Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2024.241

REVIEW ARTICLE

Application of Newcastle Disease Virus in the Treatment of Human and Canine Mammary Cancer

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ARTICLE HISTORY (23-430)

Received: September 22, 2023
Revised: December 10, 2023
Accepted: December 14, 2023
Published online: September 3, 2024

Key words:
Mammary
Neoplasm
Newcastle disease virus
Oncology

A B S T R A C T

Breast cancer is the most prevalent form of tumor worldwide and the leading cause of cancer among women globally. Similarly, mammary tumors are also common in canines, with a majority of them unfortunately being malignant. While chemotherapy is one of the most frequently employed treatments, it often comes with significant side effects. Consequently, alternative therapies are under investigation, including oncolytic viruses. The Newcastle disease virus (NDV) stands out due to its capacity to selectively target and destroy tumor cells while preserving healthy tissue, as well as its ability to trigger a robust antitumor immune response. Numerous studies support the utilization of NDV in treating various cancer types, ranging from brain tumors to gastrointestinal tract neoplasms. Other researches have also yielded promising results in the treatment of mammary neoplasms. This review aims to compile information about NDV and its application in oncolytic virotherapy for the study and treatment of human breast cancer and canine mammary tumors, employing cell lines, animal models, and clinical trials.

To Cite This Article: Coleto AF, Fonseca BB and Medeiros-Ronchi AA, 2024. Application of newcastle disease virus in the treatment of human and canine mammary cancer. Pak Vet J, 44(3): 547-554. http://dx.doi.org/10.29261/pakvetj/2024.241

INTRODUCTION

Oncolytic immunotherapy is centered around the utilization of oncolytic viruses (OVs), such as the Newcastle disease virus (NDV) (Jin *et al*., 2021), for treating various human (Martini *et al*., 2020) and animal cancers (Sánchez *et al*., 2018). Additionally, there have been encouraging findings regarding the use of NDV in murine mammary cancer cell lines (Al-Shammari *et al*., 2019; Amin *et al*., 2019; Raihan *et al*., 2019; Hassan *et al*., 2020a; Hassan *et al*., 2020b; Ramamurthy *et al*., 2021; Al-shammari *et al*., 2022; Wang *et al*., 2022) (Fig. 1).

Breast cancer exhibits a higher incidence rate in women (24.5% of cases) than other tumor types (World Health Organization, 2020a) and is identified as the primary cause of cancer-related mortalities (15.5% of cases) across all age groups (World Health Organization, 2020b). Similarly, in dogs, mammary tumors are among the most frequently diagnosed cancer types (Salas *et al*., 2015), with dogs serving as a superior experimental model compared to laboratory rodents for studying mammary tumors (Cekanova and Rathore, 2014).

Given the high prevalence of mammary tumors in both women and dogs, the imperative to develop novel cancer therapies, the potential effectiveness of the Newcastle disease virus in tumor treatment, and the role of dogs as experimental models for mammary neoplasms in women, this review aims to consolidate information regarding NDV and its application in oncolytic virotherapy for the investigation and treatment of human breast cancer and canine mammary tumors, utilizing cell lines, animal models, and clinical trials.

The Newcastle disease virus: The Newcastle disease virus (NDV) was initially isolated in 1927 from domestic chickens in Newcastle, England (Nelson, 1999). It is also referred to as APMV-1 (avian paramyxovirus 1) (Dimitrov *et al*., 2016).

This virus exhibits pleomorphism (Ganar *et al*., 2014) and possesses a spherical shape. Its capsid is helical and enveloped by a double lipid layer (Lamb and Parks, 2007). NDV has a size range of 100 to 500 nanometers (nm) (Martini *et al*., 2020) with a diameter typically falling between 200 and 300 nm (Ganar *et al*., 2014). It is pathogenic for various birds, including wild, domestic, and peridomestic species (Dimitrov *et al*., 2016).

While NDV can be pathogenic for humans (Ganar *et al*., 2014), infections in humans are exceedingly rare, and highly virulent strains tend to cause only mild symptoms such as conjunctivitis, cough, moderate fever, and flu-like symptoms (Song *et al*., 2019).

Genome, fusion and replication of NDV: The nucleic acid of NDV consists of a single-stranded RNA with negative polarity. Its genome is of non-segmented type (Lamb and Parks, 2007) and contains a minimum of six structural genes (Chambers *et al*., 1986).

