Expert Opinion on Biological Therapy

ISSN: 1471-2598 (Print) 1744-7682 (Online) Journal homepage: http://www.tandfonline.com/loi/iebt20

Oncolytic Newcastle disease virus as a prospective anti-cancer therapy. A biologic agent with potential to break therapy resistance

Volker Schirrmacher

To cite this article: Volker Schirrmacher (2015): Oncolytic Newcastle disease virus as a prospective anti-cancer therapy. A biologic agent with potential to break therapy resistance, Expert Opinion on Biological Therapy, DOI: 10.1517/14712598.2015.1088000

To link to this article: http://dx.doi.org/10.1517/14712598.2015.1088000

EXPERT OPINION

Oncolytic Newcastle disease virus as a prospective anti-cancer therapy. A biologic agent with potential to break therapy resistance

Volker Schirrmacher
Immunological and Oncological Center (IOZK), Tumor Immunology, Cologne, Germany

Introduction: Oncolytic viruses (OVs) selectively replicate in tumor cells and cause cancer cell death. Most OVs in clinical studies are genetically engineered. In contrast, the avian Newcastle disease virus (NDV) is a naturally oncolytic RNA virus. While anti-viral immunity is considered a major problem in achieving maximal tumor cell killing by OVs, this review discusses the importance of NDV immunogenic cell death (ICD) and how anti-viral immune responses can be integrated to induce maximal post-oncolytic T-cell-mediated anti-tumor immunity. Since replication of NDV is independent of host cell DNA replication (which is the target of many cytostatic drugs and radiotherapy) and because of other findings, oncolytic NDV is a candidate agent to break therapy resistance of tumor cells.

Areas covered: Properties of this avian paramyxovirus are summarized with special emphasis to its anti-neoplastic and immune-stimulatory properties. The review then discusses prospective anti-cancer therapies, including treatments with NDV alone, and combinations with an autologous NDV-modified tumor cell vaccine or with a viral oncolysate pulsed dendritic cell vaccine. Various combinatorial approaches between these and with other modalities are also reviewed.

Expert opinion: Post-oncolytic anti-tumor immunity based on ICD is in the expert's opinion of greater importance for long-term therapeutic effects than maximal tumor cell killing. Of the various combinatorial approaches discussed, the most promising and feasible for clinical practice appears to be the combination of systemic NDV pre-treatment with anti-tumor vaccination.

Keywords: anti-tumor immunity, bispecific antibodies, dendritic cells, memory T cells, oncolysis

Expert Opin. Biol. Ther. [Early Online]

1. Introduction

Oncolytic viruses (OVs) selectively replicate in tumor cells and kill them. Such oncolysis is mostly an immunogenic type of cancer cell death (ICD) [1]. This includes immunogenic apoptosis, necrosis and autophagic cell death. This is important because post-oncolytic anti-tumor activity induced by OVs is considered a key factor for an efficient therapeutic activity [2,3]. Until now, more than 20 viruses have been characterized with oncolytic activity [4]. Many have been genetically engineered to obtain their tumor selectivity, for example, the human DNA viruses Adenovirus and Herpes simplex virus. Other viruses have been armed with additional transgenes, for example, the RNA viruses measles virus (MV) and vesicular stomatitis virus (VSV). Some viruses have the advantage to be naturally oncolytic, for example,

Taylor & Francis
Taylor & Francis Group

Article highlights.

