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Review Article

An Overview of Newcastle Disease Virus as a Potential Anti-cancer Agent for Gastrointestinal Cancer

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Abstract
Gastrointestinal (GI) tumors are a major public health problem worldwide, accounting for over one-third of global cancer-associated deaths. While the current therapies, such as traditional surgery, chemotherapy, and radiotherapy show little efficacy for GI cancers, studies suggest an increasing burden for these malignancies and emphasize the necessity of further studies to present an effective strategy for the treatment of GI tumors. Oncolytic virotherapy is a novel technology in which various oncolytic viruses are used for cancer therapy. Newcastle disease virus (NDV) is one such oncolytic virus that has been frequently investigated as an oncolytic agent in various pre-clinical and clinical studies. GI cancers, as a heterogeneous class of tumors originating from the stomach, liver, colorectum, esophagus, and pancreas, are one of the most studied malignancies with promising results, which have been evaluated for potential oncolytic activities of NDV. In this way, the present review aimed to focus on describing the pre-clinical and clinical studies about the use of oncolytic NDV therapy for GI cancers, including gastric cancer, hepatocellular carcinoma, pancreatic cancer, and colorectal cancer.

Keywords: Gastrointestinal Neoplasm, Oncolytic Virotherapy, Oncolytic Viruses, Newcastle Disease Virus
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Introduction

Gastrointestinal (GI) tract cancers are a major public health problem worldwide, with approximately 3.4 million deaths all over the world in 2018, accounting for over one-third (35%) of the global cancer-associated deaths.[1] GI cancer is a term used for a heterogeneous class of tumors originating from different parts of the digestive tract, including the stomach (gastric cancer; GC), liver (hepatocellular carcinoma; HCC), colorectum (colorectal cancer; CRC), esophagus (esophageal cancer; EC), and pancreas (pancreatic cancer; PC).[2] Studies show an increasing burden for GI cancers so that the global incidence and mortality of these malignancies are estimated to increase to 7.5 and 5.6 million, respectively, by 2040, emphasizing the importance of GI cancers and the necessity of further studies to present an effective strategy for the treatment of these cancers.[1] In this way, oncolytic virotherapy, as a novel technology, has shown promising results for GI cancer treatment.[3] This strategy uses engineered viruses that directly and exclusively target tumor cells. The cells are killed directly by oncolytic virus (OV) replication via tumor cell lysis and/or apoptosis.[4] Moreover, the viruses may also eliminate the tumor cells by activating the immune system to recognize tumor antigens; a phenomenon that not only kills the tumor cells but also can be used for antitumor vaccination.[4] Oncolytic viruses are non-genotoxic, and currently, there are several OVs under pre-clinical and clinical investigations for cancer treatment. However, the use of oncolytic viruses is associated with several limitations; the most important limitation of oncolytic virotherapy is the establishment of neutralizing antibodies via the host immune system, which restricts the delivery of OVs to cancerous tissues and their therapeutic efficacy.[5] Additionally, OVs can target and infect healthy cells, which may render the immune system to target healthy tissues.[6] Among OVs, oncolytic Newcastle Disease Virus (NDV) is one of the most studied agents with promising results.[7] In this way, the present review focused on describing the pre-clinical and clinical studies about the use of oncolytic NDV therapy for GI cancers.

Oncolytic Newcastle Disease Virus

In the early nineteenth century, oncolytic viral pathogens were recognized as potential anti-tumor factors, whereby body fluids comprising viral pathogens were utilized to infect tumor tissues.[8] Since then, various OVs (i.e., adenovirus, herpes simplex virus, reovirus, and NDV) were widely investigated for therapeutic purposes.[8-12] In this way,

non-human OVs, such as NDV, may be more attractive for investigation; these viruses not only can maintain their oncolytic activities in a host that is traditionally insusceptible to that particular OV, but also at the same time, they are non-pathogenic in humans. So far, NDV has been studied successfully in numerous investigations by using different strains particularly LaSota, AF2240-I, PV701, and MTH68/H.[8,11] NDV, as an avian virus, is a member of the Paramyxoviridae family, which was first reported during an outbreak in chickens at a Newcastle-upon-Tyne farm in England in 1926.[13] The virus encodes for a negative-sense single-stranded RNA genome (with six genes namely the phosphoprotein (P), nucleocapsid protein (NP), fusion protein (F), haemagglutinin-neuraminidase (HN), matrix protein (M), and a viral RNA-dependent RNA polymerase (L)) that is associated with an NP, P, and L ribonucleotide-protein structure, surrounded via a lipid envelope (comprising HN, M, and F).[14] According to disease severity in avians, NDV is classified into three pathotypes including lentogenic (non-virulent with non-oncolytic effect), mesogenic (intermediate), and velogenic (highly-virulent) with oncolytic effects, based on the F cleavage site.[14] NDV is a potent oncolytic candidate with a promising safety profile, reported in phase I and II clinical trials.[15] In comparison to other oncolytic candidates, NDV shows some advantages; for instance, it exhibited strong genome stability. In contrast to other oncolytic candidates, a vast majority of the human population is NVD seronegative, and then there is no issue of preexisting immunity.[15] Anti-tumor activities of NVD are mediated through several direct and indirect mechanisms (Figure 1).[16] By direct mechanisms the virus results in the development of multinucleated syncytia, extrinsic and intrinsic apoptotic pathways inducing, and ER stress and MAPK pathways activation. In the indirect ones, NDV enhances the recruiting of immune mediators such as lymphocytes, NK cells, and macrophages, by inducing proinflammatory chemokines and cytokines.[16] Currently, NDV is used for cancer treatment in several ways; (i) as a direct tumor oncolytic (oncolysis),[17] (ii) used in tumor vaccines to induce anti-tumor immune responses as an adjuvant,[18] and (iii) used as a vector to express therapeutic genes.[19]

