Oncolytic Newcastle Disease Virus

Subjects: Medicine, General & Internal

Contributor: Volker Schirrmacher

Resistance to therapy is a major obstacle to cancer treatment. It may exist from the beginning, or it may develop during therapy. The review focusses on oncolytic Newcastle disease virus (NDV) as a biological agent with potential to break therapy resistance. This avian virus combines, upon inoculation into non-permissive hosts such as human, 12 described anti-neoplastic effects with 11 described immune stimulatory properties. Fifty years of clinical application of NDV give witness to the high safety profile of this biological agent. In 2015, an important milestone was achieved, namely the successful production of NDV according to Good Manufacturing Practice (GMP). Based on this, IOZK in Cologne, Germany, obtained a GMP certificate for the production of a dendritic cell vaccine loaded with tumor antigens from a lysate of patient-derived tumor cells together with immunological danger signals from NDV for intracutaneous application.

NDV viral oncolysis immunogenic cell death type I interferon dendritic cells active-specific immunotherapy bispecific antibodies gene therapy checkpoint inhibition T cell costimulation RIG-I IFNAR

1. Introduction

Oncolytic viruses (OVs) provide a new promising way to treat cancer. Such biological agents replicate selectively in tumor cells and induce tumor-selective cell death (oncolysis). Oncolytic viral therapy has an initial phase in which the virus mediates direct oncolysis of tumor cells, followed by a second phase of post-oncolytic immune response. This post-oncolytic immune response is directed towards tumor-associated antigens (TAs) and is considered as a key factor for an efficient therapeutic activity [1].

OVs adapted to the human immune system, such as native *measles virus* and *herpes-simplex virus 1* (HSV-1) exert adverse effects on human dendritic cells (DCs). These negative effects include cell viability, maturation and expression of co-stimulatory molecules. *Measles virus, mumps virus,* and *respiratory syncytial virus* are *paramyxoviruses* from man and cause serious human diseases.

Genetic engineering enabled to develop from all the mentioned viruses recombinant OV strains without pathogenicity. Reverse genetics engineering has allowed development from negative strand RNA viruses recombinant OV strains with additional transgenes [2].

A review from 2018 on oncolytic viro-immunotherapy of hematologic and solid tumors lists ten virus families from which new recombinant oncolytic strains have been generated: *Adenoviruses, flaviviruses, herpesviruses, orthomyxoviruses, paramyxoviruses, picornaviruses, poxviruses, reoviruses, rhabdoviruses, and togaviruses*. A plethora of trials are mentioned which have been initiated to assess the safety and efficacy of these OVs [3].

This review deals with a native OV from birds, *Newcastle disease virus* (NDV). This paramyxovirus is not adapted to the human immune system. Birds are permissive hosts of this virus, while cells from mammals, including man, are non-permissive. Since NDV has neither adverse effects on human cells nor any pathology, it can be used as a native OV in cancer patients. The safety profile for NDV includes lack of gene exchange via recombination, lack of interaction with host cell DNA, virus replication independent of cell proliferation and low side effects in cancer patients.

Newcastle disease is a major obstacle in poultry industry worldwide [4]. Certain strains of NDV have been developed to be used for preventive vaccination of chickens for more than 60 years [5]. In the 1960s, the phenomenon of viral oncolysis was discovered and a search began for a type of virus most suitable for clinical application in cancer patients. 1965, William A. Cassel reported about NDV as an antineoplastic agent in man [6]. Since then, NDV has been applied to cancer patients in the USA and in Europe [4][5]. Meanwhile, new regulations require a high-quality standard for NDV production as prerequisite for clinical application.

2. Basic Information

2.1. Evolution and Taxonomy of NDV

Mammals developed about 200 million years ago while a majority of bird species developed only about 66 million years ago [7]. Bird viruses thus had a relatively shorter time to adapt to the immune system of their hosts than viruses of mammals. Multicellular organisms, like birds and mammals can respond to virus infection, in particular by a type I interferon response (see below). As an avian virus, NDV has evolved viral immune escape mechanisms in birds. These interfere with the type I interferon mediated host response. Importantly, this viral escape mechanism is species specific and does not apply to non-permissive hosts.

NDV is an avian paramyxovirus type 1 (APMV-1). Such viruses have a negative sense single-stranded RNA (−ssRNA) as genome. Some strains show in non-permissive hosts a natural oncotropism (i.e., tumor selective viral replication), oncolytic potential and immune stimulatory properties.

The phylogenetic classification system of NDV has recently been updated [8]. NDV strains are classified according to their pathotypes and virulence as either lentogenic (low), mesogenic (medium) or velogenic (high). Velogenic strains are highly infectious in birds and are distinguished as viscerotropic or neurotropic pathotypes.

2.2. Molecular Biology of NDV

Genome sequences for many strains of NDV are available on the web at www.ncbi.nlm.nih.gov. All genome sizes of NDV obey to the rule of six which is characteristic for APMV-1 [9]. The genomic RNA contains a 3′-extragenic region known as leader and a 5′-extragenic region known as trailer. These are regions for control of virus transcription and replication and also for encapsidation of newly synthesized RNAs into virus particles. Leader and trailer flank the six genes (3′-N-P/V-M-F-HN-L-5′) of the viral genome. The genes code for nucleoprotein (NP), phosphoprotein (P), matrix protein (M), fusion protein (F), hemagglutinin-neuraminidase protein (HN) and large protein (L). The genome has a large capacity (>5 kb) for the incorporation of transgenes [4].

