genetic and phenotypic tumor heterogenicity, it is difficult to treat the tumors with OV as a monotherapy[5]. Therefore, the actual challenge is to design a good combinatorial therapy.

For metastatic breast cancer, several OV therapies in phase I and II clinical trials have been performed (Table 1). As mentioned in Table 1, several OVs have been tested on advanced or metastatic solid tumors, although not specifically for breast cancer, except for the PANVAC and pelareorep (Reolysin®) trials, which were tested as a combination treatment in metastatic breast cancer patients. PANVAC and pelareorep showed promising anticancer activity in pre-clinical and clinical studies, extensively reviewed by Guo et al. and Chakrabarty et al.[6,7]. These pre-clinical results have led to clinical development and testing in phase 1-2 clinical trials in a broad spectrum of malignancies. To improve the diagnosis, more research on the use of these anticancer agents in metastatic breast cancer is needed. Thus, the main research question of this thesis is: 'Are PANVAC and pelareorep suitable as an anticancer treatment for patients with metastatic breast cancer?'

The thesis will debate the mechanism of action of these OVs, the delivery to the tumor site, and the activation of the host immune system. Next, the results of several clinical trials in advanced

malignancies will be discussed as well as the results of phase II clinical trials that tested PANVAC and pelareorep in combination with a chemotherapeutic drug in patients with metastatic breast cancer. Finally, the pros and cons will be reviewed to give an answer to the research question if PANVAC and pelareorep are suitable as an anticancer treatment for metastatic breast cancer.

Table 1 The clinical trials of oncolytic viruses in patients with breast cancer.

Oncolytic virus and reference	**Method**	**Sample size (metastatic breast cancer)**	**Conclusion**
OV: HF10 (Canerpaturev (C-REV))[8] **Virus:** Herpes simplex virus-1, dsDNA, enveloped	**Combination:** No **Clinical trial:** Pilot study **Administration route:** IT **Dosage and study design:** For 3 days, 3 or 4 injections to infiltrate HF10 into the entire nodule. 1 nodule with HF10 and as a control the other nodule with 0.5 mL of sterile saline for the same number of days. Doses ranged from 10^4 pfu/0.5 mL to $5x10^5$ pfu/0.5 mL	6 (6)	The OV is safe and effective against metastatic breast cancer.
OV: ICOVIR-7[9] **Virus:** Adenovirus, dsDNA	**Combination:** No **Clinical trial:** I **Administration route:** IT **Dosage and study design:** Single round, 10 to 15 needle tracts per patient. Doses ranged from $2x10^{10}$ to $1x10^{12}$vp.	21 (3)	The OV was well-tolerated. One patient had anemia, but no other serious side effects were seen. Therefore, the OV is safe and results in anticancer activity.
OV: ONYX-015[10] **Virus:** Adenovirus, dsDNA	**Combination:** Enbrel **Clinical trial:** I **Administration route:** IV **Dosage and study design:** ONYX-015 was given on days 1, 8 and 15 of a 3-week cycle, doses were $1x10^{10}$, $1x10^{11}$, $1x10^{12}$vp per injection. Enbrel was administered twice a week, 25mg subcutaneously, 1 week and during the ONYX-015 cycle.	9 (2)	No significant adverse effects so Enbrel can be safely administered along with an OV.
OV: PANVAC[11] **Virus:** Poxvirus, dsDNA, enveloped	**Combination:** Docetaxel **Clinical trial:** II **Administration route:** SC injections **Dosage and study design:** PANVAC priming dose (vaccinia) 3 weeks prior to docetaxel. Docetaxel was given on day 2, 9 and 16 of a 28-day cycle, 35 mg/m2. All booster doses (fowlpox) on day 1 of the docetaxel cycle.	48 (48)	There may be a clinical benefit for metastatic breast cancer of the combination of PANVAC with docetaxel. The median progression-free survival was 7.9 months in the combination treatment group and 3.9 months in the chemo group.
OV: Pelareorep (Reolysin®)[12] **Virus:** Reovirus, dsRNA, no envelop	**Combination:** Paclitaxel **Clinical trial:** II **Administration route:** IV **Dosage and study design:** Paclitaxel 80mg/m^2 IV on days 1, 8, and 15 of every 4 weeks, pelareorep $3x10^{10}$ $TCID_{50}$ IV on days 1, 2, 8, 9, 15, and 16.	74 (74)	There was no difference seen in progression-free survival or response rate. But there was significantly longer overall survival for pelareorep in combination with paclitaxel.

OV: PV701[13] **Virus:** Paramyxovirus, New Castle disease virus, ssRNA	**Combination:** No **Clinical trial:** I **Administration route:** IV **Dosage and study design:** Over 2 weeks, 6 PV701 doses than 1-week rest. The first dose was $1x10^9$ pfu/m^2, the second $12x10^9$ pfu/m^2, and doses 3 to 6 were escalated by the cohort from 24 to $120x10^9$ pfu/m^2.	16 (2)	No dose-limiting toxicities, toxicities were predictable and manageable. PV701 shows a single-agent activity.
OV: T-VEC* (Talimogene laherparepvec)[14] **Virus:** Herpes simplex virus-1, dsDNA	**Combination:** No **Clinical trial:** I **Administration route:** IT **Dosage and study design:** 13 patients were in a single-dose group, where doses of 10^6, 10^7, and 10^8 pfu/mL were tested. 17 patients were in a multidose group, doses ranged from 10^6 to 10^8 pfu/mL. The volume of virus injected was based on tumor size.	30 (14)	The OV is well-tolerated, can be safely administered using a multidose protocol and shows evidence of an antitumor effect.
OV: Vaccinia virus (VV_{DD})[15] **Virus:** Western reserve strain of Vaccinia virus	**Combination:** No **Clinical trial:** I **Administration route:** IT **Dosage and study design:** Dose escalation proceeded from $3x10^7$ pfu to $3x10^9$ pfu.	16 (4)	The IT injection of the VV_{DD} was well-tolerated in patients. It resulted in selective infection and anti-tumor activity. No true clinical benefit was achieved.