The fusion and replication of NDV within the host cell to produce infective virions follows a series of intricate steps. It begins with the binding of the viral surface glycoprotein HN to the sialic acid receptor on the host cell, facilitating the interaction of HN with the viral surface protein F. The conformational change in F leads to the fusion of the viral envelope with the cell's plasma membrane, enabling the entry of the NDV nucleocapsid into the cell cytoplasm (Ganar *et al*., 2014).

Within the cytoplasm, the M protein dissociates from the RNP (ribonucleoprotein) complex. The P-L protein complex functions as the RNA polymerase responsible for initiating the transcription of viral mRNA (Dortmans *et al*., 2011). Negative-sense RNA is transcribed into a positive-sense antigenomic template (Ganar *et al*., 2014), which is subsequently translated to produce new negative-sense RNA and new structural proteins (Molouki and Peeters, 2017).

A new nucleocapsid is assembled, consisting of the newly synthesized RNA surrounded by NP, P, and L proteins. This nucleocapsid is then assembled with the M protein and the surface glycoproteins HN and F. The viral progeny is eventually released from the host cell through a process called budding (Cuadrado-Castano *et al*., 2015).

Virotherapy with NDV: "Tumor hot" types, characterized by the development of antitumoral activity (Martini *et al*., 2020), respond more favorably to therapies, including oncolytic virotherapy, which amplifies the preexisting antitumoral immune response (Hegde *et al*., 2016). On the other hand, "cold" tumors like breast neoplasms are considered non-inflamed due to limited immune cell infiltration. In such cases, the use of OVs stimulates the entry of immune cells into the tumor microenvironment, transforming them into "hot" tumors (Martini *et al*., 2020).

Numerous preclinical and clinical studies have highlighted the promising anticancer potential of oncolytic immunotherapy. Additionally, OVs generally exhibit significantly lower toxicity compared to traditional chemotherapies (Freeman *et al*., 2006).

In the 1950s, Sinkovics (1957) made a revolutionary discovery, finding that NDV, highly pathogenic to over 240 bird species, possessed oncolytic capabilities. In 1994, the National Cancer Institute began categorizing NDV virotherapy as an alternative and complementary medicine (Nelson, 1999). Since then, numerous studies have demonstrated the therapeutic potential of wild or recombinant NDV strains, either as standalone treatments or in combination with other therapies.

NDV has the remarkable ability to spare normal cells while directing its action to eliminate tumor cells (Martini *et al*., 2020), mainly because cancer cells often do not produce IFN-I (interferon type I), which is essential for controlling viral infections (Marelli *et al*., 2018).

This virus meets the three major criteria for effective anticancer therapy: firstly, it exhibits selective cytotoxicity, allowing for the selective destruction of cancer cells while protecting the patient's healthy cells (Yurchenko *et al*., 2018). Additionally, lytic strains directly lyse tumor cells, while non-lytic strains induce a robust antitumoral immune response by depositing viral proteins on the neoplastic cell membrane (Kalyanasundram *et al*., 2018).

A significant advantage of using avian viruses for cancer treatment in mammals is the absence of prior exposure and, consequently, the lack of neutralizing antibodies (Sánchez *et al*., 2016). This is because the vast majority of the population does not have preexisting immunity to Newcastle disease, unlike other viruses pathogenic to humans, such as herpesviruses, adenoviruses, and smallpox virus (Schirrmacher *et al*., 2019). Another advantage is that NDV does not integrate its genome into the host cell genome (Niu *et al*., 2015). Furthermore, it can induce cancer cells to undergo apoptosis, leading to a transformation of the tumor microenvironment from immunosuppressive to pro-inflammatory (Schirrmacher *et al.*, 2015a). These mechanisms will be discussed in more detail throughout this review.

Mechanisms of induction of oncolysis and antitumor immune response: NDV selectively eliminates tumor cells while simultaneously triggering an antitumor immune response (Song *et al*., 2019). One of the mechanisms behind this effect is the induction of immunogenic cell death (ICD) following viral entry and replication. NDV primarily induces ICD through immunogenic apoptosis processes (Keshavarz *et al*., 2020).

ICD takes place after the synthesis of viral proteins is halted, leading to the exposure of viral HN and F antigens on the cell surface (Song *et al*., 2019). Following ICD, several other mechanisms come into play, including the release of viral progeny, which infect neighboring neoplastic cells (Ji *et al*., 2017). Additionally, there is local and systemic stimulation of the innate and adaptive immune response through the release of tumor and viral antigens, which are recognized by macrophage-activating DCs, CD4+, CD8+, and NK cells (Martini *et al*., 2020).