Infection of cells by NDV involves as a first step binding of the virus to the host cell's surface via the cell-binding domain of the HN molecule [10]. α2,6-linked sialic acid was demonstrated to serve as high-affinity receptor for HN and for binding of NDV to cells [11]. Cell surface binding of the virus is followed by activation of the fusion protein F. A concerted action of HN and F leads to fusion of the viral and the host cell membrane so that the viral genome can enter the cytoplasm of the host cell. NDV can also utilize a clathrin-mediated endocytosis route and macropinocytosis as alternative endocytic pathway to enter cells [12]. In the cytoplasm, the −ssRNA is transcribed into messenger RNA. The viral mRNAs remain polyadenylated and carry 5′-phosphate groups (5′-PPP) in contrast to mammalian mRNAs which are either capped or contain base modifications. The viral mRNAs are then translated into the corresponding viral proteins.

The second step relates to viral replication using a newly produced nucleocapsid as positive-strand "anti-genome" [13]. The viral nucleocapsid consists of a single species of viral RNA (15,186 to 15,198 nucleotides in length) and replicon complex proteins (NP, P, L). Once produced, the nucleocapsid as "antigenome" is used as template for viral replication. The viral proteins P and L associate to form the enzyme RNA-dependent RNA polymerase, which is essential for viral replication. Viral replication can produce 1000 to 10,000 copies per cell. The newly produced viral genomes become encapsulated at the plasma membrane. A membrane budding process then enables the release of new virus particles.

Infection of normal cells from non-permissive hosts (e.g., mouse or man) usually does not proceed to the second step [14]. In NDV-infected non-activated or activated T cells, intracellular viral RNA (− and + strands and double-stranded (ds) RNA) can be detected within 1 to 12 h [14]. The viral replication cycle is prevented by *interferon-induced genes* (ISGs) such as *myxovirus (influenza*) resistance A (MxA), 2′-5′-oligoadenylate synthetase/ribonuclease L (OAS) and dsRNA-activated protein kinase R (PKR) (13). Mammalian and avian cells use OAS/RNase L to degrade cellular and viral RNA and retinoic acid inducible protein I (RIG-I) to enhance interferon induction as the first line of defense against viral infection. Viruses have developed diverse strategies to escape antiviral effects of OAS. NS1 protein of *Influenza virus A* acts upstream of its pathway while other viral proteins such as *Theiler's virus* L protein act downstream.

NDV was recently reported to be able to establish persistent infection in tumor cells in vitro. There was a contribution of the cleavage site in F and of a second sialic acid binding site of HN. Persistent infection avoids oncolysis and immunogenic cell death (ICD) (see below) and facilitates viral spread from cell to cell as a potential mechanism to escape host antiviral responses [15].

OVs such as naturally occurring and attenuated NDV can be used alone or in combination with cancer vaccines for human cancer therapy [16]. OVs can also be used as vectors for gene therapy of cancer. Basic research in molecular biology and virology allowed to elucidate paramyxovirus replication and pathogenesis. Such studies were primarily performed with the rodent paramyxovirus *sendai virus*. Reverse genetics is a method by which infectious negative strand RNA viruses can be generated entirely from cloned DNA (c-DNA) [3][17][18][19]. This molecular engineering technology has allowed to develop recombinant oncolytic paramyxovirus strains with additional transgenes.

Harnessing OV-mediated anti-tumor immunity by therapeutic transgenes, pharmacological agents or bispecific antibodies has been the topic of a special issue of Frontiers in Oncology [1].

2.3. The Type I Interferon Response in Birds and Its Inhibition

Type I interferon (IFN-I) responses serve as a first line of host defense against microbe invasion. Type I interferons can induce tumor cell apoptosis and anti-angiogenesis via signaling through the common type I interferon alpha receptor (IFNAR). IFN-I can also exert direct effects on cells of the immune system [20].

It is of great interest to compare the interferon response to NDV in permissive cells from birds as natural hosts to the response in cells from non-permissive hosts including humans. Infection of chickens with virulent NDV is associated with severe pathology, morbidity and mortality. Recently, immune responses were described of mature chicken bone-marrow-derived dendritic cells (BM-DC) upon infection with NDV strains of differing pathogenicity. Gene expression profiling revealed increased expression of *melanoma differentiation associated gene 5* (MDA-5), of the helicase LGP2, the Toll-like receptors (TLR) 3 and 7, of type I interferon (IFN-α, IFN-β), IFN-γ, of interleukins (Ils) IL-1β, IL-6, IL-10, IL 12, IL-18, of chemokine ligand CCL5, and of major histocompatibility molecules MHC-I and MHC-II. Velogenic NDV showed a stronger replication capacity in BM-DCs than lentogenic NDV [21].

Of importance for the pathogenicity of NDV in birds is the capacity of certain viral products to antagonize the interferon response. In avian cells, NDV has developed a frameshift variant of the viral phosphoprotein P to escape type I IFN mediated anti-viral responses [22]. The incorporation of two G nucleotides at the RNA editing site of the P gene results in the frameshift variant V protein. Recently it was demonstrated that the interferon antagonistic activities of the V proteins of NDV correlated with their virulence [22]. The V protein interacts specifically with bird proteins [22]. It thereby can inhibit IFN signaling by targeting signal transducers and activators of transcription 1 (STAT1) for degradation. It can also interact with MDA-5, leading to the inhibition of interferon regulatory factor (IRF)-*3* activation and IFN-β induction [23].

Upon virus infection, MDA-5 (or RIG-I in humans) recognizes ds viral RNA with 5′-triphosphate as being foreign and distinct from self-RNA. This initiates a strong type I interferon response [24][25]. Mitochondrial antiviral-signaling protein (MAVS) is an essential adaptor protein in RIG-I mediated antiviral innate immunity. Recently, a MAVS gene from goose (goMAVS) was identified. This bird derived MAVS mediated the activation of the type I interferon pathway in a species-specific manner [26]. Cells from birds can activate [26] or inhibit [23][27] a type I interferon

response quite similar to cells from mammals. However, decisive protein–protein interactions in the interferon signaling pathway are bird-specific.