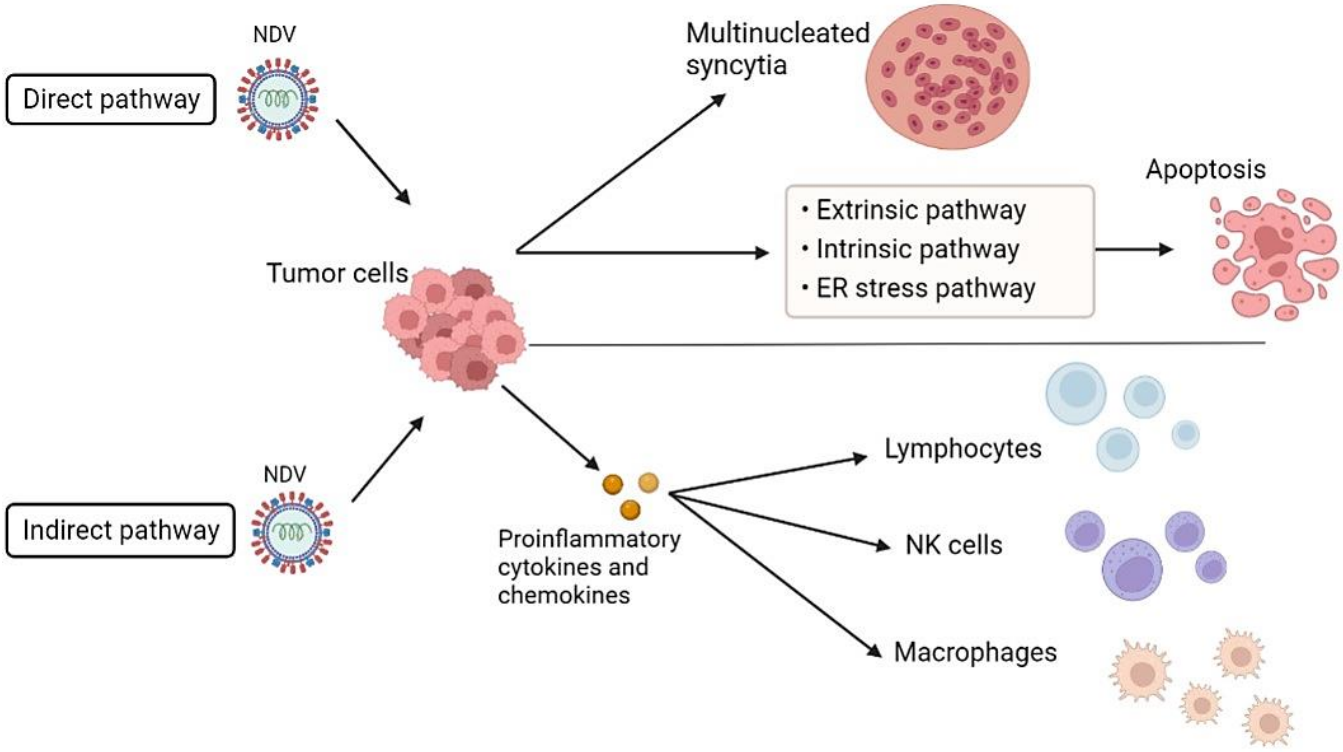

Figure 1. The Main Anti-tumor Mechanisms of NDV as Direct and Indirect Pathways. By direct mechanisms, NDV results in the development of multinucleated syncytia, extrinsic/intrinsic apoptotic pathways activation, and ER stress and MAPK pathways activation. In the indirect ones, NDV enhances the recruiting of immune mediators such as lymphocytes, NK cells, and macrophages, by inducing proinflammatory chemokines and cytokines.

Oncolytic NDV and Gastric Cancer

Gastric cancer is regarded as the fifth most prevalent cancer and the third most important cause of tumor-associated deaths, worldwide. For this cancer, the curative treatments are surgery, immunotherapy, targeted therapy, radiotherapy, and chemotherapy. Nonetheless, these procedures are associated with poor patient treatment, demanding a novel method for the treatment of patients with this devastating malignancy.[20] Regarding this, oncolytic virotherapy is one such promising curative approach, which has been investigated

by several OVs (such as herpes simplex virus-1, adenovirus, and NDV) in pre-clinical studies for GC treatment (Table 1).[21] In this way, an investigation by Song et al. assessed a genetically engineered NDV as an OV for GC treatment.[22] In that study, a novel virus, known as NDV-F3aa, was engineered by reverse genetics from NDV-Hitchner B1 strain; so that, three amino acids were removed from the fusion protein, leading to improved antitumor activity of this agent both *in vitro* (MKN-74 cells) and *in vivo*. Indeed, their results showed that this OV was an effective anti-cancer therapy to reduce the tumor burden of human GC in a xenograft mice model, without any toxicity.[22] These findings suggested NDV-F3aa as a promising candidate for further studies and future clinical trials of GC. A subsequent investigation assessed an NDV-D90 strain as an oncolytic in GC and suggested that the virus may have potential anti-tumor activities against this cancer; the study reported that NDV-D90 induced apoptosis, reduced cell growth and invasion in gastric cancer BGC-823, SGC-7901, and MKN-28 cell lines by modulation of p38, ERK1/2, and Akt signaling. Moreover, *in vivo* injection of NDV-D90 into mice decreased tumor growth and increased intratumoral necrosis.