In veterinary literature, it has been reported that the virus can potentially destroy tumor cells by releasing perforins and granzymes from NK cells, recognizing antigens presented by MHC I. OVs may also destroy neoplastic cells by releasing perforins and granzymes from CD8+ lymphocytes, recognizing antigens presented by MHC I. These, in turn, can activate other CD8+ effector cells and generate CD8+ memory cells through CD127 receptors (interleukin-7 receptor-α) and CD62L (L-selectin). Other potential mechanisms include infection, direct oncolysis by the virus, and the secretion of various pro-inflammatory cytokines such as IFN-β, IL-1, IL-6, IL-18, IP-10, MCP-1 (CCL2), MCP-2 (CCL8), MCP-5 (CCL12), M-CSF and TNF.

It is still unclear whether there is oncolytic activity of NK and CD8+ cells in uninfected cancer cells. These different pathways of OVs cytotoxicity can occur simultaneously (Sánchez *et al*., 2018).

Virotherapy with NDV for the treatment of human breast cancer

In vitro research: Twenty-one studies conducted between 2003 and 2020 examined eight different pure or modified NDV strains (73T, AF2240, AMHA1, Anhinga,

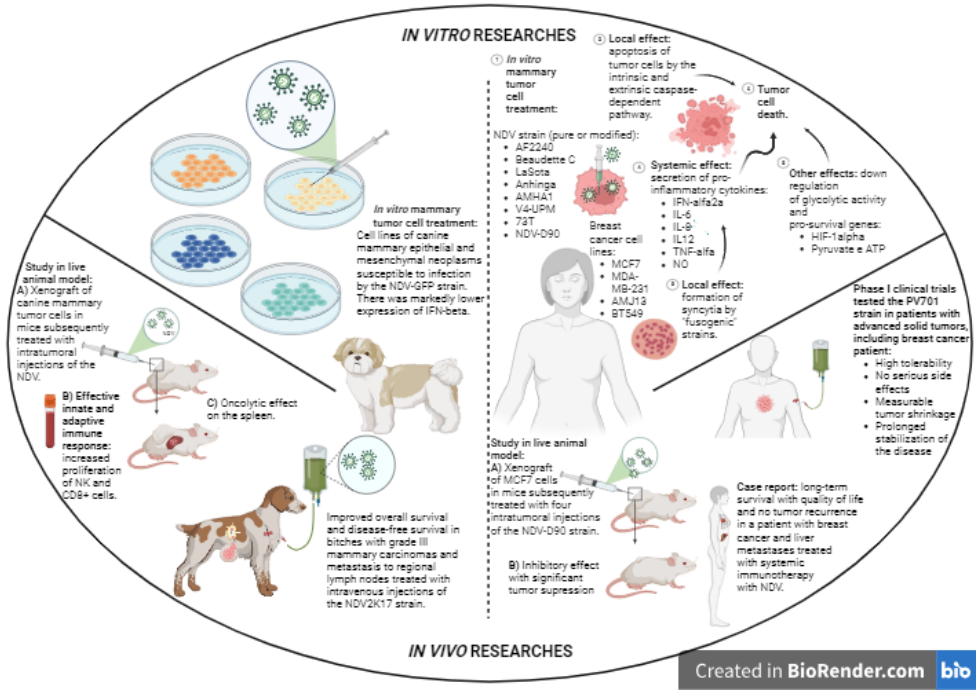

Fig. 1: Graphical abstract of *in vitro* and *in vivo* research using Newcastle disease virus for the treatment of human and canine mammary cancers. Source: the authors.