2.4. The Type I Interferon Response in Mammalian Cells and Its Inhibition

In mammalian cells, NDV induces a strong interferon response which involves an early and a late phase. This leads to inhibition of virus replication. The early phase response is initiated by cytoplasmic RIG-I-like receptors (RLR) upon recognition of foreign viral RNA while the late phase is initiated by membrane-bound IFNAR upon recognition of their respective ligands [20][27]. RLR is a family of cytoplasmic RNA helicases that includes RIG-I and MDA-5. These RNA sensors signal through the mitochondrial adaptor MAVS, recruiting kinases to activate the nuclear transcription factors NF-κB and IRF-3, and induce the transcription of IFN-I and proinflammatory cytokines.

In RIG-I-deficient fibroblasts, cytokine production is abrogated in response to NDV, *sendai virus, vesicular stomatitis virus*, *hepatitis C virus* or *influenza A and B virus*, thus demonstrating the importance of this foreign RNA sensing cytoplasmic receptor [28].

Other pathogen recognition receptors (PRR) are TLRs. These consist of more than 10 members and are expressed on the cell surface membrane or on endosomes. TLRs appear to be required to induce an interferon response in plasmacytoid dendritic cells (pDC) [29], while RLRs are critical to sense NDV by conventional myeloid DCs (mDC), macrophages, and fibroblasts. Details about the interferon signaling cascades and their regulation have been described [30][31]. Cytosolic nonself DNA recognition via the cyclic GMP-AMP synthase (cGAS) pathway also leads to IFN-I induction [32]. Upon sensing DNA viruses, RNA polymerase III is reverse transcribed to produce short RNAs, which are recognized by RIG-I. Latest updates on RIG-I also reveal long noncoding RNAs (LncRNAs) as being involved in regulation of RLR pathways. Antiviral IFN-I signaling is also regulated by covalent protein modification via ligation of small ubiquitin-like modifier (sumoylation) [32]. Not only foreign nucleic acids but also “unmasked”, misprocessed, or mislocalized host-derived RNA or DNA molecules can be recognized by RLRs or cGAS thus leading to proinflammatory or autoimmune disease [32].

During the late phase (8–18 h) of the IFN response [25], the induced type I IFN molecules (IFN-α and -β) secreted during the early phase, interact with the cell surface expressed IFNAR. These cytokine receptors are expressed by cells of all lineages, but not on mature erythrocytes. Nearly 20 four-helix bundle cytokines exist in humans and mice to interact as ligands with IFNAR. IFNAR consist of two chains, R1 and R2. The cytoplasmic domains of these chains are physically associated with Janus-family tyrosine kinases (JAKs) and Tyrosine-kinase (Tyk)-2. Ligand–receptor interaction initiates an amplification loop of the IFN response, which involves signaling via STAT proteins and IRF-7 [33].

Human mDCs were infected by NDV to study an uninhibited cellular response to virus infection [34]. The new approach integrated genome-wide expression kinetics and time-dependent promoter analysis. Interestingly, the anti-viral cell-state transition during the first 18 h post-infection could be explained by a single convergent regulatory network. A network of 24 transcription factors was predicted to regulate 779 of the 1351 up-regulated genes. It was concluded that the proposed network is effective in changing the cells underlying biology. The timing

of this step-wise transcriptional signal propagation appeared as highly conserved. The effect of NDV on human DCs was also analyzed by the release of cytokines important for Th1 or Th2 polarization. Human monocyte-derived DCs were found to become polarized towards DC1 [35]. Signaling through RIG-I and IFNAR was found in further studies to be of importance for immune activation of DCs and other immune cells by NDV in mouse or human [25].

In contrast, signaling through RIG-I and IFNAR is successfully antagonized by respective inhibitory proteins from *ebola virus* (EBOV). EBOV, belonging to the family of *filoviridae*, was first discovered in 1976. It infects cells from primates (*gorilla, shimpanzee, human*). Primary target cells are macrophages and DCs. Zoonotic transmission of EBOV to humans causes severe and often lethal hemorrhagic fever. Disease characteristics are a systemic inflammatory response syndrome (SIRS), disseminated intra-vascular coagulation, systemic hemorrhage and multiple organ failure. Two of the 8 viral proteins of EBOV are involved in immunosuppression. In multiple ways they prevent type I interferon signaling. VP35 antagonizes the early phase of the interferon response. VP24 is an antagonist of the late phase. The molecular details of this virus-inhibited cellular response to infection have been described elsewhere [36]. Recently, a novel EBOV glycoprotein (GP) modified recombinant NDV (rNDV) was constructed as potential vaccine. The EBOV GP was expressed at a high level. Upon immunization, guinea pigs developed high levels of neutralizing GP-specific IgG and IgA antibodies [37].

3. Over 50 Years of Clinical NDV Application

3.1. 1960s to 1970s: Post-Operative Treatment with Oncolysate Vaccines

As early as 1965, Cassel and Garret from Atlanta (GA, USA) reported on oncolytic NDV (strain *73 T*) as an antineoplastic agent [6]. Thereafter they observed the development of post-oncolytic immunity [38]. These authors were pioneers in developing NDV-based viral oncolysate vaccines for post-operative active-specific immunization of stage II malignant melanoma patients (two Phase II clinical studies involving 32 and 51 patients). A ten-year follow up of these 83 treated patients revealed that over 60% were alive and free of recurrent disease [39].

Later, a similar Phase II study with autologous NDV-modified tumor lysate vaccines has been conducted in Germany involving 208 patients with locally advanced renal cell carcinoma. Kirchner et al. concluded that the results demonstrated improved disease-free survival (DSF) in comparison with survival data published for similar patients who were treated by surgery alone [40].

3.2. 1990s to 2000s: Post-Operative Treatment with Autologous Live NDV-Modified Tumor Cell Vaccine (ATV-NDV)

To increase the immunogenicity of virus-modified tumor vaccines, a new concept was developed at the German Cancer Research Center (DKFZ) in Heidelberg, Germany. The idea was to develop an irradiated virus-infected autologous live cell tumor vaccine (ATV-NDV). The concept was tested with success in various metastatic animal tumor model systems [41][42]. After a series of tests with human tumor cell lines or with tumor cells from fresh

operation specimens, human tumor cell modification by virus infection (NDV lentogenic strain *Ulster*) was found to be an efficient and safe way to produce a homologous human ATV-NDV cancer vaccine. This had pleiotropic immune stimulatory properties [43].