dsDNA: double-stranded DNA; IT: intratumoral; pfu: plaque-forming units; vp: viral particles; IV: intravenous; SC: subcutaneous; $TCID_{50}$: Median Tissue Culture Infectious Dose; ssRNA: single-stranded RNA

*Herpes simplex virus expressing $OncoVEX^{GM-CSF}$

2. PANVAC

PANVAC (CEA-MUC-1-TRICOM) is a poxviral-based cancer vaccine. It is a Vaccinia virus (VV) with a linear, double-stranded DNA genome[6]. The PANVAC vaccine contains a triad of T cell costimulatory molecules (TRICOM; B7-1, LFA-3, and ICAM-1), and the two human antigens: carcinoembryonic antigen (CEA) and MUC-1[16,17] (Fig. 2). Since the late 1980s, VV treatment is used as a smallpox vaccine, so there is extensive clinical experience and knowledge of the virus.[6]

2.1 Vaccinia virus mechanism of action

The VV life cycle takes place in the cytoplasm of mammalian cells (Fig. 1). The VV can enter the mammalian cell via virion fusion with the cell membrane of the host cell[18]. VV has both an outer envelope and an internal membrane, with enzymes for initiation of viral transcription which happens post-infection. The viral transcription is classified into early, intermediate and late transcription, with each stage having its own specific promotors and transcription factors. The viral core contains components needed for the early transcription as well as DNA-dependent RNA polymerase, leading to the synthesis of early messenger RNA (mRNA). Translation of these RNA yields proteins are involved in the uncoating of viral DNA, DNA replication and transactivation of intermediate mRNA.[6,19]

VV replication takes place in the cytoplasm of infected cells and the entire replication cycle is completed in 1 hour. VV DNA replication occurs in endoplasmic reticulum enclosed cytoplasmic mini-nuclei, called poxvirus factories[20]. Intermediate mRNA encodes for transactivators, which can generate late mRNA synthesis. Late proteins include structural proteins for membrane formation and early transcription factors, which are incorporated into new virus particles. The use of the host translation apparatus by the virus contributes to enhanced viral replication and the suppression of host protein synthesis, thereby facilitating the viral invasion of infected cells[21]. Soon after viral entry, the virus induces a cytopathic effect. The viral enzymes produced during the early phase of the life cycle can shut down the cell function of the host cell. For instance, within 6 hours after infection, the host protein synthesis is shut down. Consequently, there is enough facilitation in the host cell for the expression of viral genes and viral replication.[6,22]

The VV consists of two infectious forms, the intracellular mature virus (IMV) and the extracellular enveloped virus (EEV). The immature virus becomes IMV after condensation of the core and processing of core proteins, after which it is transported to sites where it becomes wrapped with two membrane layers derived from the Golgi network. These virus particles then travel to the cell surface, where its membrane fuses with the plasma membrane, thus exposing the IMV virus particles on the cell surface. These IMV particles are transported to the cell periphery via microtubules, where they become cell-associated enveloped virus (CEV) by fusing with the cell plasma membrane[23].

There are 3 mechanisms for spreading of the virus, which are fast and efficient. The first is cell-to-cell spread. Viral protein F11 acts as a scaffold, by using its PDZ domain to unite myosin IXa and RHOA, it can inhibit RHOA signaling and eventually promote viral spread[24]. The last two spreading mechanisms are for distant spread. One for the production and release of the extracellular enveloped virus (EEV) form[25] and the last for the repulsion of superinfecting virions[26].