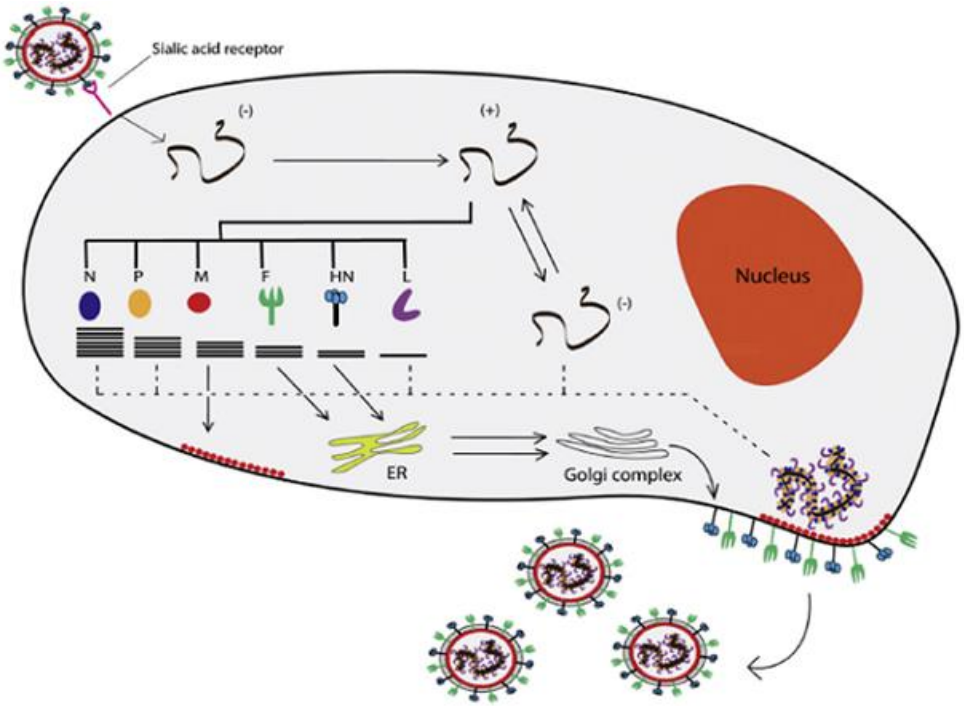

Fig. 2: NDV fusion and replication. (-): negative sense RNA. (+): positive sense RNA. ER: endoplasmic reticulum (GANAR *et al*., 2014).

Beaudette C, LaSota, NDV-D90, or V4-UPM) in human breast cancer cell lines, including AMJ13 (invasive breast cancer, no special type, estrogen and progesterone receptor negative (ER-PR-)), BT549 (invasive ductal carcinoma), MCF7 (invasive breast cancer, estrogen and progesterone receptor positive (ER+ PR+)), and MDA-MB-231 (HER2-ER-PR- invasive ductal carcinoma) (Table 1).

Several studies assessed the oncolytic potential of the AF2240 strain in MCF7 or MDA-MB-231 strains. Meyyappan (2003) employed both AF2240 and V4-UPM strains in MCF7 and MDA-MB-231 cell lines, both of which exhibited apoptosis characteristics such as cell retraction, cytoplasmic vacuolization, nuclear fragmentation, chromatin condensation, and apoptotic body formation. Notably, the AF2240 strain demonstrated significantly greater apoptotic activity, and the MDA-MB-231 cell line displayed a higher response to both strains. Kalid *et al*. (2010) detected up-regulation of pro-apoptotic genes MYBL2 and PUMA in MCF7 cells infected with AF2240. Othman *et al*. (2010) observed a significant increase in apoptotic cells in the MCF7 cell line infected with the AF2240 strain three days' post-infection. Additionally, Jamal *et al*. (2012) noted 50% cell death in cisplatin-resistant MCF7 cells within the first 12 hours' post-infection with the AF2240 strain. Furthermore, Lam *et al*. (2011) reported that human peripheral blood mononuclear cells activated with the AF2240 strain