One clinical study evaluated the effect of vaccine quality parameters (i.e., cell number and cell viability) on the survival of primary operated breast cancer patients treated post-operatively with ATV-NDV [44]. Overall survival (OS), four years after surgery, was 96% for patients who received a high-quality vaccine (n = 32) compared with an OS of 68% for those who had received a low-quality vaccine (n = 31), a difference that was statistically significant [45].

A Phase II trial involved patients with locally advanced colorectal carcinoma (n = 57). Two years after surgery, OS for patients treated post-operatively with ATV-NDV was 98% compared to 74% for historical control subjects [44]. Using ATV-NDV vaccine obtained from cell culture, a further Phase II study was performed with patients suffering from glioblastoma multiforme. The median OS of vaccinated patients (n = 23) was 100 weeks versus 49 weeks in 87 non-vaccinated control subjects from the same clinic and the same time period ($p < 0.001$) [46].

A similar Phase II trial involved 20 patients with stage III and IV head and neck squamous cell carcinomas (HNSCC). The study demonstrated feasibility and safety of the ATV-NDV vaccine regimen and suggested prolongation of five-year survival [47].

Finally, a prospectively randomized Phase II/III trial (n = 50) was performed in stage IV colorectal carcinoma patients after resection for hepatic metastases. The objective was to investigate the efficiency of ATV-NDV as a tertiary prevention method. Evaluation was performed after an exceptionally long follow-up period of 9–10 years. While there was no significant difference between control and vaccinated rectal cancer, a significant benefit was seen in the colon cancer subgroup with regard to metastasis-free survival and overall survival: In the vaccinated arm, only 30.8% had died, while in the control arm 78.6% had died [48]. The trial provides evidence for the clinical value of the vaccine ATV-NDV. Its mechanism of function has been reviewed [49].

In 2003, a Phase III trial from China reported results from 310 patients suffering from Gastrointestinal Carcinoma (stage I-IV) which were treated with autologous tumor vaccine and NDV vaccine (strain *La Sota*). In comparison to 257 patients in which resection was performed without further vaccination, a significantly improved mean and median survival was observed for the vaccination group (7 years versus 4.46 years) [50].

3.3. 2000s: Systemic Application Studies with Oncolytic NDV

A selected case series study of four high grade GBM was performed in Hungary by Csatary and colleagues by i.v. application of an attenuated oncolytic veterinary vaccine strain, which was termed MTH-68/H. It was reported in 2004 on radiographically-documented responses and on long survival with improved symptomatology [51]. About ten years earlier, the same group had reported on a placebo-controlled Phase II clinical trial in which the virus was applied to patients (n = 26) with various advanced chemorefractory cancers. Interestingly, the virus had been applied at high doses via inhalation as a means to target lung metastases [52]. The high virus doses had been well

tolerated. The 2-year survival rates had been 21% in the virus treatment group versus 0% in the placebo group. Although the study had not been randomized, the authors suggested a decrease in cancer-related symptoms and better survival [52].

Another case-series study in GBM was performed in Israel by Freeman, Zakay-Rones and colleagues with the lentogenic strain NDV-HUJ. The virus was administered i.v. using intra-patient dose-escalation followed by three cycles of 55 billion infectious units. Toxicity was minimal and a maximal tolerated dose was not reached. One patient experienced a complete response, although of only transient duration (about three months), while the others developed progressive disease [53].

The oncolytic NDV strain *PV701* was applied in the United States by Wellstat Biologics in patients with advanced cancers that were unresponsive to standard therapy. This strain had been extensively tested in vitro [54] and in human tumor xenotransplanted mice [55]. Seventy-nine late-stage cancer patients were given escalating doses of virus i.v. Doses of 12×10^9 to 12×10^{10} infectious particles (plaque forming units/m^2) were well tolerated [56]. 3 Phase I trials were undertaken, involving 113 patients, to evaluate effects, such as virus dose, schedule and i.v. infusion rate. Adverse events were flu-like, tumor-site-specific or those occurring during infusion. When patients were desensitized with a lower initial dose, the maximal tolerated dose (MTD) could be increased 10-fold. In 95 evaluable patients, there were 10 responses (six major and four minor) with progression-free survival ranging from 4 to 31 months [57].

Another interesting activity of NDV relates to the clinically important process of liver fibrosis. Activated hepatic stellate cells (HSCs) represent a crucial factor in the development of liver fibrosis and are involved in the development of hepatocellular carcinoma (HCC). NDV was reported to be able to repress the activation of human HSCs and to be capable, upon systemic application, to revert the development of hepatic fibrosis in mice [58].

3.4. 2010s: Combining Oncolytic Virus Modified Vaccines (ATV-NDV) with Costimulatory Bispecific Antibodies

Most of the clinical trials performed with the vaccine ATV-NDV showed promising results with improvements in OS by about 30% of the treated patients. The remaining about 70% have to be considered as immunological non-responders. Since T cell anergy (non-responsiveness of TA-specific T cells) is a major problem in cancer patients and since this is often due to insufficient costimulation, a strategy was developed, as early as 1999, to augment T cell costimulatory signals in the vaccine ATV-NDV [59].

The strategy consisted of adding NDV-specific single chain antibodies with dual specificity (bispecific scFv antibodies, bsAb) to the vaccine ATV-NDV. In comparison to other bispecific antibodies, such as bispecific T cell engagers (BITES), which target TAs, the above bsAbs target viral antigens (VAs). They can thus be applied to any type of tumor cell modified by NDV. ATV-NDV tumor vaccine with attached anti-HN-anti-CD3 (αHN-αCD3) and anti-HN-anti-CD28 (αHN-αCD28) bsAb exerted in vitro, upon stimulation of allogeneic human peripheral blood mononuclear cells (PBMC), strong and durable antitumor effects against human tumor cell monolayers [60]. This anti-tumor activity was independent from recognition of TAs.