- The bird virus Newcastle disease virus (NDV) is described with its immunostimulatory properties in man and its selective replication in human tumor cells.
- Following virus replication, tumor cells are killed, a process called oncolysis which is characterized by immunogenic cell death (ICD).
- Vaccines containing virus-infected tumor cells (e.g., ATV-NDV) and viral oncolysate (e.g., VOL and VOL-DC) are described to be highly immunogenic and to provide to the immune system information about the tumor (tumor antigens) and about immunological danger (foreign viral RNA).
- Two tables summarize clinical studies with NDV-modified tumor vaccines.
- Vaccination leads immediately to innate immunity activation and later to induction of adaptive tumor-specific immune responses with establishment of specific T-cell-mediated immunological memory.
- Post-oncolytic anti-tumor immunity based on ICD and on reactivation of a broad reservoir of cancer-reactive memory T cells is in the expert's opinion of greater importance for long-term therapeutic effects than maximal tumor cell killing.
- The article proposes several combinatorial approaches of NDV, of which one, the combination of systemic NDV application with anti-tumor active specific vaccination, has already entered one clinical practice in Germany.
- For the future, it is suggested among others to use oncolytic NDV to break tumor resistances to therapy, including resistance to immune checkpoint inhibitors.

This box summarizes key points contained in the article.

the avian viruses Newcastle disease virus (NDV) and Sindbis virus, minute virus of mice, parvovirus of rats and the mammalian reovirus. It is of interest that three clinically promising oncolytic RNA viruses (NDV, VSV, MV) belong to the same family of viruses [5]. These paramyxoviruses have a similar genome, a single strand RNA of negative orientation (-ssRNA). This review focuses on the bird virus NDV, of which various strains (MTH68/H [6], Ulster [7], NDV-HUJ [8]) have been and are being used for application in cancer patients.

The first report about the antineoplastic activity of NDV appeared about 50 years ago [9]. The many observations made since then, either *in vitro* or *in vivo*, in animal tumor models as well as in cancer patients, demonstrate the special anti-neoplastic and immunostimulatory properties of this avian paramyxovirus. Its high safety profile in cancer patients suggests to use NDV as a new biological agent against cancer [10,11].

2. Properties of NDV

2.1 Taxonomy and structure

NDV is an enveloped virus of 100 – 300 nm diameter with a negative-sense single stranded (ss)RNA genome of roughly 16,000 nucleotides. It is classified as an avian paramyxovirus-1 (APMV-1) in the Avulavirus genus of the family Paramyxoviridae [12]. The RNA contains six genes coding for the viral proteins NP, P, M, F, HN and L.

2.2 Virus infection and replication

Infection of permissive avian cells can be divided into two sequential steps: i) binding, fusion, transduction of the viral genome and transcription of viral genes, ii) viral replication using (+) strand full-length template for viral genome amplification, viral encapsulation and budding and finally host cell lysis.

Step (i) of infection takes place in a broad range of cell types from permissive and non-permissive hosts. Target cell binding occurs through viral HN protein and sialic acid-containing host cell receptors. This triggers F protein conformational changes and releases fusion peptides to fuse the viral and cellular membranes. NDV penetrates target cells mostly by endocytosis. In non-permissive hosts, step (ii) occurs only in tumor cells since it is stopped rapidly in normal cells through a defense mechanism involving IFN-α and -β [13]. In comparison to normal cells, tumor cells often have a weaker type I IFN response and a weaker sensitivity to type I IFN-receptor (IFNR)-mediated signaling [14].

2.3 The cellular anti-viral response

The anti-viral response of normal cells is initiated by the recognition of foreign viral RNA containing 5′ phosphate [15]. Mammalian mRNA is either capped or contains base modifications. Single-stranded (ss) and double-stranded (ds) viral RNA is recognized by two types of pathogen recognition receptors (PRR): i) Toll-like receptors (TLRs), especially TLR3, 7, 8, 9 and ii) RIG-like receptors (RLRs). Among the RLRs, RIG-I was demonstrated to be a cytoplasmic receptor for NDV RNA in mammalian cells [16].

2.4 Pathology in birds, safety in man

NDV strains are separated into lentogenic, mesogenic or velogenic strains depending on their virulence in birds. Like other viruses, NDV has developed immune escape mechanisms in its natural host, the bird. It uses for this purpose the viral protein V, which interferes with signal transducer and activator of transcription-mediated type I IFN signals. This immune evasion mechanism is species-restricted and functions only in birds [17]. Mesogenic and velogenic strains can induce fatal respiratory disease in birds (e.g., chicken pest) [18].