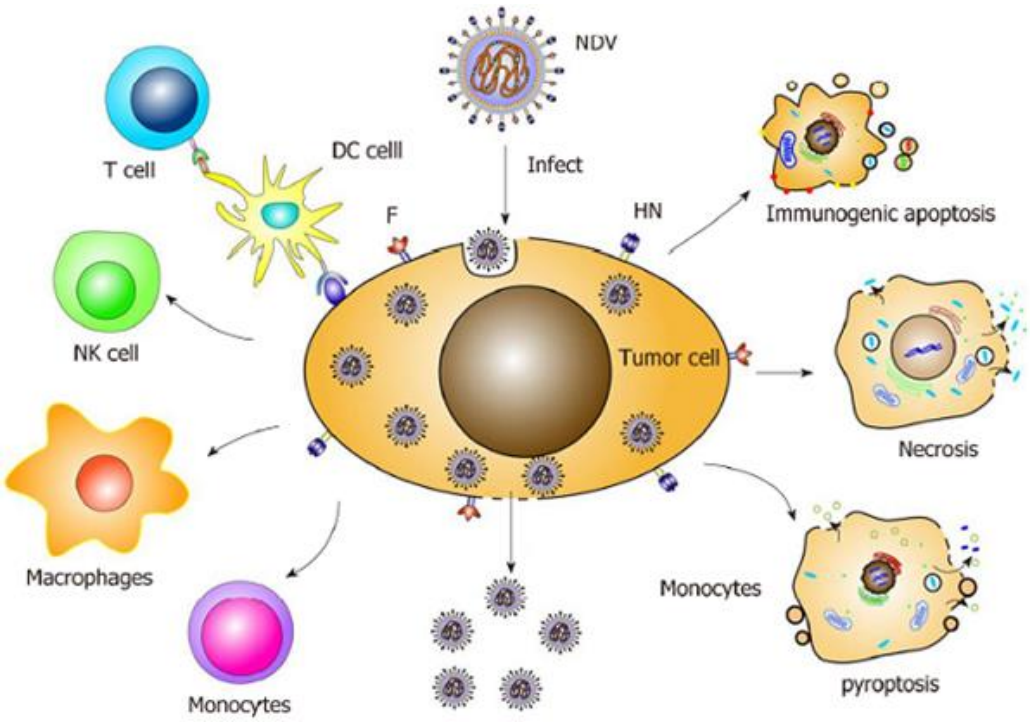

Fig. 3: Main mechanisms of NDV virotherapy in fight against cancer. NK cell: natural killer cell. DC cell: dendritic cell (SONG *et al*., 2019).

displayed high cytolytic efficiency in MCF7 cells. Ahmad *et al*. (2015) also employed the same strain in MDA-MB-231 cells and observed apoptosis induction.

Researchers assessed NDV-induced apoptosis pathways in human breast cancer cell lines. Elankumaran *et al*. (2006) edited and recombined Beaudette C and LaSota strains into rLaSota, rLaSotaV.F., rBC, rBC-EGFP, and rBC-Edit strains. All of these strains exhibited cytotoxicity towards MCF7, except for rLaSotaV.F. The authors emphasized their dependence on the caspase-dependent apoptosis pathway, with early oncolysis involving caspase-9 and caspase-3. Caspase-8 cleavage occurred later and was induced by TRAIL. Low replication and the absence of oncolytic activity of NDVr strains were observed in caspase-3 null MCF7 cells, demonstrating the caspase-dependent nature of NDV-induced apoptosis. Ghrici *et al*. (2013a, 2013b) demonstrated that the expression of the HN gene of the AF2240 strain induced apoptosis in MCF7 cells, and the apoptotic activity of the NP gene was independent of viral replication and viral protein synthesis, possibly occurring in the initial phase of the virus life cycle and being activated by the intrinsic pathway. Hassan *et al*. (2020) and Hassan *et al*. (2020) also observed apoptosis activated by the intrinsic pathway, marked by a significant increase in caspase-9, in AMJ13 and MCF7 cells treated with the LaSota strain. Shan *et al*. (2021) reported that the NDV-D90 strain significantly killed BT549 and MCF7 cells in a time- and dose-dependent manner, inducing apoptosis through the intrinsic and extrinsic signaling pathway in the BT549 lineage and the intrinsic pathway in the MCF7 lineage, with no observed necrosis.

The secretion of inflammatory cytokines by human breast cancer cells has also been a focus of research. Zhao *et al*. (2008) observed high levels of IL-2 expression in human tumor cell lines, including MCF7, using a LaSota-derived recombinant strain containing the IL-2 gene (rNDV/IL2). Burke *et al*. (2020) reported a robust immune response in MDA-MB-231 cells using the recombinant strain MEDI5395, derived from 73T. They observed positive regulation of PD-L1, CD80, and HLA-DR receptors, along with the production of IFN-α2a, IL-6, IL-8, and TNF-α. Ahmed *et al*. (2014) found that treatment with the AF2240 strain in MDA-MB-231 cells resulted in relevant production of NO and TNF-α in vitro, contributing to the cytotoxic activity of RAW 264.7 macrophages. Amin *et al*. (2019) observed cytotoxic effects on MCF7 and MDA-MB-231 cells using a recombinant strain derived from AF2240 containing the IL-12 gene (rAF-IL12).

Hypoxic cancer cells can overexpress HIF-1α, which controls pro-survival genes under hypoxia conditions. Abd-aziz *et al*. (2016) tested the AF2240 strain in MCF7 cells and reported decreased accumulation of HIF-1α induced by hypoxia, suggesting NDV as a promising agent for eliminating hypoxic cancer cells.