The activity of the bsAbs could be further augmented by introducing the cytokine IL-2 which binds to its high affinity receptor CD25 on T cells. For this purpose, a trispecific immunocytokine reagent (αHN-IL-2-αCD28) was constructed, produced and tested in vitro as above [61]. A transcriptome analysis and cytokine profiling of naive T cells stimulated by ATV-NDV tumor vaccine via attached αHN-αCD3 (for signal 1) and αHN-IL2 (for signal 2) revealed unsuspected costimulatory activity of the cytokine IL-2 [62]. The above trispecific immunocytokine, attached to ATV-NDV, provided the strongest T cell costimulatory activity in combination with a suboptimal amount of attached αHN-αCD3. This effect was most likely due to the concomitant transmission of signal 1 via CD3 and costimulatory signals 2a and 2b via the two T cell co-receptors CD28 and CD25 [60].

A Phase I clinical study evaluated the recombinant bispecific protein αHN-αCD28. In this autologous situation, the ATV-NDV vaccine provided TAs for signal 1 and αHN-αCD28 for signal 2. This dose-escalation study, in which the vaccine was modified by increasing amounts of the bsAb, involved 14 colorectal carcinoma patients with late-stage disease (stage IV with liver metastases). There were no severe adverse events. Before the vaccination, none of the patients had detectable levels of cancer-reactive blood circulatory T cells (ELISPOT test). Interestingly, after vaccination, all patients became positive in the ELISPOT assay, at least once during the course of 5 vaccinations. Furthermore, there was a dose-response relationship with the bsAb. A partial response of metastases was documented in 4 patients. The study suggests that the bsAb modified vaccine ATV-NDV-bsαHN-αCD28 is safe and can re-activate possibly anergic T cells from advanced-stage cancer [63]. A further potentiation of the costimulatory effect can be expected from a vaccine of the type ATV-NDV-αHN-*IL-2*-αCD28.

Bispecific antibodies and also trispecific immunocytokines have been proposed to have great potential in future for targeting the immune system against cancer [64]. While NDV infection of tumor cells could break T cell tolerance in vitro with human cells [65], it was not sufficient to have a similar effect in vivo in late-stage cancer patients. To overcome anergic T cells in this situation and to reactivate them required stronger costimulation as exemplified with the bispecific anti-CD28 antibody attached to ATV-NDV [63]. Not only the costimulatory signal can be augmented by bsAb but also signal 1. If the TA mediated signal 1 is very weak, it could be intensified by a suboptimal amount of bsαHN-αCD3. The ATV-NDV-bsαHN-αCD28 vaccine would be further modified by attachment of bsαHN-αCD3.

The concept of increasing T cell costimulatory signals is complementary to the concept of decreasing T cell inhibitory signals via checkpoint inhibitory antibodies. It is likely that the ratio of positive to negative signals will be decisive for the T cell response.

Table 1 contains a summary of the mentioned studies of clinical application of NDV.

Table 1. Clinical application of NDV (Part I).

1. Post-operative application of NDV oncolysate vaccines in Stage II melanoma (*n* = 83) by WA Cassel and DR Murray (1977) (oncolytic strain 73 T)
2. Systemic treatment of advanced chemorefractory cancers; Phase II placebo-controlled trial (*n* = 33 versus 26 controls) by LK Csatary and S Eckhardt (1993) (oncolytic strain MTH/68)

3. Systemic treatment of glioblastoma multiforme (GBM) (*n* = 14); Intra-patient dose escalation followed by 3 cycles of 55 billion infectious units by AI Freeman and Z Zakay-Rones (2006) (lentogenic strain HUJ)

4. Phase I dose-escalation trials of intravenous virus administration in patients with advanced solid cancers resistant to standard therapy (*n* = 113) by AL Pecora and RM Lorence (2002, 2003) (oncolytic strain PV71)

5. Post-operative treatment with the irradiated live tumor cell vaccine Autologous Live NDV-Modified Tumor Cell Vaccine (ATV-NDV):

(i) Early breast cancer (*n* = 63), metastatic breast cancer (*n* = 27) and metastatic ovarian cancer (*n* = 31) by T Ahlert and V Schirrmacher (1997) (lentogenic strain Ulster)

(ii) Phase II trial in patients with locally advanced colorectal carcinoma (CRC) (*n* = 57) by D Ockert and V Schirrmacher (1996) (lentogenic strain Ulster)

(iii) Phase II trial in patients with GBM (*n* = 23 versus 87 non-vaccinated patients from the same clinic in the same time interval) by HH Steiner and C Herold-Mende (2004) (lentogenic strain Ulster)

(iv) Phase I/II trial patients with Stage III and IV head and neck squamous cell carcinomas (HNSCC) (*n* = 20) by J Karcher and G Dyckhoff (2004) (lentogenic strain Ulster)

6. Prospectively randomized Phase II/III trial to investigate the efficiency of ATV-NDV vaccination after liver resection for hepatic metastases of CRC as a tertiary prevention method (*n* = 51) by T Schulze and PM Schlag (2009) (lentogenic strain Ulster)

References

1. Fournier, P.; Schirrmacher, V. Harnessing oncolytic virus-mediated antitumor immunity. Front. Oncol. 2014, 4, 1–109.
2. Heidbuechel, J.P.W.; Engeland, C.E. Paramyxoviruses for tumor-targeted immuno-modulation: Design and evalution ex vivo. J. Vis. Exp. 2019, 143, e58651.
3. Pol, J.G.; Lévesque, S.; Workenhe, S.T.; Gujar, S.; Le Boeuf, F.; Clements, D.; Fahmer, J.E.; Fend, L.; Bell, C.J.; Mossman, K.L.; et al. Trial Watch: Oncolytic viro-immunotherapy of hematologic and solid tumors. Oncoimmunology 2018, 7, e1503032.
4. Schirrmacher, V. Immunobiology of Newcastle Disease Virus and its use for prophylactic vaccination in poultry and as adjuvant for therapeutic vaccination in cancer patients. Int. J. Mol. Sci. 2017, 18, 1103.
5. Schirrmacher, V. Fifty years of clinical application of Newcastle disease virus: Time to celebrate! Biomedicines 2016, 4, 16.
6. Cassel, W.A.; Garrett, R.E. Newcastle disease virus as an antineoplastic agent. Cancer 1965, 18, 863–868.