Table 1. Pre-clinical Investigations of NDV in GI Cancers

Cancer	Virus Strain/Modified virus	Cell Lines	*In vivo*	Results	Ref.
GC	Hitchner B1/ NDV(F3aa)-GFP	MKN-74	Mice model	The Effective killing of MKN-74 cells *in vitro*; reduced tumor burden *in vivo*	(1)
	NDV-D90	BGC-823, SGC-7901 and MKN-28	Mice model	Induced apoptosis, reduced cell growth and invasion *in vitro*; decreased tumor growth *in vivo*	(2)
	LaSota/rL-RVG	SGC-7901 and HGC	-	Induced autophagy and apoptosis *in vitro*	(3)
	Hitchner B1/ NDV-GFP	MKN-74, Rat hepatocytes, Peritoneal lavage samples of GC patients	-	Specific infection and detection of GC cells	(4)
HCC	NDV-HK84	SK-HEP-1 and Hep3B	Mice model	Induced apoptosis, suppressed proliferation, migration, and invasiveness *in vitro*; reduced tumor growth *in vivo*	(5)
	LaSota/NDFLtag-EGFP	FHCC-98 and LX-2	Mice model	Reduced carbon tetrachloride- induced hepatic fibrosis in mice model	(6)
	Hitchner B1/ L289A	Huh7 and HepG2, and rat HCC cell line	Mice model	Increased tumor-specific syncytia formation and necrosis *in vitro*; prolonged survival of L289A-treated mice	(7)
	Anhinga strain/ NDV/Anh-EGFP	HepG2 and H22	Mice model	Suppressed HCC development both in HepG2 cells and in H22- bearing mice model	(8)
	Italien strain /rNDV-18HL	SMMC-7721	Mice model	Suppressed tumor cell migration and invasion *in vitro*; enhanced tumor necrosis, decreased metastasis, and prolonged survival of studied mice	(9)
	Anhinga strain/ NDV/Anh-TRAIL	HepG2 and H22	Mice model	Inhibition of HCC both in HCC cells and mice models	(10)
	rNDV-P53	HepG2 and H22	Mice model	Increased the apoptosis rate in HepG2 cells and mice model	(11)
PC	LaSota	PANC-1, SU.86.86, PL45, CFPAC-1, BxPC3, Capan-1, and Panc 10.05	-	Human PC cell lines were more than 700 times more sensitive to NDV killing than control cell lines	(12)
	NDV	PANC-1, MIA PaCa-2, BxPC-3, Hs 700T, Hs 766T, CFPAC, SU.86.86, AsPC-1, Capan-1, Capan-2 and HPAF-II	-	All the cell lines were susceptible to NDV	(13)
	NDV R75/98	DT6606PDA and Panc02	Mice model	NDV led to PC rejection and inhibited tumor relapse *in vivo*	(14)
	Hitchner-B1 and Ulster	PANC-1, SU.86.86, PL45, CFPAC-1, BxPC3, Capan-1, and Panc 10.05	-	Human PC cell lines were more sensitive to NDV killing than control cell lines	(15)
CRC	AF2240	SW620, HT29, ddd-1, Dks8, HCT116, and HCT116	-	In 70-90% of cells, cell death was observed	(16)
	V4-UPM and AF 2240	HT29	-	Cytolytic activities by inducing apoptosis	(17)
	Mukteshwar	SW620	Mice model	Suppressed tumor growth and prolonged the survival of tested mice	(18)
	73-T strain	SW620, HT29 and MM17387	Mice model	Suppressed tumor growth in the studied mice	(19)
	AF2240-I/ rAF-IL12	HT29	Mice model	Significant tumor regression	(20)
	rNDV-mOX40L	CT26	Mice model	Suppressed tumor cell growth	(21)
	Hitchner-B1 /rNDV-F3aa	CT26	Mice model	Long-lasting tumor remission	(22)
	Hitchner-B1 /rNDV-F3aa-IL-2	CT26	Mice model	Long-lasting tumor remission	(23)
	Clone30/rNDV-VEGF-Trap	EA.hy926 and CT26	Mice model	Reduced cell growth and migration *in vitro*; decreased tumor volume and angiogenesis *in vivo*	(24)

Additionally, NDV-D90 was reported to decrease GC vascularization, by inhibition of factors such as Matrix Metallopeptidase 2 and vascular endothelial growth factor A.[23] In another study by Bu et al., the anti-cancer activity of a recombinant avirulent NDV LaSota strain, known as rL-RVG, that expressed the rabies virus glycoprotein, was evaluated against GC SGC-7901 and HGC cells *in vitro*.[24] Accordingly, it was found that rL-RVG can induce autophagy and apoptosis in these cells via activating ER stress.[24,25] Furthermore, migration of GC was shown to be suppressed by rL-RVG through modulating α7-nicotinic acetylcholine receptors/ERK- EMT.[20] In addition to cancer treatment, NDV has also been shown to be a potential candidate for GC detection; so in a study by Wong et al., an NDV expressing the enhanced green fluorescent protein (NDV-GFP) was found to specifically infect and detect GC cells, suggesting this agent as a more sensitive technology in compared to current conventional cytology.[26] Taken together, these studies show that NDV can be regarded as a promising agent for GC treatment and detection by future investigations