The avian virus NDV is not a pathogen in man. The majority of the human population is seronegative for NDV. This avoids the problem of pre-existing immunity seen with many human candidate OVs [19]. When applied to humans, NDV induces only mild fever for a day or conjunctivitis. This virus shows a high tolerability in man. In rodent and mammalian normal (non-malignant) cells, the NDV-induced type I IFN response is very strong and capable of preventing

viral replication. In a non-permissive host such as man, NDV shows tumor selectivity with respect to virus replication and oncolysis. This tumor selectivity is due to tumor cell defects in anti-viral and apoptotic pathways [20]. The ubiquitous nature of the NDV receptor allows use of NDV against a large variety of cancers. The high safety profile in man [10,11,21] and the tumor selectivity [20] may obviate the need for specific tumor targeting.

There are other advantageous characteristics of NDV which are related to its molecular biology: The modular nature of gene transcription, the undetectable rate of recombination, the lack of a DNA phase in the replication cycle [22], the robust virus production and a manufacturing system based on eggs or cell culture.

2.5 Oncolysis, tumor selectivity and ICD

NDV has been demonstrated to mediate its oncolytic effect by both intrinsic and extrinsic caspase-dependent pathways of cell death [23]. NDV-induced apoptosis is dependent on up-regulation of TNF-related apoptosis-inducing ligand (TRAIL) and caspase activation [24]. This causes opening of mitochondrial permeability transition pores and loss of mitochondrial membrane potential, leading to activation of the apoptosis process [25]. MAPK and endoplasmic reticulum (ER) stress pathways also play important roles in NDV-mediated oncolysis [20,23]. Interestingly, NDV can exert oncolytic activity also against hypoxic cancer cells, which is of clinical relevance and corroborates its potency as therapeutic agent [26].

Identified mechanisms of selectivity of NDV for tumor cells in non-permissive hosts have been summarized [20] and include the following:

1) Defects in activation of anti-viral signaling pathways [27-30];
2) Defects in type I IFN signaling pathways [27,31,32];
3) Defects in apoptotic pathways [33,34];
4) Activation of Ras signaling and expression of Rac1 protein [35].

NDV interacts with Rac-1 upon viral entry, syncytium induction and actin reorganization of the infected cell as part of the replication process. These findings support the proposition to use NDV as a novel biological agent to specifically target aberrant signaling (proliferation and invasion pathways) in glioblastoma multiforme (GBM) [36]. It is in this context that the question of immune suppression during OV therapy of GBM is being discussed [37].

An important new paradigm of oncolytic virus-mediated immunotherapy is the concept of ICD [1-3]. Classical physiological apoptosis is non-immunogenic. It is characterized by membrane integrity, cell shrinkage, membrane blebbing, release of small apoptotic bodies, nuclear condensation and DNA fragmentation. Immunogenic modes of cell death include immunogenic apoptosis, necrosis and pyroptosis. Immunogenic apoptosis differs from classical apoptosis by translocation to the plasma membrane of calreticulin and heat shock proteins (HSPs) prior to apoptosis. Such changes can be induced by some chemotherapeutic agents and by OVs. Damage-associated molecular patterns (DAMPs) are released in the late phase. Necrosis is characterized by organelle swelling, reactive oxygen species, nuclear swelling, membrane swelling, membrane rupture and release of intracellular contents, including DAMPs such as ATP, high mobility group box 1, uric acid, etc. Pyroptosis is characterized by nuclear condensation, DNA fragmentation, membrane swelling, release of membrane vesicles, membrane rupture and release of intracellular contents including DAMPs. Basically, cancer cells dying by ICD involve elements of the DNA damage response, elements of the ER stress response [38] as well as elements of the apoptotic response [39].