7. Jarvis, E.D.; Mirarab, S.; Aberer, A.J.; Li, B.; Houde, P.; Li, C.; Ho, S.Y.; Faircloth, B.C.; Nabholz, B.; Howard, J.T.; et al. Whole-genome analyses resolve early branches in the tree of life of modern birds. Science 2014, 346, 1320–1331.
8. Dimitrov, K.M.; Abolnik, C.; Afonso, C.L.; Albina, E.; Bahl, J.; Berg, M.; Briand, F.X.; Brown, I.H.; Choi, K.S.; Chvala, I.; et al. Updated unified phylogenetic classification system and revised nomenclature for Newcastle disease virus. Infect. Genet. Evol. 2019.
9. Miller, P.J.; Koch, G. Newcastle disease. In Diseases of Poultry, 13th ed.; Swayne, D.E., Ed.; Wiley-Blackwell: Hoboken NJ, USA, 2013; pp. 89–138.
10. Fournier, P.; Zeng, J.; Von der Lieth, C.W.; Washburn, B.; Ahlert, T.; Schirrmacher, V. Importance of serine 200 for functional activities of the hemagglutinin-neuraminidase protein of Newcasle disease virus. Int. J. Oncol. 2004, 24, 623–634.
11. Li, Q.; Wei, D.; Feng, F.; Wang, X.L.; Li, C.; Chen, Z.N.; Bian, H. α2,6-linked sialic acid serves as a high-affinity receptor for cancer Oncolytic virotherapy with Newcastle disease virus. J. Cancer Res. Clin. Oncol. 2017, 143, 2171–2181.
12. Tan, L.; Zhang, Y.; Zhan, Y.; Yuan, Y.; Sun, Y.; Qiu, X.; Meng, C.; Song, C.; Liao, Y.; Ding, C. Newcastle disease virus employs macropinocytosis and Rab5a-dependent intracellular trafficking to infect DF-1 cells. Oncotarget 2016, 7, 86117.
13. Samal, K.S. Newcastle disease and related avian paramyxoviruses. In The Biology of Paramyxoviruses, 1st ed.; Samal, S.K., Ed.; Caister Academic Press: Norfolk, UK, 2011; pp. 69–114.
14. Fiola, C.; Peeters, B.; Fournier, P.; Arnold, A.; Bucur, M.; Schirrmacher, V. Tumor selective replication of Newcastle disease virus: Association with defects of tumor cells in antiviral defence. Int. J. Cancer 2006, 119, 328–338.
15. Rangaswamy, U.S.; Wang, W.; Cheng, X.; Mc Tamney, P.; Carroll, D.; Jin, H. Newcastle disease virus establishes persistent infection in tumor cells in vitro: Contribution of the cleavage site of fusion protein and second sialic acid binding site of hemagglutinin-neuraminidase. J. Virol. 2017, 91, e00770-17.
16. Lech, P.J.; Russel, S.J. Use of attenuated paramyxoviruses for cancer therapy. Expert. Rev. Vaccines 2010, 9, 1275–1302.
17. Feng, H.; Wei, D.; Nan, G.; Cui, S.J.; Chen, Z.N.; Bian, H. Construction of a minigenome rescue system for Newcastle disease virus strain Italien. Arch. Virol. 2011, 156, 611–616.
18. Ayllon, J.; Garcia-Sastre, A.; Martinez-Sobrido, L. Rescue of recombinant Newcastle disease virus from cDNA. J. Vis. Exp. 2013, 80, e50830.

19. Cardenas-Garcia, S.; Afonso, C.L. Reverse genetics of Newcastle disease virus. Methods Mol. Biol. 2017, 1602, 141–158.
20. Hervas-Stubbs, S.; Perez-Gracia, J.L.; Rouzaut, A.; Sanmamed, M.F.; Le Bon, A.; Melero, I. Direct effects of type I interferons on cells of the immune system. Clin. Cancer Res. 2011, 17, 2619–2627.
21. Xiang, B.; Zhu, W.; Li, Y.; Gao, P.; Liang, J.; Liu, D.; Ding, C.; Liao, M.; Kang, Y.; Ren, T. Immune responses of mature chicken bone-marrow-derived dendritic cells infected with Newcastle disease virus strains with differing pathogenicity. Arch. Virol. 2018, 163, 1407–1417.
22. Wang, X.; Dang, R.; Yang, Z. The interferon antagonistic activities of the V proteins of NDV correlated with their virulence. Virus Genes 2019, 55, 233–237.
23. Alamares, J.G.; Elankumaran, S.; Samal, S.K.; Iorio, R.M. The interferon antagonistic activities of the V protein from two strains of Newcastle disease virus correlate with their known virulence properties. Virus Res. 2010, 147, 153–157.
24. Hornung, V.; Ellegast, J.; Kim, S.; Brzózka, K.; Jung, A.; Kato, H.; Poeck, H.; Akira, S.; Conzelmann, K.K.; Schlee, M.; et al. 5′-Triphosphate RNA is the ligand for RIG-I. Science 2005, 314, 994–997.
25. Fournier, P.; Wilden, H.; Schirrmacher, V. Importance of retinoic acid-inducible gene I and of receptor for type I interferon for cellular resistance to infection by Newcastle disease virus. Int. J. Oncol. 2012, 40, 287–298.
26. Sun, Y.; Mao, X.; Zheng, H.; Wu, W.; Rehman, Z.U.; Liao, Y.; Meng, C.; Qiu, X.; Tan, L.; Song, C.; et al. Goose MAVS functions in RIG-I-mediated IFN-ß signaling activation. Dev. Comp. Immunol. 2019, 93, 58–65.
27. Li, X.; Hanson, R.P. In vivo interference by Newcastle disease virus in chickens, the natural host of the virus. Arch. Virol. 1989, 108, 229–245.
28. Ng, C.S.; Kato, H.; Fujita, T. Fueling type I interferonopathies: Regulation and function of Type I interferon antiviral responses. J. Interferon Cytokine Res. 2019, 39.
29. Kumagai, Y.; Kumar, H.; Koyama, S.; Kawai, T.; Takeuchi, O.; Akira, S. Cutting edge: TLR-dependent viral recognition along with type I IFN positive feedback signaling masks the requirement of viral replication for IFN-(alpha) production in plasmacytoid dendritic cells. J. Immunol. 2009, 182, 3960–3964.
30. Tough, D.F. Type I interferon as a link between innate and adaptive immunity through dendritic cell stimulation. Leuk. Lymphoma 2004, 45, 257–264.
31. Ivashkiv, L.B.; Donlin, L.T. Regulation of type I interferon responses. Nat. Rev. Immunol. 2014, 14, 36–49.