Regarding the redirection of glycolysis-dependent metabolism, Al-Shammari *et al*. (2019) observed an antitumor effect of the AMHA1 strain combined with 2-DG in AMJ13 cells, resulting in significant inhibition of tumor progression in vitro, compared to single treatments in vivo. Al-Ziaydi *et al*. (2020) evaluated the effects of the AMHA1 strain on the energy metabolism of AMJ13 and MCF7 cells. The researchers noted effectiveness in viral replication, induction of apoptosis, and a considerable decrease in glycolytic activity, leading to reduced pyruvate and ATP levels.

Liu *et al*. (2021) tested two recombinant strains in the MDA-MB-231 and MCF7 strains. In MCF7 cells, they observed few syncytia and moderate susceptibility. In the MDA-MB-231 strain, rClone30-Anh(F) and rClone30-Anh(HN-F) strains mediated cell death through rapid fusogenic capacity, resulting in the formation of a greater number of syncytia. rClone30-Anh(F) up-regulated the expression of ATG5, Beclin 1, MAP1LC3B, and alpha-sialic acid acyltransferase, while down-regulating interferon.

Table 1: Human breast cancer cell lines treated with Newcastle disease virus strains.

Reference	Lineage	Strain	Country	Objectives
Meyyappan (2003)	MCF7	AF2240 V4-UPM	Malaysia	Assessment of apoptotic capacity
	MDA-MB-231	AF2240 V4-UPM		
Elankumaran *et al.* (2006)	MCF7	Beaudette C modified (rBC, rBC-EGFP, rBC-Edit) LaSota modified (rLaSota, rLaSotaVF)	EUA	Assessment of apoptotic capacity
Zhao *et al.* (2008)	MCF7	LaSota modified (rNDV/IL2)	Germany	Assessment of inflammatory cytokine secretion
Elankumaran *et al.* (2010)	MCF7	Recombinant Beaudette C (contains a wild-type IFN-antagonistic V protein (rBC), and an isogenic mutant V protein (rBC-Edit virus) that induces robust IFN in infected cells) Recombinant LaSota (with a virulent F protein cleavage site that is as sensitive to interferon as the rBC-Edit virus (rLaSota V.F. virus)	EUA	Compare the oncolysis abilities of 2 recombinant strains (Beaudette C and LaSota)
Kalid *et al.* (2010)	MCF7	AF2240	Malaysia	Assessment of apoptotic capacity
Othman *et al.* (2010)	MCF7	AF2240	Malaysia	Assessment of apoptotic capacity
Jamal *et al.* (2012)	MCF7	AF2240	Malaysia	Anti-tumor effects: oncolysis
Ghrici 2013a *et al.* (2013)	MCF7	AF2240	Malaysia	Assessment of apoptotic capacity
Ghrici 2013b *et al.* (2013)	MCF7	AF2240	Malaysia	Assessment of apoptotic capacity
Ahmed *et al.* (2014)	MDA-MB-231	AF2240	Malaysia	Assessment of inflammatory cytokine secretion
Lam *et al.* (2014)	MCF7	AF2240 (PBMC treated with the strain)	Malaysia	Assessment of the immune response and oncolysis
Ahmad *et al.* (2015)	MDA-MB-231	AF2240	Malaysia	Assessment of apoptotic capacity
Abd-aziz *et al.* (2016)	MCF7	AF2240	Malaysia	HIF pro-survival gene assessment (hypoxia-inducible factor)
Al-shammari *et al.* (2019)	AMJ13	AMHA1 combined with 2-DG glucose competitor molecules	Iraq	Anti-tumor effects: redirection of glycolysis-dependent metabolism, using different treatments: NDV and/or 2-DG
Amin *et al.* (2019)	MDA-MB-231 MCF7	AF2240 combined with the IL-12 gene (rAF-IL12)	Malaysia	Assessment of inflammatory cytokine secretion
Al-ziaydi *et al.* (2020)	AMJ13 MCF7	AMHA1	Malaysia	Anti-tumor effects: redirection of glycolysis-dependent metabolism
Burke *et al.* (2020)	MDA-MB-231	73T modified (MEDI5395)	EUA	Assessment of inflammatory cytokine secretion
Hassan *et al.* (2020)	AMJ13 MCF7	LaSota	Iraq	Assessment of apoptotic capacity
Hassan *et al.* (2020)	AMJ13 MCF7	LaSota	Iraq	Assessment of apoptotic capacity
Liu *et al.* (2021)	MDA-MB-231 MCF7	Recombinant Anhinga (rAnh); Recombinant anhinga expressing red fluorescence (rAnh-RFP); Recombinant Clone30 (rClone30); Recombinant clone30 expressing red fluorescence protein (rClone30-RFP). Chimeric viruses rClone30-Anh(HN), rClone30-Anh(F), rClone30-Anh(HN-F)	China	Anti-tumor effects with different cells and strains
Shan *et al.* (2021)	BT549 MCF7	NDV-D90	China	Assessment of apoptotic capacity

In vivo research: In 2020, Chinese researchers conducted a study where they tested the NDV-D90 strain in six-week-old female mice that had been xenografted with MCF7 cells. The treatment involved four intratumoral injections. Remarkably, just eight days after initiating the therapy, they observed a significant inhibitory effect leading to substantial tumor suppression (Shan *et al*., 2021).