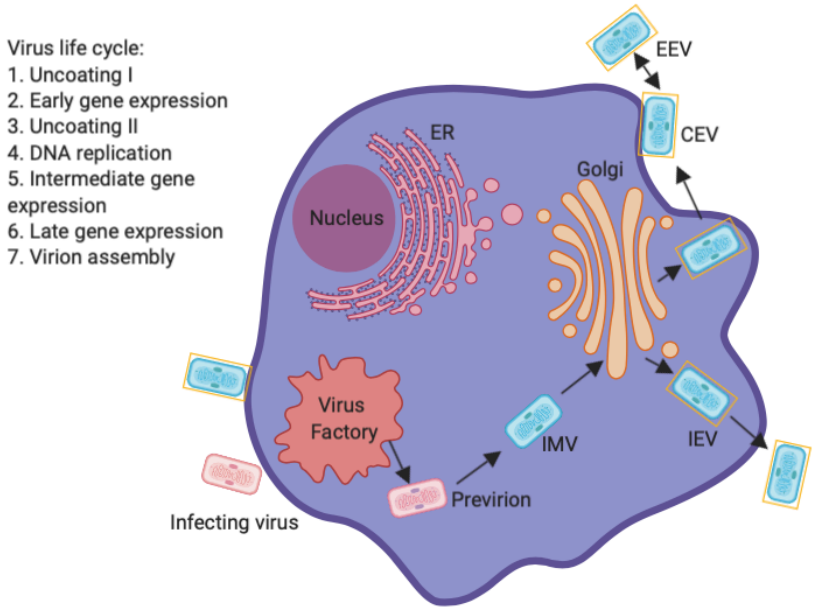

Fig. 1 The life cycle of the Vaccinia virus. A diagram of an infected cell is shown, including the nucleus, ER (endoplasmic reticulum), Golgi apparatus and the stages of the viral life cycle. After late gene expression, the IMV (intracellular mature virus) forms. The IMV targets the Golgi, thereafter the virus particle gets an envelope, to form the IEV (intracellular enveloped virus). The IEVs go to the cell surface by polymerization of actin filaments. After release, the virus can attach to the membrane as a CEV (cell-associated enveloped virus) after being released into the medium as an EEV (extracellular enveloped virus). This figure was adapted from Guo et al. (2019)[6].

2.2 Activation of the host immune system

The majority of breast tumors express the antigens CEA and MUC-1, this is an important target for the PANVAC vaccine. To activate the host immune system, the PANVAC vaccine uses a triad of T cell costimulatory molecules (TRICOM), and the two human antigens: carcinoembryonic antigen (CEA) and MUC-1. This way the vaccine induces a T-cell response in the host[16] (Fig. 2). For the activation of T cells, two signals are needed that are expressed on the same cell. First, an antigen, which is delivered to the T-cell receptor via a peptide-MHC complex of antigen-presenting cells (signal 1). Next to that, an costimulatory signal, involves the interaction of costimulatory molecules, like TRICOM, of the antigen-presenting cells with its ligand on the T cell (signal 2)[16].

TRICOM in combination with poxvirus recombinant vectors, which encodes the transgenes for an antigen, results in strong activation of antigen-specific CD4 and CD 8 T-cells and antitumor activity[16].

A majority of the tumors express CEA and MUC-1, including breast carcinomas, and could therefore ne an important target for breast cancer therapy[27,28]. By adding the two human antigens, CEA and MUC-1, the vaccine will cause tumor antigen-specific immune responses[16] and could be suitable for the treatment of breast cancer.

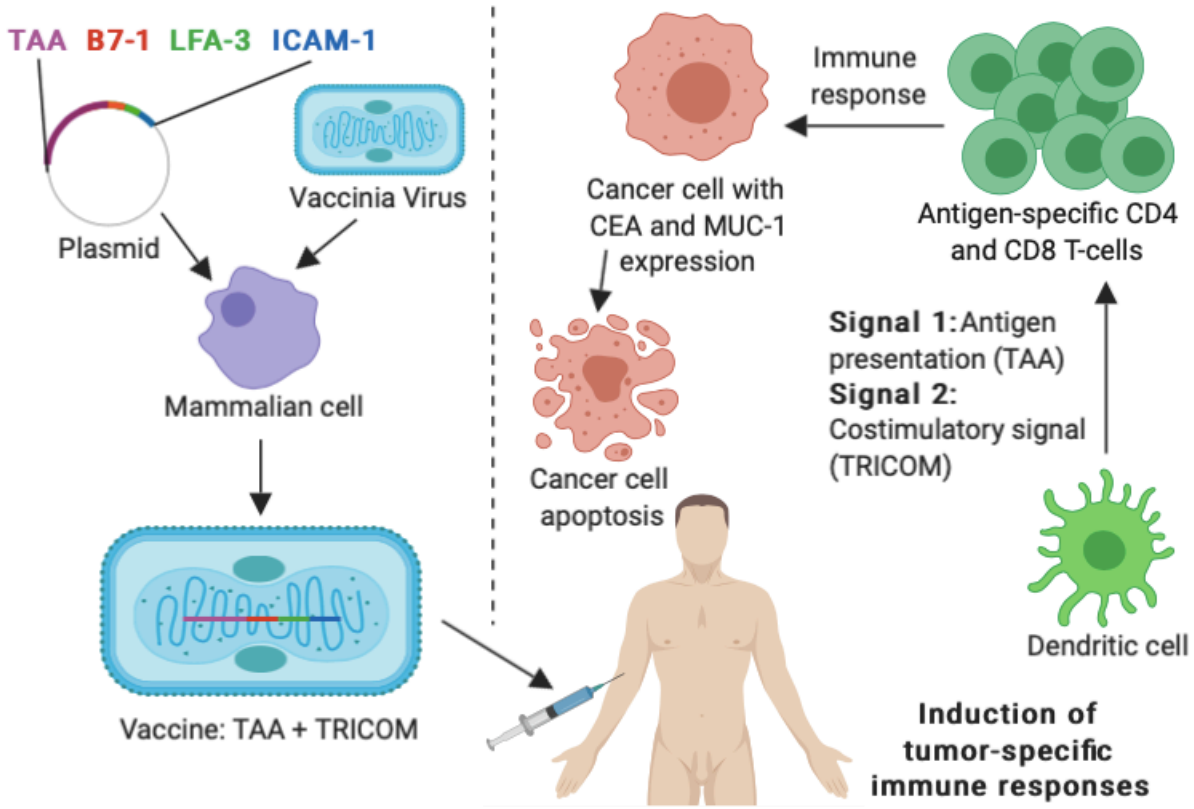

Fig. 2 Production of vaccine and immune response. On the left, the vaccine production, starting with a plasmid with tumor antigen genes (TAA; CEA and MUC-1) and costimulatory molecules genes (TRICOM; B7-1, LFA-3, and ICAM-1). Together with Vaccinia Virus, the vaccine is made in mammalian cells. On the right, the induction of tumor-specific T cell responses; the activation of CD4 and CD8 T cells to kill cancer cells.