OVs induce multimodality ICD and release or present pathogen-associated molecular patterns (PAMPs) as danger signaling molecules. The PAMPs of NDV is described in Section 2.6.

OVs can also induce autophagy in cancer cells. Autophagy mediates sequestration, degradation and recycling of cellular organelles and proteins, and intracellular pathogens. Autophagy can also enhance tumor immunogenicity via the release of DAMPs. NDV was shown to trigger autophagy in glioma cells to enhance virus replication [40]. Mitophagy was reported to promote oncolytic NDV replication by blocking intrinsic apoptosis in lung cancer cells [41]. OV-induced autophagy in cancer cells also promotes cross-presentation of tumor-associated antigens (TAAs), thereby facilitating anti-tumor immune responses [42].

Table 1 summarizes the various features of ICD induced by NDV in tumor cells. These include an ER stress response, immunogenic apoptosis, necrosis and autophagy. Such ICD features lead to shutdown of protein synthesis, surface exposure of calreticulin and HSPs, induction of danger signals, release of pro-inflammatory cytokines and improved antigen presentation.

Tumor selectivity, oncolysis and ICD are not separate but interconnected phenomena that occur locally in time and space. Virus infection is associated with increased production of viral proteins from within the cell and at the cell surface. This has additional effects on cellular processes and pathways.

2.6 Immunostimulatory properties

NDV, as a strong inducer of type I IFN, is expected to have a strong effect on the immune system. Both, the innate and the adaptive immunity system become activated and cross-talks between them are initiated counteracting regulatory T-cell (Treg) activity [43].

2.6.1 *Innate immunity activation*

PRRs recognize PAMPs. In case of NDV, these are viral 5′ phosphate ssRNA and dsRNA recognized by RIG-I [32] and TLR3. The viral surface protein HN also carries immunostimulatory properties. It is recognized by Nkp46 of NK cells

Table 1. Immunogenic tumor cell death features induced by NDV.

In vitro ICD	Mechanism
ER stress response [38]:	
Activation of PERK	
Phosphorylation of eIF2a	Shutdown of protein synthesis
Immunogenic apoptosis [1-3,23,39,41,55]:	
Ecto-CRT	Surface exposure of calreticulin
HSPs	Surface exposure of heat shock proteins
Viral RNA	Danger signals via PAMPs
Necrosis/necroptosis [46,55]:	
Release of DAMPs (e.g., high mobility group box 1)	Danger signals to DCs
Release of pro-inflammatory cytokines	Inflammation
Autophagy [43,45]:	Clearance of viral pathogen, improved processing of viral antigens
Mitophagy [44]:	Weakening RIG-I receptor signals
Immune response:	
Up-regulation of MHC I [52]	Improved antigen presentation
Activation of NK cells [40]	NKp46; increased anti-tumor cytotoxicity
Activation of monocytes [47,48]	NF-κB; NO; TNF; increased anti-tumor cytotoxicity
Activation of DCs [92,93]	Polarization towards DC1
Costimulation of CD4 and CD8 T cells [50,51]	Increase of tumor infiltrating lymphocytes [55]
	Increased secretion of IFN-γ [55]
	Decrease in MDSCs [55]
Induction of tumor-specific immune memory and induction of long-term survival [55]	
Dependency on functional adaptive immune system (RAG2, CD8 T cells) [55]	