Clinical trials have also been conducted by researchers. Two phase I clinical trials investigated the PV701 strain in patients with advanced solid tumors. Pecora *et al*. (2002) administered single or repeated intravenous injections to 79 patients, eight of whom had breast cancer. These patients demonstrated a significantly improved tolerance to the virus, with some enduring doses ten times higher than the initial injection. In one patient with breast carcinoma, there was measurable but less than 50% reduction in the total tumor burden. However, a patient with breast cancer and bilateral pleural effusions experienced grade 3 dyspnea as a side effect.

On the other hand, Laurie *et al*. (2006) conducted a two-step desensitization protocol in 16 patients, two of whom had breast cancer. Notably, this therapy was better tolerated than the rapid bolus method used in previous studies, and no severe side effects were reported. Among the patients with breast carcinoma, one experienced prolonged disease stabilization, with no progression for at least 6 months as a positive outcome of the treatment.

Furthermore, in 2015, a noteworthy case was reported involving a patient who had breast cancer with liver metastases. The treatment approach for this patient consisted of 13 sessions of systemic immunotherapy with NDV, radiofrequency hyperthermia applied to the liver, and five vaccinations utilizing dendritic cells (DCs) derived from breast cancer cells that had been infected with NDV (oncolyzed). Importantly, this treatment was well-tolerated by the patient.

Remarkably, at the time of the report's publication, the woman had achieved a remarkable long-term survival of 66 months from the initial diagnosis. Even more encouraging was the fact that she maintained a high quality of life, and there were no signs of tumor recurrence. This promising clinical outcome was

Table 2: Viruses, viral strains and canine models treated with oncolytic virotherapy.

Virus	Viral strain	Canine model	Cell line	Reference
Canine Adenovirus (CAV)	ICOCAV17	Sarcomas		Gómez *et al.* (2020)
Measles	rMV-SLAMblind	Canine transitional cell carcinoma (canine cell line and mouse xenograft)	TCC-NU1 nectin-4+	Iizuka *et al.* (2020)
Sendai virus (SV)	Pure Sendai virus	Mast cell tumor		Ilyinskaya *et al.,* (2018)
Vesicular stomatitis virus (VSV)	VSV-IFNβ-NIS	Canines with cancer		Naik *et al.* (2018)
Myxoma virus (MYXV)	MYXVΔserp2	Spontaneous soft tissue sarcomas		MacNeill *et al.* (2018)
Reovirus	Pelareorep(Reolysin®)	Cutaneous mast cell tumor	CM-MC HRMC	Mahalingam *et al.* (2017)
Semliki Forest Virus	VA7	Osteosarcoma	Abrams D17	Nishiya *et al.* (2016)
Vaccinia virus (VACV)	LIVP6.1.1	Soft tissue sarcoma (cell lineage and mouse xenograft)	STSA-1	Adelfinger *et al.* (2015)
Canine distemper virus (CDV)	FXNO YSA-TC MD-77	Histiocytic sarcoma	CTT	Puff *et al.* (2009)
Canine parvovirus	Canine parvovirus	Fibroma	A27	Singh *et al.* (2006)
Canary pox virus	ALVAC	Canine spontaneous melanoma		Frey *et al.* (2002)

attributed to virotherapy with NDV, as the patient had not undergone any conventional treatments and had made no significant lifestyle changes (Schirrmacher *et al*., 2015b).

Oncolytic potential of viruses in the treatment of canine cancer: In the present day, neoplasms are a leading cause of death in small animals, primarily due to their increased lifespan, delayed diagnoses, and limited availability of effective treatments (Sarver *et al*., 2022). Mammary gland tumors are among the most commonly diagnosed neoplasms in dogs (Salas *et al*., 2015), and unfortunately, complete remissions for surgically non-incisable tumors remain rare (Wang *et al*., 2016). Chemotherapy may be overestimated by caregivers, who sometimes perceive it as a curative therapy, when in fact, it is a palliative treatment. However, it can be physically taxing and unpleasant for the animal, and it involves exposure to potentially teratogenic, mutagenic, and carcinogenic drugs (Stephens, 2019). Therefore, OVs can provide benefits to canine cancer patients (Sánchez *et al*., 2018).