2.3 PANVAC in advanced carcinomas

PANVAC with the antigens CEA, and MUC-1, as well as the TRICOM costimulatory molecules, has been tested in several clinical trials with advanced-stage carcinomas, including those of the breast, ovary, and pancreas (Table 2).

The first study of Kaufman et al. is a phase 1 clinical trial, where they tested PANVAC in 10 patients with advanced pancreatic cancer. This study was conducted to determine safety, tolerability and to obtain data on immune response and survival. They used PANVAC-V (Vaccinia virus with CEA, MUC-1, and TRICOM) to prime the patients, followed by three booster vaccinations of PANVAC-F (fowlpox virus with CEA, MUC-1, and TRICOM). Moreover, they used the granulocyte-macrophage colony-stimulating factor (GM-CSF) as a local adjuvant after each vaccination and for three days after injection. This study, with a small sample size, concluded that prime-boost vaccination with PANVAC

is safe and well-tolerated. The vaccination also generated antigen-specific immune responses against the viral vectors in patients with advanced pancreatic cancer.[29]

The next study performed by Gulley et al. is a pilot study of 25 patients with CEA- or MUC-1-expressing metastatic carcinomas. The primary endpoint of this study was vaccine safety, the secondary endpoints were determining the immunologic and clinical responses. This study uses PANVAC-V as a prime vaccination and PANVAC-F as a booster vaccination. Gulley et al. concluded that this vaccine was demonstrated to be safe. It was successful in generating CD8 and CD4 antigen-specific immune responses and showed evidence of clinical activity.[30]

As an extension to the prior described study, a pilot study was conducted by Mohebtash et al. with PANVAC in 26 patients with metastatic breast and ovarian cancer. The primary endpoint of this study was to determine the clinical response and the secondary endpoint was to determine the immunologic response. Mohebtash et al. concluded that some patients with limited tumor burden and minimal prior chemotherapy could benefit from the vaccine, provided that their immune system was not compromised.[31]

A recent article by Gatti-Mays et al. demonstrated a phase 1 trial of BN-CV301. This vaccine is a poxviral-based vaccine targeting MUC-1 and CEA with costimulatory molecules. PANVAC was redesigned to make it safer and more antigenic. The endpoints of this study were safety and dosage, as well as clinical and immune responses. In conclusion, the BN-CV301 vaccine could be safely used in patients with advanced cancer.[32]

Table 2 The clinical trials of PANVAC in patients with advanced carcinomas.

Oncolytic virus and reference	Method	Results and conclusion
PANVAC[29]	**Clinical trial:** I **Administration route:** SC injection **Patients:** 10, advanced pancreatic cancer **Dosage and study design:** Day 0: primer PANVAC-V $2x10^8$ pfu; Day 14, 18, 42: booster PANVAC-F $1x10^9$ pfu; GM-CSF 100 µg after each immunization and for 3 consecutive days thereafter.	Most treatment-related side effects were mild injection-site reactions. In all patients: antibody response against VV. 5 out of 8 evaluable patients: anti-CEA and/or MUC-1 specific T cell response. These patients had a significant increase in overall survival (15.1 vs. 3.9 months). In conclusion, prime-boost vaccination with PANVAC is safe and well-tolerated.
PANVAC[30]	**Clinical trial:** Pilot study **Patients:** 25, CEA- or MUC-1-expressing metastatic carcinomas **Administration route:** SC injection **Dosage and study design:** primer PANVAC-V $2x10^8$ pfu; on day 15, 29, and 43, then every 28 days while on study: booster PANVAC-F. Sargramostim 100 µg on the day of vaccination and the following 3 consecutive days.	The side-effects were limited to injection-site reactions. CD8 and CD4 immune responses to MUC-1 and/or CEA were observed, following vaccination in 9 of the 16 tested patients. One breast cancer patient had a >20% decrease in the size of a large liver metastasis. In conclusion, this vaccine was demonstrated to be safe and shows evidence of clinical activity.

PANVAC[31]	**Clinical trial:** Pilot study **Patients:** 26, metastatic breast (n=12) and ovarian cancer (n=14) **Administration route:** SC injection **Dosage and study design:** primer PANVAC-V 2x10^8 pfu; on day 15, 29, and 43, then every 28 days while on the study: booster PANVAC-F. Sargramostim 100 µg on the day of vaccination and the following 3 consecutive days.	The side effects were limited to mild injection-site reactions. For the breast cancer patients, median time to progression was 2.5 months and the median overall survival was 13.7 months. Patients with stable or responding disease had fewer prior therapies and lower tumor marker levels compared to the patients with no evidence of response. For the ovarian cancer patients, median time to progression was 2 months and the median overall survival was 15.0 months. In conclusion, some patients with limited tumor burden and with minimal prior chemotherapy could benefit from the vaccine.
BN-CV301[32]	**Clinical trial:** I **Patients:** 12, advanced carcinomas **Administration route:** SC injection **Dosage and study design:** 1, 2 or 4 injections of 4x108 Inf.U/0.5 mL prima MVA-BN-CV301on weeks 0 and 4. Boost FPV-CV301 1x109 Inf.U/0.5 mL every 2 weeks for 4 doses, then every 4 weeks.	No dose-limiting toxicities were observed. Side-effects were more common with the prime doses. All adverse events due to the treatment were temporary, self-limiting, grade 1/2, and included injection site reaction and flu-like symptoms. Most patients generated antigen-specific T cells to CEA and MUC-1. It resulted in prolonged stable disease in multiple patients, especially in KRAS mutant gastrointestinal tumors. In conclusion, the BN-CV301 vaccine can be safely used in patients with advanced cancer.