32. Lee, J.-H.; Chiang, C.; Gack, M.U. Endogenous nucleic acid recognition by RIG-I-like receptors and cGAS. J. Interferon Cytokine Res. 2019, 39, 450–458.
33. Waldmann, T.A.; Chen, J. Disorders of the JAK/STAT pathway in T cell lymphoma pathogenesis: Implications for immunotherapy. Annu. Rev. Immunol. 2017, 35, 533–550.
34. Zaslavsky, E.; Hershberg, U.; Seto, J.; Pham, A.M.; Marques, S.; Duke, J.L.; Wetmur, J.G.; Tenoever, B.R.; Sealfon, S.C.; Kleinstein, S.H. Antiviral response dictated by choreographed cascade of transcription factors. J. Immunol. 2010, 184, 2908–2917.
35. Fournier, P.; Arnold, A.; Schirrmacher, V. Polarization of human monocyte-derived dendritic cells to DC1 by in vitro stimulation with Newcastle disease virus. J. BUON 2009, 14, S111–S122.
36. Schirrmacher, V. Signaling through RIG-I and type I interferon receptor: Immune activation by Newcastle disease virus in man versus immune evasion by Ebola virus (Review). Int. J. Mol. Med. 2015, 36, 3–10.
37. Yoshida, A.; Kim, S.H.; Manoharan, V.K.; Varghese, B.P.; Paldurai, A.; Samal, S.K. Novel avian paramyxovirus-based vaccine vectors expressing Ebola virus glycoprotein elicit mucosal and humoral immune responses in guinea pigs. Sci. Rep. 2019, 9, 5520.
38. Cassel, W.A.; Garrett, R.E. Tumor immunity after viral oncolysis. J. Bacteriol. 1966, 92, 792.
39. Cassel, W.A.; Murray, D.R. A ten-year follow-up on stage II malignant melanoma patients treated postsurgically with Newcastle disease virus oncolysate. Med. Oncol. Tumor Pharmacother. 1992, 9, 169–171.
40. Kirchner, H.H.; Anton, P.; Atzpodien, J. Adjuvant treatment of locally advanced renal cancer with autologous virus-modified tumor vaccines. World J. Urol. 1995, 13, 171–173.
41. Heicappell, R.; Schirrmacher, V.; von Hoegen, P.; Ahlert, T.; Appelhans, B. Prevention of metastatic spread by postoperative immunotherapy with virally modified autologous tumor cells. I. Parameters for optimal therapeutic effects. Int. J. Cancer 1986, 37, 569–577.
42. Schirrmacher, V.; Heicappell, R. Prevention of metastatic spread by postoperative immunotherapy with virally modified autologous tumor cells. II. Establishment of specific systemic immunity. Clin. Exp. Metastasis 1987, 5, 147–156.
43. Schirrmacher, V.; Haas, C.; Bonifer, R.; Ahlert, T.; Gerhards, R.; Ertel, C. Human tumor cell modification by virus infection: An efficient and safe way to produce cancer vaccine with pleiotropic immune stimulatory properties when using Newcastle disease virus. Gene Ther. 1999, 6, 63–73.
44. Ockert, D.; Schirrmacher, V.; Beck, N.; Stoelben, E.; Ahlert, T.; Flechtenmacher, J.; Hagmüller, E.; Buchcik, R.; Nagel, M.; Saeger, H.D. Newcastle disease virus-infected intact autologous tumor

cell vaccine for adjuvant active specific immunotherapy of resected colorectal carcinoma. Clin. Cancer Res. 1996, 2, 21–28.