Oncolytic NDV and Hepatocellular Carcinoma

Hepatocellular carcinoma, regarded as a major public health concern, is one of the top five most common cancers and a leading cause of tumor-associated death worldwide.[27] Nevertheless, despite impressive advances in HCC treatment, the current therapies, such as traditional surgery, chemotherapy, and radiotherapy indicated little efficacy, so that a vast majority of patients show an advanced stage and only about 10-20% of HCC cases can be cured.[28] Hence, it is crucial to investigate novel methods for HCC treatment. In this way, oncolytic virotherapy (in particular NDV), as a targeted therapy, has demonstrated promising findings for HCC (Table 1). In an investigation conducted by Chen et al., the antitumor activity and biosafety of the wild-type NDV HK84 strain (NDV/HK84) and 10 other NDV strains were assessed both *in vivo* and *in vitro* (SK-HEP-1 and Hep3B cells).[29] Accordingly, NDV/HK84 showed the highest oncolytic effectiveness; the virus-induced apoptosis, and suppressed proliferation, migration, and invasiveness of HCC cells *in vitro*. Furthermore, it interestingly reduced tumor growth in studied nude mice.[29] Similarly, Li et al. by using a recombinant NDV (known as NDFLtag-EGFP) carrying an enhanced green fluorescent protein (EGFP) derived from the LaSota NDV strain, showed that NDV can reduce carbon tetrachloride-induced hepatic fibrosis in mice model.[30] In a study, an NDV vector containing an L289A mutation within the F gene was engineered by Altomonte and colleagues, and was assessed for membrane fusion and HCC cytotoxicity *in vitro* (Huh7 and HepG2 cells) and immune-competent Buffalo rats with multifocal, orthotopic liver tumors.[27] The vector increased the fusion and cytotoxicity of the cells *in vitro* and also led to tumor-specific syncytia formation and necrosis without any reported toxicity for neighboring parenchyma. Moreover, it resulted in significantly prolonged survival of L289A-treated mice compared with control NDV, suggesting L289A as a potent candidate for clinical studies in HCC patients.[27] To increase efficacy, a genetically engineered NDV Anhinga strain harboring an insertion of EGFP, known as NDV/Anh-EGFP, was generated and tested both *in vitro* and *in vivo* for hepatocarcinoma by Wu et al.[28] They showed that NDV/ Anh-EGFP could be regarded as a novel candidate for clinical investigations so that it significantly suppressed hepatocarcinoma development both in HepG2 cells and in the H22-bearing mice model.[28] In another study by Wei and colleagues a recombinant NDV, named rNDV-18HL, containing an intact mouse-human chimeric HAb18 antibody (cHAb18) gene against CD147 (a tumor-associated antigen frequently found in HCC) was engineered using Italien strain.[31] In that study, rNDV-18HL increased the suppression of tumor cell migration and invasion *in vitro* (SMMC-7721 cells). Moreover, it specifically targeted orthotopic SMMC-7721 xenografts, resulting in enhanced tumor necrosis, decreased metastasis, and prolonged survival among studied mice.[31] So far, several investigations have used recombinant NDV encoding interferon and/or pro-inflammatory cytokines, such as rNDV -IL12-IL2, rNDV-TRAIL, rNDV-P53, for HCC therapy with reported promising antitumor effects *in vitro* and animal models.[32] Wu and colleagues assessed a recombinant NDV Anhinga strain encoding soluble tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) (NDV/Anh-TRAIL); TRAIL acts as a factor to selectively target cancer cells without toxicity for adjacent normal cells.[33] NDV/Anh-TRAIL indicated an efficient inhibition of HCC without significant toxicity both in HCC cells and mice models.[33] Similarly, the other recombinant NDVs indicated stronger anti-cancer activities than wild-type NDVs in both HepG2 cells and in H22-bearing mice.[32] An and colleagues showed that a recombinant NDV expressing P53 (as a vital tumor suppressor) significantly increased the apoptosis rate in HepG2 cells and the tumor tissues of rNDV-P53-treated H22-bearing mice than that of control animals, suggesting it is an ideal agent for the hepatoma therapy.[34] In short, both *in vitro* and *in vivo* studies suggest NDV as a potent candidate for cancer treatment particularly for hepatocarcinoma.