SC: subcutaneous; PANVAC-V: Vaccinia virus with CEA, MUC-1, and TRICOM; pfu: plaque-forming units; PANVAC-F: Fowlpox virus with CEA, MUC-1, and TRICOM; GM-CSF: Granulocyte-macrophage colony-stimulating factor; VV: Vaccinia virus; Sargramostim: Human recombinant GM-CSF; Inf.U: Infectious Units; MVA-BN-CV301: recombinant Modified vaccinia Ankara with CEA, MUC-1, and TRICOM; FPV-CV301: recombinant fowlpox with CEA, MUC-1, and TRICOM

2.4 PANVAC and docetaxel in metastatic breast cancer

The phase II clinical trial by Heery et al. in 2015[11] showed remarkable results for breast cancer patients. In their research, they combined PANVAC and the chemotherapeutic drug docetaxel. Docetaxel is a chemotherapeutic drug, commonly used in the management of metastatic breast cancer. In pre-clinical studies, docetaxel showed a synergistic effect with vaccines and the addition of docetaxel to a vaccine did not inhibit the immune response compared to administration of the vaccine alone[33]. Furthermore, docetaxel can alter carcinoma cell phenotypes, so they are more amenable to T cell-mediated killing[11].

This study enrolled 48 patients with metastatic breast cancer. There was no statistically significant difference in baseline characteristics. The group that received the combination of PANVAC and docetaxel consisted of 25 patients (group A), the group that received docetaxel alone consisted of 23 patients (group B). The primary endpoint was progression-free survival. Secondary endpoints were safety and immunologic correlative studies.

Regarding the safety of PANVAC and docetaxel, there were higher rates of injection site reaction and edema seen in group A, caused by the cumulative toxicity of docetaxel. This can be explained by the fact that group A received more doses of docetaxel. However, there were no overall differences in toxic effects between the two groups. Both agents are safe, as monotherapy as well as a combination.

A median progression-free survival of 7.9 months in group A was observed, which was longer than 3.9 months found in group B. This indicates a clinical benefit of the addition of PANVAC. There was no statistically significant difference in the development of antigen-specific T-cell response and time to progression seen between group A and B. However, after 3 cycles of chemotherapy, there was a clear trend in group A where a greater increase in CD4+/Treg was suggested in comparison to the docetaxel group. This was however not statistically significant.

This study concludes that PANVAC can be safely combined with standard-of-care chemotherapy like docetaxel and can result in an improvement in progression-free survival. Therefore, PANVAC and docetaxel may have a clinical benefit for patients with metastatic breast cancer.

3. Pelareorep

Pelareorep is a linear double-stranded RNA reovirus. The RNA gene segments are enclosed in two icosahedral symmetric capsids[7,34]. The pelareorep vaccine consists of a live, replication-competent, naturally occurring Reovirus Type 3 Dearing strain. Many human cancers have RAS-activating mutations. Reovirus uses activated RAS-signaling pathway to practice its cytotoxic effect on[7]. For this reason, reovirus is proposed as a treatment for human tumors[35].

3.1 Reovirus mechanism of action

Reoviruses attach to cell-surface carbohydrates like sialic acid residues and junction-adhesion molecule-A (JAM-A) (Fig. 3). Functional signal-transducing Epidermal growth factor receptor (EGFR) enhances efficient reovirus infection[36]. Hereafter, the reovirus will be internalized by β1 integrins, most likely by clathrin-dependent endocytose. In the endocytic compartment, the reovirus outer capsid will be removed. After that, the virus will pierce the endosomal membrane and deliver transcriptionally active reovirus core particles into the cytoplasm, causing oncolysis.[34]

For the oncolysis, the reovirus has different abilities. Reovirus can activate dendritic cells which will trigger the production of pro-inflammatory cytokines, natural killer cells, and T-cell independent viral replication. Next to that, to enhance cell death in a p53-dependent manner, earlier research showed that the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) transcription factor was induced when cells were treated with reovirus and Nutlin-3a or a certain chemotherapeutic agent[37]. For the enhancement of cell death, NF-kB activation and expression of p53-target genes are important. Therefore, the p53-status affects the efficacy of combination treatment with reovirus.

Besides the apoptotic pathway, reovirus can injure the host cell via necroptosis. Via different mechanisms, reovirus can induce the RIP1 (receptor-interacting serine/threonine-protein kinase 1) dependent necroptosis pathway[38]. Necroptosis can also occur via the death receptor signals, pathogen-associated molecular patterns and loss of inhibitors of apoptosis.