45. Ahlert, T.; Sauerbrei, W.; Bastert, G.; Ruhland, S.; Bartik, B.; Simiantonaki, N.; Schumacher, J.; Häcker, B.; Schumacher, M.; Schirrmacher, V. Tumor-cell number and viability as quality and efficacy parameters of autologous virus-modified cancer vaccines in patients with breast and ovarian cancer. J. Clin. Oncol. 1997, 15, 2763.
46. Steiner, H.H.; Bonsanto, M.M.; Beckhove, P.; Brysch, M.; Geletneky, K.; Ahmadi, R.; Schuele-Freyer, R.; Kremer, P.; Ranaie, G.; Matejic, D. Antitumor vaccination of patients with glioblastoma multiforme: A pilot study to assess feasibility, safety, and clinical benefit. J. Clin. Oncol. 2004, 22, 4272–4281.
47. Karcher, J.; Dyckhoff, G.; Beckhove, P.; Reisser, C.; Brysch, M.; Ziouta, Y.; Helmke, B.H.; Weidauer, H.; Schirrmacher, V.; Herold-Mende, C. Antitumor vaccination in patients with head and neck squamous cell carcinomas with autologous virus-modified tumor cells. Cancer Res. 2004, 64, 8057–8061.
48. Schulze, T.; Kemmner, W.; Weitz, J.; Wernecke, K.D.; Schirrmacher, V.; Schlag, P.M. Efficiency of adjuvant active specific immunization with Newcastle disease virus modified tumor cells in colorectal carcinoma patients following resection of liver metastases. Results of a prospective randomized trial. Cancer Immunol. Immunother. 2009, 58, 61–69.
49. Schirrmacher, V.; Fournier, P.; Schlag, P. Autologous tumor cell vaccines for post-operative active-specific immunotherapy of colorectal carcinoma: Long-term patient survival and mechanism of function. Expert Rev. Vaccines 2014, 13, 117–130.
50. Liang, W.; Wang, H.; Sun, T.M.; Yao, W.Q.; Chen, L.L.; Jin, Y.; Li, C.L.; Meng, F.J. Application of autologous tumor cell vaccine and NDV vaccine in treatment of tumors of the digestive tract. World J. Gastroenterol. 2003, 9, 495–498.
51. Csatary, L.K.; Gosztonyi, G.; Szeberenyi, J.; Fabian, Z.; Liszka, V.; Bodey, B.; Csatary, C.M. MTH-68/H oncolytic viral treatment in human high-grade gliomas. J. Neurooncol. 2004, 67, 83–93.
52. Csatary, L.K.; Eckhardt, S.; Bukosza, I.; Czegledi, F.; Fenyvesi, C.; Gergely, P.; Bodey, B.; Csatary, C.M. Attenuated veterinary virus vaccine for the treatment of cancer. Cancer Detect. Prev. 1993, 17, 619–627.
53. Freeman, A.I.; Zakay-Rones, Z.; Gomori, J.M.; Linetsky, E.; Rasooly, L.; Greenbaum, E.; Rozenman-Yair, S.; Panet, A.; Libson, E.; Irving, C.S.; et al. Phase I/II trial of intravenous DNV-HUJ oncolytic virus in recurrent glioblastoma multiforme. Mol. Ther. 2006, 13, 221–228.
54. Reichard, K.W.; Lorence, R.M.; Cascino, C.L.; Peeples, M.E.; Walter, R.J.; Fernando, M.B.; Reyes, H.M.; Greager, J.A. Newcastle disease virus selectively kills human tumor cells. J. Surg. Res. 1992, 52, 448–453.

55. Phuangsab, A.; Lorence, R.M.; Reichard, K.W.; Peeples, M.E.; Walter, R.J. Newcastle disease virus therapy of human tumor xenografts: Antitumor effects of local or systemic administration. Cancer Lett. 2001, 172, 27–36.
56. Pecora, A.L.; Rizvi, N.; Cohen, G.I.; Meropol, N.J.; Sterman, D.; Marshall, I.L.; Goldberg, S.; Gross, P.; O'Neil, J.D.; Groene, W.S. Phase I trial of intravenous administration of PV701, an oncolytic virus, in patients with advanced cancer. J. Clin. Oncol. 2002, 20, 2251–2266.
57. Lorence, R.M.; Pecora, A.L.; Major, P.P.; Hotte, S.J.; Laurie, S.A.; Roberts, M.S.; Groene, W.S.; Bamat, M.K. Overview of phase I studies of intravenous administration of PV701, an oncolytic virus. Curr. Opin. Mol. Ther. 2003, 5, 618–624.
58. Li, Y.L.; Wu, J.; Wie, D.; Zhang, D.W.; Feng, H.; Chen, Z.N.; Bian, H. Newcastle disease virus represses the activation of human hepatic stellate cells and reverses the development of hepatic fibrosis in mice. Liver Int. 2009, 29, 593–602.
59. Haas, C.; Herold-Mende, C.; Gerhards, R.; Schirrmacher, V. An effective strategy of human tumor vaccine modification by coupling bispecific costimulatory molecules. Cancer Gene Ther. 1999, 6, 254–262.
60. Haas, C.; Lulei, M.; Fournier, P.; Arnold, A.; Schirrmacher, V. A tumor vaccine containing anti-CD3 and anti-CD28 bispecific antibodies triggers strong and durable antitumor activity in human lymphocytes. Int. J. Cancer 2006, 118, 658–667.
61. Aigner, M.; Janke, M.; Lulei, M.; Beckhove, P.; Fournier, P.; Schirrmacher, V. An effective tumor vaccine optimized for costimulation via bispecific and trispecific fusion proteins. Int. J. Oncol. 2008, 32, 777–789.
62. Fournier, P.; Aigner, M.; Schirrmacher, V. Transcriptome analysis and cytokine profiling of naive T cells stimulated by a tumor vaccine via CD3 and CD25. Int. J. Oncol. 2010, 37, 1439–1452.
63. Schirrmacher, V.; Schlude, C.; Weitz, J.; Beckhove, P. Strong T-cell costimulation can reactivate tumor antigen-specific T cells in late-stage metastasized colorectal carcinoma patients: Results from a phase I clinical study. Int. J. Oncol. 2015, 46, 71–77.
64. Fournier, P.; Schirrmacher, V. Bispecific antibodies and trispecific immunocytokines for targeting the immune system against cancer: Preparing for the future. Biodrugs 2013, 27, 35–53.
65. Zeng, J.; Fournier, P.; Schirrmacher, V. Induction of interferon-alpha and tumor necrosis factor-related apoptosis-inducing ligand in human blood mononuclear cells by hemagglutinin-neuraminidase but not F protein of Newcastle disease virus. Virology 2002, 297, 19–30.