Oncolytic NDV and Pancreatic Cancer

Pancreatic cancer is one of the most highly resistant cancers to treatment; so, approximately in all patients with this cancer, the disease is associated with metastases, and traditional chemo- and radiation therapy show little efficacy for this cancer.[35] This malignancy is therapeutically non-responsive and investigations for new treatment methods, such as

oncolytic virotherapy, are necessary.[35] NDV is one such agent that has been investigated for PC by pre-clinical studies (Table 1). In a study, Walter et al. studied the efficacy of this novel method for pancreatic cancer treatment by NDV.[36] The authors found that NDV may have therapeutic effects on PC. They showed that human PC cell lines (PANC-1, SU.86.86, PL45, CFPAC-1, BxPC3, Capan-1, and Panc 10.05) were more than 700 times more sensitive to the lentogenic LaSota strain of NDV (NDV-LS) killing than control cell lines.[36] Similarly, Buijs and colleagues assessed the responses of 11 different pancreatic adenocarcinoma cell lines (PANC-1, MIA PaCa-2, BxPC-3, Hs 700T, Hs 766T, CFPAC, SU.86.86, AsPC-1, Capan-1, Capan-2, and HPAF-II) to NDV and interferon treatment, where all the cell lines were found to be susceptible to NDV.[37] In the same way, Schwaiger et al. indicated that NDV leads to PC rejection and inhibits tumor relapse via modulating adaptive immunity in the orthotopic tumor mice model. The rejection and recurrence prevention were directed through NK cell and adaptive immunity activation, respectively.[38] That study highlighted the ability of NDV to activation of an adaptive anti-tumor immune response to direct long-term pancreatic tumor surveillance. The cytotoxicity of NDV for pancreatic cancer has been also demonstrated in another study by Walter et al., who reported that the avirulent Hitchner-B1 (B1) and Ulster (U) strains of NDV "may have meaningful potential in treating pancreatic cancer".[39] Indeed, the evaluated pancreatic tumor cells (PANC-1, SU.86.86, PL45, CFPAC-1, BxPC3, Capan-1, and Panc 10.05) in that study were significantly more sensitive to NDVs than normal cells (For example, to kill control cells, about 1,500 times more B1 was required than pancreatic tumor cells).[39]

Oncolytic NDV and Colorectal Cancer

Colorectal cancer is regarded as one of the most common causes of cancer-associated deaths (the third most prevalent malignancy and the fourth most common cause of tumor-mediated death) in men and women, globally.[40] Nevertheless, despite this fact, it is currently well-known that various CRC subtypes are mostly resistant to traditional treatments; a phenomenon that, similar to other GI cancers, emphasizes the importance of investigations for the development of novel cancer therapies.[41] Although novel treatment modalities such as oncolytic virotherapy are still limited, CRC is one of the most studied GI cancers in this regard, which has been investigated in various pre-clinical and clinical studies.[42]

Oncolytic NDV Pre-clinical Studies and CRC

So far, extensive pre-clinical studies have demonstrated the potential efficacy of NDV for the treatment of patients with colorectal cancer (Table 1). In a study, Chia and colleagues incubated several human CRC cell lines (including SW620, HT29, ddd-1, Dks8, HCT116, and HCT116) with AF2240 strain. In 70-90% of cells, cell death was observed to introduce a concept for subsequent *in vivo* studies.[43] In the same way, the impact of NDV strains V4-UPM and AF 2240 on the HT-29 human colorectal adenocarcinoma cells was assessed by Assayaghi et al.; in a result, the use of both strains led to cytolytic activities against the cancer cells (but not control mouse fibroblasts) by inducing apoptosis (the viruses increased and decreased Bax and Bcl-2 expression levels, respectively). These observations highlighted the ability of NDV for targeting and killing cancer cells without any effect on normal cells.[44] In an investigation, the anti-cancer ability of the NDV Mukteshwar strain was evaluated by developing a nude mouse model for human colon cancer SW620 cell lines. In that work, the virus suppressed tumor growth and prolonged the survival of tested mice.[45] Ockert and colleagues engineered a human colon cancer cell (SW620, HT29, and MM17387) -bearing mice model and evaluated it with NDV 73-T strain.[46] They found that multi-dose injection of the virus suppressed tumor growth in the studied mice and reached 77-96%. Interestingly, to improve the anti-cancer efficacy of NDV against CRC, the novel idea of recombinant NDV encoding a therapeutic gene has also been evaluated by previous studies. In this regard, to increase the immunostimulatory activities of NDV, an AF2240-I NDV strain expressing the IL-2 gene, known as rAF-IL12, was developed by Najmuddin et al., study.[41] *In vitro*, rAF-IL12 led to a higher cytotoxicity efficacy against HT29 cancer cell lines compared to the parental AF2240-i. *In vivo*, the rAF-IL12- treated HT29 tumor-bearing NCr-Foxn1nu nude mice indicated significant tumor regression. Furthermore, rAF-IL12 significantly elevated the level of IL-2, -12, IFN-γ, and apoptosis-associated factors such as Smad3, Fas, BID, caspase-8, granzyme B, and BAX both *in vitro* and *in vivo*.[41] Similarly, Tian et al., generated a recombinant NDV encoding the murine immune co-stimulator OX40 ligand (OX40L), named rNDV-mOX40L, to increase NDV antitumor immunity;[47] OX40L enhances anti-cancer immunity via delivering a strong costimulatory signal to various immune cells such as CD4+ and CD8+ T. Vaccination with the recombinant virus suppressed the growth of CT26 colorectal cells *in vivo*. It stimulated vigorous infiltration of various tumor-specific cells such as CD4+, CD8+ T, and particularly OX40+ T cells to exert antitumor effects. Moreover, higher synthesis of IFN-γ protein in the tumor site was reported compared to the NDV-treated mice. Vigil et al. modified NDV to contain a highly fusogenic F protein (NDV-F3aa) and constructed several recombinant NDV-F3aa expressing GM-CSF, tumor necrosis factor (TNF)-α, IFN-γ, or IL-2 to evaluate them for enhanced therapeutic efficacy.[48] In that study, mice bearing CT26 cells treated with NDV-F3aa showed enhanced tumor growth suppression and prolonged survival compared to unmodified control NDV. On the other hand, compared to NDV-F3aa,