For an oncolytic virus to be successful it must be spread or carried to the tumor site, where it has to escape the host immune system. To reach the tumor site, reovirus must evade neutralizing antibodies. Research showed that a reovirus can be carried by granulocytes and platelets via JAM-1 (junctional adhesion molecule 1), and plasma and blood mononuclear cells. Therefore, the virus travels along these cells to evade the host immune system.[39] Moreover, dendritic cells and T cells can deliver reovirus to the tumor, protecting the virus from neutralizing antibodies.

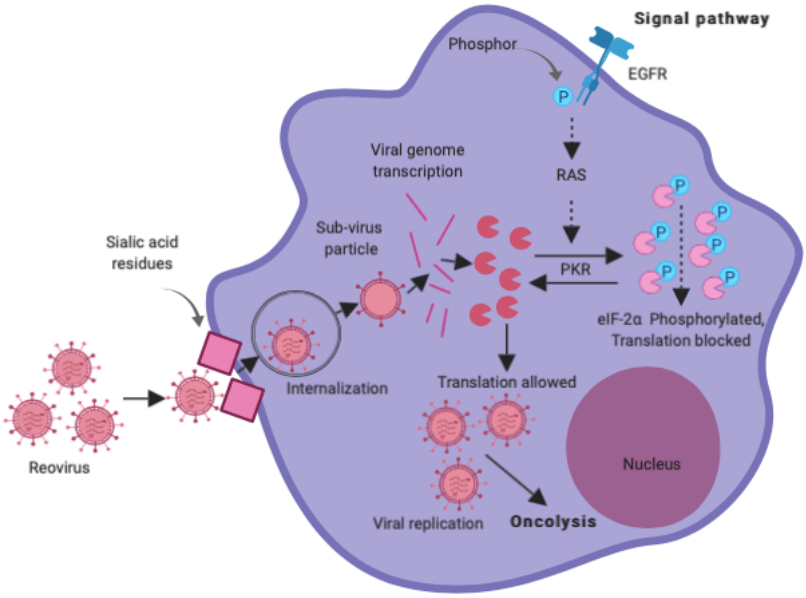

Fig. 3 Reovirus mechanism of action. A diagram of an infected cell is shown, including the nucleus and the stages of the mechanism of action of reovirus. Reovirus attaches to sialic acid residues on the host cell. Epidermal growth factor receptor (EGFR) will enhance reovirus infection. After clathrin-dependent endocytose, the virus will be internalized. In the endocytic compartment, the reovirus outer capsid will be removed. After that, the virus will pierce the endosomal membrane and deliver transcriptionally active reovirus core particles into the cytoplasm, this will cause oncolysis. This figure was adapted from Chakrabarty et al. (2015)[7].

3.2 Activation of the host immune system

Most of the tumors have different immune evasion strategies, which is why the immune system often fails to recognize tumor antigens. For example, as tumors are alterations of self-tissue and they produce immunosuppressive cytokines[40]. Therefore, the oncolytic reovirus is qualified as an anticancer therapeutic agent as it might cause direct lysis of tumor cells, facilitated by different mechanisms, as well as it generates an innate and adaptive immune response to the tumor microenvironment[40,41]. For example, reovirus can induce pro-inflammatory cytokines to enhance maturation and tumor antigen presentation capacity of antigen-presenting cells. In order to activate T cells. Next to this, the host immune system can react against reovirus. It will induce an antiviral innate and adaptive immune response and inhibits replication and spread of the reovirus. This anti-reovirus T cell-mediated immune response then destructs the tumor cells because the reovirus prefers to infect cancer cells[40]. Thus, this contributes to the oncolytic activity of reovirus.

3.3 Pelareorep in advanced carcinomas

Pelareorep has been tested in several phase II clinical trials with advanced-stage carcinomas, including melanomas, pancreas adenocarcinomas, and colorectal tumors (Table 3).

Galanis et al. studied the effects of pelareorep administration on patients with metastatic melanoma. Their primary objective was to assess the antitumor effect in terms of response rate and clinical benefit rate and to assess the toxicity profile of pelareorep. Secondary objectives were to assess the progression-free and overall survival, the viral replication and the impact of pre-existing anti-reovirus immunity on the efficacy and toxicity of the pelareorep treatment. Overall, the pelareorep treatment was well-tolerated in metastatic melanoma patients and showed antitumor immune responses.[42]

A randomized phase 2 study of Noonan et al., examined whether the addition of pelareorep to carboplatin and paclitaxel was of clinical benefit in patients with metastatic pancreatic adenocarcinomas. The primary endpoint was progression-free survival. The secondary endpoints were objective response rate, overall survival, and correlative endpoints included immune studies and KRAS mutational analysis. It was concluded that pelareorep can be administered safely and was well-tolerated, but does not improve progression-free survival when combined with carboplatin and paclitaxel, regardless of KRAS mutational status.[43]

The phase II clinical trial of Mahalingam et al. tested the antitumor effect of pelareorep in combination with the chemotherapeutics carboplatin and paclitaxel in patients with advanced malignant melanomas. The primary objective of this study was to assess the antitumor effect of the treatment regimen in terms of objective response rate. Secondary objectives were to assess progression-free survival, overall survival, disease control rate and duration, and the safety and tolerability of the treatment regimen. Mahalingam et al. concluded that pelareorep in combination with carboplatin and paclitaxel is safe and is a potentially efficacious treatment for advanced malignant melanomas.[44]