The most recent studies involving canine models treated with various viral strains are presented in Table 2. Studies categorize OVs as potential antitumor agents for animals (Gómez *et al*., 2020; Iizuka *et al*., 2020), including their combined use with conventional therapies, such as immune checkpoint inhibitors (Cejalvo *et al*., 2018). This aspect makes them an intriguing alternative in the field of Veterinary Oncology (Sánchez *et al*., 2018).

Virotherapy with NDV for the treatment of canine mammary tumors

In vitro research: To date, only one study has examined the oncolytic potential of NDV in canine mammary cells. In their 2021 research, Santos *et al*., 2021 chose two canine mammary epithelial neoplasm cell lines (E20 and E37), two canine mammary mesenchymal neoplasm cell lines (M5 and M25), and a non-cancerous cell line (CF41.Mg). They assessed the antitumor activity of the genetically modified NDV LaSota strain, designed to express the GFP protein (NDV-GFP).

The results showed that all four neoplastic cell lines were more susceptible to NDV-GFP infection when compared to the normal cell line. However, the oncolytic effect was notably more pronounced in the M5 neoplastic mesenchymal lineage, which is considered more aggressive, than in the tumorigenic epithelial lineage (E20). Furthermore, a negative correlation was observed between the expression of the IFN pathway and susceptibility to NDV-GFP. The most sensitive cell line (M5) exhibited significantly lower IFN-β expression compared to the most resistant line (E20), indicating that the predominant mechanism of oncolysis in the experiment was associated with the IFN pathway.

In vivo research: To date, one study has tested the oncolytic capacity of NDV in canine with mammary carcinomas. Das *et al*. (2017) selected 12 bitches with grade III mammary carcinomas and metastasis to regional lymph nodes, diagnosed by fine needle aspiration; all subsequently underwent mastectomy. Group I consisted of six bitches diagnosed with carcinomas; all received four intravenous doses of the purified NDV2K17 strain postoperatively. Group II was composed of six bitches diagnosed with carcinomas; postoperatively, none received adjuvant treatment with NDV. All bitches in group I had higher overall survival (OS) and disease-free survival (DFS) (MEAN 365 days), compared to group II (OS MEAN 283 days; DFS MEAN 221 days). They observed that all animals developed a fever (104 ± 0.5˚F) and anorexia lasting for 3 to 4 days after the initial injection. Mild conjunctivitis was observed in two animals, which improved after supportive therapy. Reductions in body weight were also noted in all the female dogs during the viral therapy period, normalizing after the conclusion of virotherapy. Overall, all dogs receiving viral therapy survived the study period without any difficulties.

NDV beyond mammary tumor: It is necessary to emphasize that the oncolytic properties of NDV are not restricted to mammary tumors, as demonstrated by its efficacy against several types of human cancers, including advanced mesothelioma (Pecora *et al*., 2002), head and neck squamous cell carcinoma (Karcher *et al*., 2004), glioblastoma multiforme (Freeman *et al*., 2006), colorectal carcinoma (Schirrmacher *et al*., 2015a), prostate cancer (Shobana *et al*., 2013), as well as in the treatment of dogs with intracranial meningioma (King, 2017), which further endorses the credibility of this promising oncolytic virus.

Conclusions: In conclusion, oncolytic immunotherapy using Newcastle disease virus (NDV) holds great promise for the treatment of both breast cancers in humans and mammary tumors in dogs. Research has demonstrated the oncolytic potential of various strains of NDV in both in vitro and in vivo settings, with encouraging results in terms of tumor suppression and induction of apoptosis. Furthermore, NDV virotherapy has shown favorable safety profiles, with generally manageable adverse effects such as fever and mild conjunctivitis in animal studies.

While challenges and questions remain, including ideal administration protocols and long-term outcomes, the review presented here highlights the potential of NDV as a valuable tool in the fight against breast cancer in women and mammary tumors in dogs. Further studies and clinical trials are warranted to refine treatment strategies and assess the full scope of therapeutic benefits of NDV in these contexts.

Authors contributions: Arlinda Flores Coleto conceived and designed the review. Alessandra Aparecida Medeiros-Ronchi corrected and edited the final manuscript. All authors interpreted the data, critically revised the manuscript for important intellectual contents and approved the final version.

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