these responses were significantly increased via the administration of recombinant NDV expressing IL-2; so that, a majority of the mice demonstrated complete and long-lasting tumor remission.[48] Engineering NDV to encode a therapeutic gene was further assessed by these authors. They constructed an NDV Hitchner B1 encoding an MHC-I specific antigen of β-galactosidase (β-gal), a model tested and expressed via murine CT26 cell lines.[49] Treatment of CT26 tumor-bearing mice in that study led to complete tumor regressions. Interestingly, these observations were further enhanced via co-administration of a recombinant NDV encoding IL-2.[49] Meng et al. inserted VEGF-Trap, as a key player in anti-angiogenesis, into the genome of NDV (rNDV-VEGF-Trap).[50] It decreased cell growth and migration *in vitro*. In CT26-bearing mice, rNDV-VEGF-Trap decreased tumor volume, weight, and angiogenesis and enhanced the level of P53, BAX expression, and cleaved caspase-3 in the tumor tissue. These findings proposed that rNDV-VEGF-Trap may have potential anti-CRC efficacy, particularly through inducing anti-angiogenesis and apoptosis responses.[50] Taken together, the promising results of CRC pre-clinical studies not only warrant more investigation of NDV for this cancer (particularly those recombinant NDVs expressing other tumor-specific antigens and/or therapeutic genes) but also show that combinatorial methods introducing OVs targeting various mechanisms (e.g., tumor-associated antigens and adaptive immunity) may be needed to enhance optimal anti-cancer activities.

Oncolytic NDV Clinical Studies and CRC

Having seen that NDV could lead to a significant anti-tumor response in pre-clinical investigations, studies next engaged this virus in various clinical trials with CRC patients to assess the clinical efficacy of this OV (Table 2).[42] Currently, the use of NDV for cancer treatment in the clinical phases is conducted based on three general strategies: (i) application of free infectious NDV alone; (ii) application of intact cancer cells previously infected with NDV in cell culture; (ii) application of protein lysates obtained from NDV-infected cancer cells *ex vivo*; and (iv) combined application.[42,51]

Table 2. Clinical Investigations of NDV in CRC

Virus (Strain)	Clinical Phase	Type of Tumor	Number of Patients	Results	Ref.
ATV-NDV (Ulster)	PhaseI	CRC	16 subjects	12 patients showed an increased specific anti-tumor sensitivity	(25)
ATV-NDV (Ulster)	Phase I	CRC	20 subjects	16 patients responded with a delayed-type hypersensitivity (DTH) reaction	(26)
ATV-NDV	Phase II	CRC	57 subjects	Improving the 2-year survival rate of patients (97.9%) compared to control (73.8%)	(19)
ATV-NDV (Ulster)	Phase I	CRC	23 subjects	An enhanced sensibilization against ATV determined via DTH	(27)
ATV-NDV (Ulster)	Phase II	CRC	23 subjects	A lower tumor recurrence rate (61%) in the ATV-NDV vaccine-treated cases compared to untreated ones	(28)
ATV-NDV (LaSota)	Phase III	CRC	310 subjects	The remission rate of advanced tumors was enhanced	(29)
ATV-NDV (Ulster)	Phase III	CRC	50 subjects	Prolonged overall patient survival rate	(30)
ATV-NDV (Ulster)	Phase I	CRC	14 subjects	A partial diminishment of metastases in 4 patients	(31)

The anti-tumor activities of protein lysates and infected intact tumor cells are mediated by the adjuvant effect of NDV proteins, which are expressed in the infected tumor cells (Figure 2). However, the intact cell vaccines have higher anti-tumor efficacy compared to oncolysate. Since NDV infection of cancer cells can result in various immune responses (such as tumor-specific natural killer and CD8+ cells) against tumor antigens, autologous tumor vaccines (ATV-NDV) are generated by using patient-specific tumor cells.[42,51] In a study of CRC, Bohle and colleagues evaluated 16 patients after tumor resection via a tumor cell vaccine modified by a live and nontoxic NDV, for the first time.[52] In that study, 12 out of the patients showed an increased specific antitumor sensitivity. Lehner et al. in a phase I investigation with 20 CRC patients tested an ATV-NDV vaccine. Overall, no severe side effect was reported in that immunotherapy, except for mild fever in 4/20 cases.[53] Similarly, 16 patients responded to the vaccine with an active specific immune response (a delayed-type hypersensitivity (DTH) skin reaction), more effectively 24 h following ATV-NDV vaccination, to provide more beneficial data for future studies. Studies showed that the ATV-NDV vaccine quality plays a critical role in anti-tumor efficiency and increasing the quality of the ATV-NDV vaccine should be regarded vital by investigations.[54] For instance, in a clinical study of 57 patients with CRC, Ockert and colleagues improved the ATV-NDV vaccine preparation via purification of the tumor cells by Percoll density centrifugation, and then removed tumor-infiltrating lymphocytes through immunomagnetic beads.[46] In that study, no serious side effects were observed and the vaccine improved the 2-year survival rate of patients (97.9%), compared to the control (73.8%).[46] Currently, in patients with CRC, metastatic disease is one of the most common causes of CRC-mediated mortality and recurrence occurs in the majority of cases.[55] Hence, introducing effective methods to inhibit this phenomenon is vital. In this way, several clinical studies have investigated NDV in CRC patients with liver metastasis.[55] In a study, human primary