Finally, a clinical trial of Jonker et al. examined the anticancer effect of FOLFOX6/Bevacizumab with or without the oncolytic virus pelareorep. In this randomized phase II study, the primary endpoint was progression-free survival. The secondary endpoints were overall survival, objective response rate, quality of life and correlative analyses. In conclusion, pelareorep can be safely combined with FOLFOX6/Bevacizumab. Jonker et al. noticed an increase in objective response rate and progression-free survival in the pelareorep group.[45]

Table 3 The clinical trials of pelareorep in patients with advanced carcinomas

Oncolytic virus and reference	Method	Results and conclusion
Pelareorep[42]	**Clinical trial:** II **Patients:** 21, metastatic melanoma **Administration route:** IV **Dosage and study design:** Days 1-5 of 28-days cycle: $3x10^{10}$ $TCID_{50}$ pelareorep.	Treatment was well-tolerated. The median time to progression was 45 days and median survival was 165 days. Biopsies of 13 patients contained metastatic tumor, productive reovirus replication was detected in 2 of the 13 tumors, despite increase in neutralizing antibodies. These patients had a longer progression-free survival (80 and 87 days, respectively) as compared to the median survival of 45 days in this study. In summary, the reovirus treatment was well-tolerated in metastatic melanoma patients as viral replication was demonstrated in biopsy samples.
Pelareorep, paclitaxel, and carboplatin[43]	**Clinical trial:** II **Patients:** 73, metastatic pancreatic adenocarcinomas **Administration route:** IV **Dosage and study design:** Day 1 of each 21-days cycle: 175 mg/m^2 paclitaxel, and carboplatin at a dose area under the concentration-time curve of 5mg/mL/minute over 30 minutes. Followed by $3x10^{10}$ $TCID_{50}$/day pelareorep in the pelareorep arm (n=36) on days 1-5 of each cycle.	There was no difference in progression-free survival between the groups and the majority of this progression-free survival time there was no toxicity or progression noted. KRAS status did not impact the outcome. Soluble immune biomarkers like IL-6, IL-8, and VEGF, as well as increased circulation T and natural killer cell subsets were associated with treatment outcome. Finally, pelareorep was associated with higher levels of 14 proinflammatory plasma cytokines/chemokines and cells with immunosuppressive phenotype. In conclusion, pelareorep can be used safely but does not improve progression-free survival when combined with carboplatin and paclitaxel, regardless of KRAS mutational status.
Pelareorep, paclitaxel, and carboplatin[44]	**Clinical trial:** II **Patients:** 14, advanced malignant melanoma **Administration route:** IV **Dosage and study design:** First, patients received premedication for paclitaxel; corticosteroids and H1 and H2 antagonists. Day 1 of each cycle, 200 mg/m^2 paclitaxel, followed by carboplatin at a dose of AUC 6mg/mL/min calculated by Calvert's formula, and then followed by $3x\ 10^{10}$ $TCID_{50}$ pelareorep. Day 2-5: only pelareorep at the same dose as on day 1. Cycles were repeated every 21 days for up to 8 cycles in total.	No grade 4 adverse events or deaths were observed. Furthermore, manageable grade-3 toxicities commonly attributed to pelareorep were noticed. 3 patients showed partial responses, and no complete responses were observed. The median progression-free survival was 5.2 months, and the overall survival was 10.9 months, with a 1-year overall survival rate of 43%. In conclusion, pelareorep can be safely combined with carboplatin and paclitaxel and has potential clinical benefit.
Pelareorep and FOLFOX6/ Bevacizumab[45]	**Clinical trial:** II **Patients:** 103, Metastatic colorectal cancer, no previous chemotherapy for advanced disease. **Administration route:** IV	At 13 months, the progression-free survival was significantly higher in the pelareorep arm (median 7 vs. 9 months). There was no statistical difference in overall survival (median, 19.2 vs. 20.1 months).

	Dosage and study design: Every 2 weeks, FOLFOX6/Bevacizumab: bevacizumab 5mg/kg (1 hour), oxaliplatin 85 mg/m^2 and leucovorin 400 mg/m^2 (2 hours), and fluorouracil 400 mg/m^2 bolus, and 2400 mg/m^2 (46 hours). FOLFOX6/bevacizumab with (n=51) or without (n=52) pelareorep 3x10^{10} $TCID_{50}$ on days 1 to 5 (cycles 1, 2, 4, and alternate cycles hereafter)	An increased objective response rate was noticed with pelareorep, but with a shorter median duration of response. The patient in the pelareorep arm experienced more hypertension and proteinuria and was more likely to quit bevacizumab before progression. In conclusion, FOLFOX6/bevacizumab in combination with pelareorep was tolerable. It is likely that the decreased treatment intensity with standard agents in the pelareorep contributed to the lack of benefit with pelareorep.

$TCID_{50}$: Median Tissue Culture Infectious Dose; KRAS: Kirsten rat sarcoma viral oncogene; IL= interleukin; VEGF: vascular endothelial growth factor; FOLFOX6: leucovorin/5-FU/oxaliplatin

3.4 Pelareorep and paclitaxel in metastatic breast cancer

The phase II clinical trial by Bernstein et al. in 2018[12] tested the OV pelareorep in combination with the chemotherapeutic drug paclitaxel in patients with previously treated metastatic breast cancer. Paclitaxel is a commonly used chemotherapeutic in different cancers, among which metastatic breast cancer.