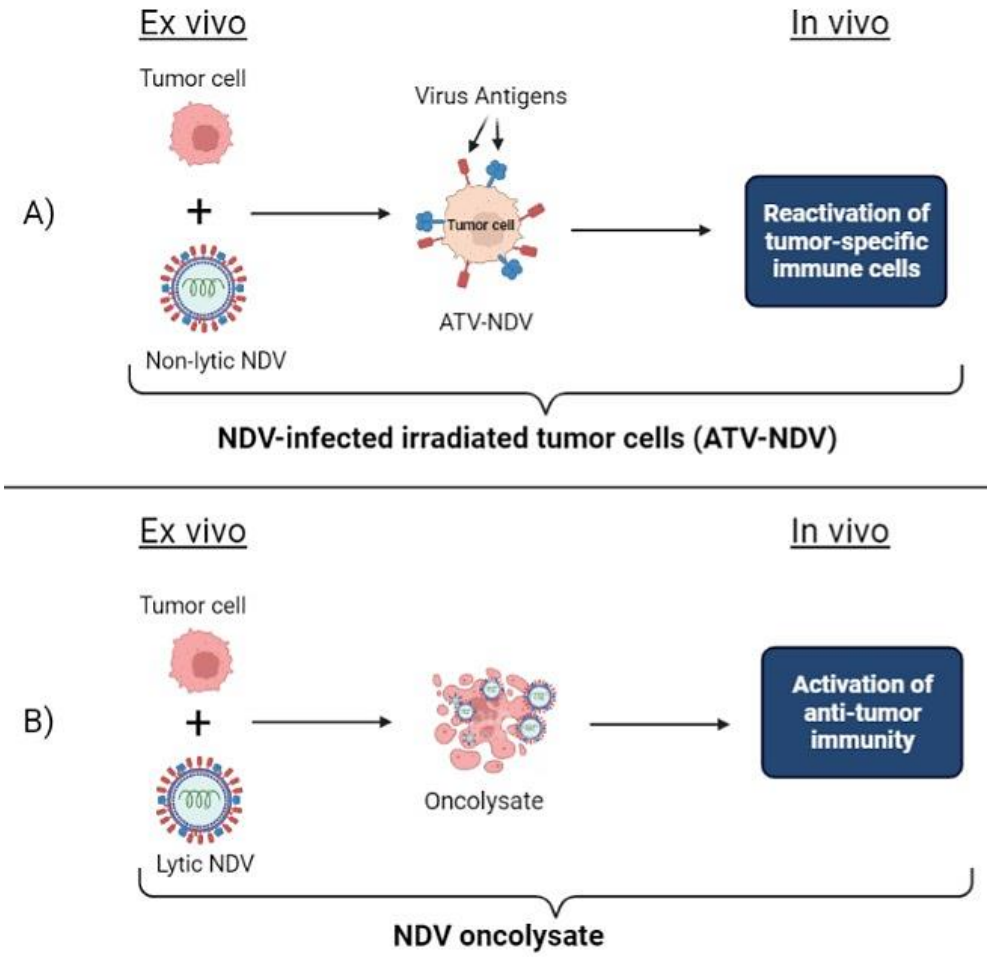

Figure 2. Vaccination with ATV-NDV Vaccine (A) and NDV Oncolysate (B) as Two Promising Methods. For ATV-NDV, the irradiated tumor cells are infected with non-lytic NDVs *ex vivo* to express viral antigen, then after the modified cell are injected to patients. For NDV oncolysate, tumor cells are infected with lytic NDVs *ex vivo* and the infected cells are lysed to provide oncolysate.

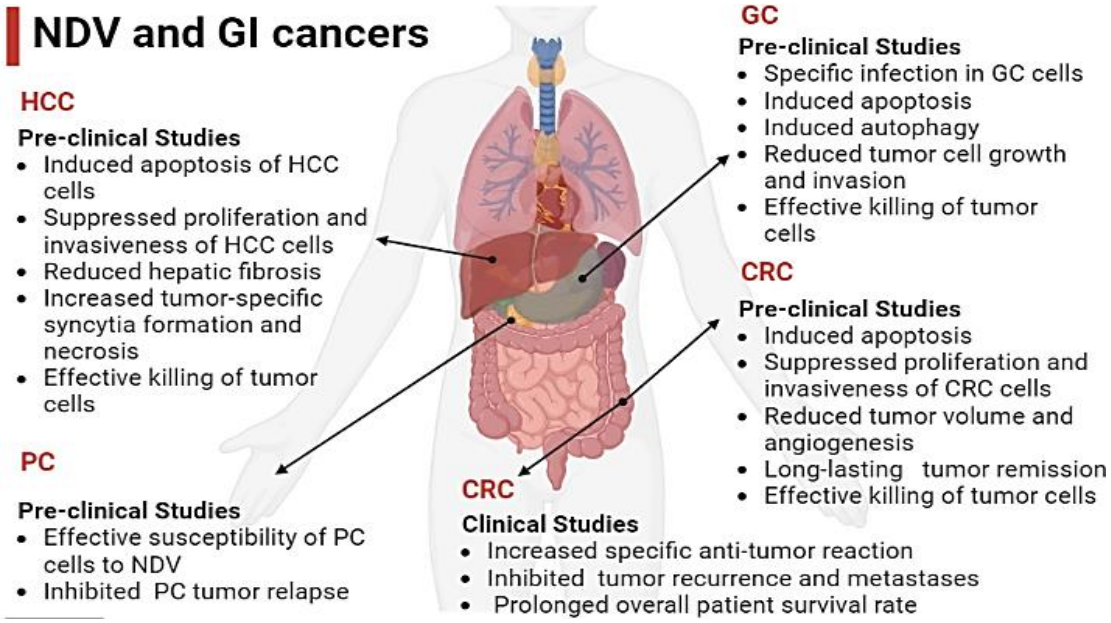

Figure 3. The Effects of NDV on GI Cancers. GC: Gastric cancer; HCC: Hepatocellular carcinoma; PC: Pancreatic cancer; CRC: Colorectal cancer.

CRC tumor cells were obtained and admixed with a nonlytic Ulster strain of NDV to prepare the ATV-NDV vaccine by Liebrich and colleagues.[56] Vaccination was performed in 23 cases with CRC liver metastasis and the findings demonstrated an enhanced sensibilization against ATV, determined via DTH skin reactivity in the patient; so that can be used for cancer therapy.[56] Subsequently, a phase II clinical study was carried out by Schlag et al. to assess postoperative active specific immunotherapy (ASI) with ATV-NDV for 23 patients with CRC after liver metastases resection.[57] Promising findings were reported in that study, where about 18-month follow-ups indicated a lower tumor recurrence rate (61%) in the ATV-NDV vaccine-treated cases compared to untreated ones.[57] Liang et al. studied 310 CRC patients with resection using the ATV-LaSota NDV strain vaccine;[58] it was found that vaccination with this vaccine significantly enhanced the remission rate of advanced tumors. In the vaccinated cases, the seven-year survival rate was increased (56.5%) compared to patients with resection alone (43.42%). The total effective rate in that study was reported as 24%, with one subject with complete remission. Furthermore, the vaccination enhanced immune responses by elevating the number of NK cells.[58] In one phase III clinical study, 50 cases of CRC with liver metastases were randomly categorized into two case and control groups.[55] After vaccination with six doses of ATV-NDV in the experimental groups, the patients were followed up for about 10 years. It was found that ATV-NDV can prolong the overall patient survival rate.[55] In a study by Schirrmacher et al., an ATV-NDV tumor vaccine containing a bispecific anti-CD28 fusion protein (bsHN-CD28), named ATV-NDV-bsHN-CD28, was developed to increase T cell costimulatory signals in 14 patients with advanced CRC.[59] Accordingly, all the treated cases indicated an immune response of tumor-reactive T cells at least once during the treatment course by vaccination. Moreover, four cases showed a partial reduction of metastases. In that study, ATV-NDV-bsHN-CD28 was reported as a safe vaccine, which "can reactivate possibly anergized T cells from a chronic disease like advanced-stage cancer".[59] In short, the NDV-based approaches, in particular the ATV-NDV tumor vaccine, seem to be safe and effective for the treatment of patients with CRC (Figure 3). More investigations should be performed to enhance the quality of this promising method to increase the survival of CRC patients.

Conclusion

The numerous pre-clinical investigations and limited clinical studies of NDV show the potential efficacy of this strategy for the treatment of different GI cancers, in particular CRC and metastases. Nonetheless, to achieve greater success in future large-scale clinical studies, there are some issues requiring to be resolved. For instance, further preclinical studies are needed to determine how NDV infects and spreads in human tumor tissues. This virus is an avian pathogen, which may have diverse tissue tropism in various hosts such as humans and mice, with dissimilar immune responses. Hence, the mouse model-bassed pre-clinical studies may not properly simulate the course of human infection with NDV and their underlying interplays. To resolve this, it is suggested to use the patient organ culture methods, containing both human tumor and normal tissues to assess the mode of NDV infection, tropism, spread, and particularly the underlying mechanisms of oncolytic activities of this virus in these relevant human cancerous tissues. Studies have proposed direct (selective targeting of tumor cells) and indirect (inducing the host anti-tumor responses) oncolytic activities as two major modes of action for NDV, which are induced after treatment with this virus. Accordingly, further investigations are required to show the role of each pathway in future clinical studies. Regarding the choice of proper NDV strain, the pathogenic replicative viruses (e.g., MTH-68/H and PV701) can appropriately spread in tumor tissues and kill the cells; nonetheless, these strains may lead to adverse side effects. On the other hand, the attenuated ones (e.g., Ulster, Hitchner-B1, and HUJ) target tumor cells with limited spreading activity; in fact, these strains seem to activate anti-tumor immune responses more efficiently without adverse side effects. Finally, rNDVs encoding therapeutic genes and ATV-NDV vaccines are currently two promising methods. More studies are needed to find the proper therapeutic genes and increase the quality of the ATV-NDV vaccine to enhance NDV oncolytic activities.

Authors' Contributions

All the authors have contributed substantially to this work.

Conflict of Interest Disclosures

The authors declare that they have no conflicts of interest.

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