As for the method, 74 women were randomized into two groups; 36 women received a combination treatment of pelareorep and paclitaxel (Arm A) and 38 woman received paclitaxel alone (Arm B). The patients in Arm B had less advantageous baseline prognostic variables. The primary endpoint was progression-free survival and secondary endpoints were objective response rate, overall survival, circulating tumor cell counts, safety and exploratory correlative analyses. The survival analyses were adjusted for prior paclitaxel treatment.

Pelareorep was well-tolerated, apart from a few mild side-effects. The most common pelareorep-related adverse events found were fever, fatigue, diarrhea, chills, nausea and flu-like symptoms. This corresponds with results found in previous research. As for the clinical outcomes, there was almost no difference in progression-free survival, median progression-free survival in Arm A was 3.78 months vs 3.38 months in Arm B. Between Arm A and B, there were no significant differences in response rate and in circulating tumor cell counts. Interestingly, there was a statistically significant longer overall survival in Arm A, the combination group, with 17.4 months vs 10.4 months in Arm B.

In conclusion, this study found no benefit of the addition of pelareorep to paclitaxel measured in progression-free survival, objective response rate and circulating tumor cell count. There was a statistically significant improvement of the overall survival, but this might have occurred due to imbalances in prognostic factors. The combination of pelareorep and paclitaxel needs more investigation before it can be used as a treatment for metastatic breast cancer.

4. Discussion

PANVAC and Pelareorep were tested in clinical trials for various cancers. These OVs have been tested both as monotherapy and in combination with several chemotherapeutics. Unfortunately, these OV treatments do not seem to be promising therapies for metastatic breast cancer yet.

The PANVAC vaccine is suitable as an anti-breast cancer agent because, first of all, PANVAC is very safe in use. The Vaccinia virus has been used for many years already, so there is a lot of knowledge about and clinical experience with the virus. Moreover, PANVAC causes hardly any side-effects, only injection-site reactions are noticed. As an anticancer agent, the virus is suitable due to its efficient life cycle, fast cytopathic effect, and fast-spreading. It promotes the host-T cell response by activating antigen-specific CD4 and CD8 T cells. Because of its selectivity for CEA and MUC-1 antigens, PANVAC is suitable for breast cancer because these carcinomas overexpress these antigens. Finally, the trial of Heery et al. showed evidence of increased median progression-free survival in the combination group[11]. In summary, the VV in PANVAC is suitable to use as a cancer treatment, but the clinical trial of Heery et al. shows not very convincing anti-metastatic breast cancer activity[11].

There are a few reasons why PANVAC is not suitable as an anticancer agent for metastatic breast cancer. First of all, in the small sample size clinical trial of Heery et al. patients experienced a few side-effects, namely the high rates of injection-site reactions and flu-like symptoms. Next to this, the trial found no statistically significant immune response, which raises the question if the vaccine is effective or not against metastatic breast cancer.[11]

Moreover, PANVAC is only tested in advanced tumors, most of the times these patients already had chemotherapy which affected their immune system. Therefore, the results of all clinical trials are based on the vaccine as a palliative treatment. It is possible that the vaccine cannot work optimally because of the impaired function of the immune system. Maybe if the vaccine is used in an earlier stage of cancer, it works better and has more potential. Moreover, the vaccine is not very universal. It only affects tumors that express MUC-1 and/or CEA. This makes it only applicable to specific tumors. So far, the clinical trials of PANVAC are not that promising, especially not for metastatic breast cancer since it is only been tested in 1 trial.

The reovirus-based cancer vaccine pelareorep seems to be suitable as an anti-breast cancer vaccine due to the characteristics of the reovirus. First of all, the reovirus has different ways to induce apoptosis or necroptosis in a fast way. For oncolysis, it uses mutations in the RAS-signaling pathway of tumor cells, this is of interest since various human cancers have mutations in their RAS-signaling pathway. Next to that, reovirus is capable of evading the host immune system traveling along the on mononuclear blood cells to move to the tumor site. Furthermore, its effect on the host immune system

is remarkable: it activates the host immune system against both the tumor cells and the reovirus itself. This way, the host immune system will destruct the tumor cells because reovirus is inside these cancerous cells. In summary, reovirus can cause fast lysis of tumor cells and activate the host immune system in different ways.

According to the clinical trials on different kinds of carcinomas, pelareorep is well-tolerated and thus safe to use both as mono- and combination therapy. It has shown no promising results in all advanced carcinomas including metastatic breast cancer. The trial of Bernstein et al.[12] concluded no clinical benefit of the addition of pelareorep to paclitaxel. The same conclusion was drawn by Noonan et al.[43] in their trial of pelareorep in combination with carboplatin and paclitaxel in patients with metastatic pancreatic adenocarcinomas. No improvements in progression-free survival, regardless of KRAS mutational status, were found. The other clinical trials concluded as well that pelareorep was of minimal clinical benefit.

In conclusion, both PANVAC and pelareorep are not suitable as a treatment for metastatic breast cancer yet. They can have clinical benefits but are, so far, not suitable to be of real benefit for patients with metastatic breast cancer. Further research is needed to improve the vaccines of PANVAC and pelareorep. Furthermore, it is important to find out which chemotherapeutic or other therapy is the best co-therapy with PANVAC and pelareorep. Finally, it would be interesting to perform clinical trials on patients who did not had prior cancer treatment, as for the oncolytic virus to work optimally, it has to make use of a well-functioning patients' immune system, which is likely to be affected if the patient has received prior immune- or chemo therapy.

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