ER (endoplasmic reticulum) stress response: early activation of the ER resident enzyme PERK precedes increased phosphorylation of the translation initiation factor eIF2a that leads to virus-induced shutdown of protein synthesis; Immunogenic apoptosis: Cell surface exposure of calreticulin (ecto-CRT) and heat shock proteins (HSPs) precedes apoptosis, cytoplasmic foreign viral RNA is recognized as danger via retinoic acid inducible gene I (RIG-I) and via Toll-like receptors (TLRs); PAMP: Pathogen associated molecular pattern; Necrosis/necroptosis: Release of damage associated molecular patterns (DAMPs) and of pro-inflammatory cytokines, in particular TNF-α and type I IFN; Autophagy: Self-digestion of organelles after inclusion in cytosolic lysosomes (autophagolysosomes); plays a critical role in triggering immune responses. Immune responses: MHC I: Major histocompatibility complex molecules expressing tumor-associated antigens (TAAs) recognized by CD8 T lymphocytes; NK: Natural killer cell activated via hemagglutinin-neuraminidase (HN) of NDV through binding to its receptor NKp46; NO: Nitric oxide, DC: Dendritic cell, MDSC: Myeloid-derived suppressor cell; RAG2: A recombinase enzyme for V(D)J T-cell receptor rearrangement; ICD: Immunogenic cell death.

and transmits cytotoxicity-inducing signals [44]. Cell surface exposed HN but not F molecules are also capable of inducing in human peripheral blood mononuclear cells a strong type I IFN response [45]. In addition, the characterization of NDV protein sequences recently revealed pro-apoptotic Bcl-2 homology-3 domain-like regions in NDV M, L and F proteins [46]. Upon contact with NDV, not only NK cells but also monocytes become activated. They then express TRAIL and produce TNF-α and nitric oxide [47,48].

2.6.2 *Adaptive immunity activation*

Through its receptor binding activity, HN molecules at the surface of infected tumor cells introduce new cell adhesive strength for interaction with lymphocytes [49] and for T-cell co-stimulation, including CD4 helper [50] and CD8 cytotoxic T cells [51]. In addition, human tumor cell infection by NDV leads to up-regulation of human leukocyte antigen and intercellular adhesion molecule 1 molecules [50]. Further events are the induction of IFNs, chemokines (IP10, RANTES) and finally ICD [52].

3. Prospective anti-cancer therapies

3.1 NDV as single agent

3.1.1 *Intratumor inoculation*

Intratumoral and intraperitoneal injection of oncolytic NDV (strain PV-701) caused durable, complete tumor regression in athymic mice bearing human neuroblastoma and fibrosarcoma xenografts [53]. The insertion of exogenous reporter genes did not affect NDV replication. Recombinant NDVs (strain Italien) induced syncytium formation and cell death, prolonged the survival of tumor-bearing athymic mice and suppressed loss of body weight after intratumoral injection [54]. Orthotopic glioma studies in immunocompetent mice recently revealed that intratumoral virotherapy with NDV induced through ICD tumor-specific immune memory and long-term survival [55].

3.1.2 *Locoregional treatment*

Intra-nasal NDV application follows the natural route of virus infection via respiratory and alimentary tract mucosal surfaces. In mice, this way of application induced in lung cells the secretion of pro-inflammatory cytokines and type I IFNs [46].

For locoregional treatment of liver malignancies, injection of viruses such as NDV into the hepatic artery would be logic but, for mice, only the intraportal route is feasible. Since not only gut, but also spleen drains into the portal system, a split-spleen reservoir model was used for multiple portal venus injections. Separate splenic veins were used: i) for production of diffuse artificial liver metastases with a luciferase-gene-transfected murine colon carcinoma (CT26-luc) line and ii) for independent locoregional application of oncolytic NDV. *In vivo* bioluminescence imaging revealed a significant retardation of tumor growth upon NDV application. Also, overall survival (OS) was improved. Since CT26-luc cells were resistant to the oncolytic effect of the virus, it was concluded that the effect was host mediated, most likely involving type I IFN induction [56]. Monocytes, NK cells and T cells could have been involved because of IFN I-induced TRAIL expression [47].

In an orthotopic immunocompetent liver tumor rat model, administration of an engineered fusogenic NDV via hepatic arterial infusion resulted in significant syncytia formation and necrosis. This translated into a significant 20% prolongation of survival [57].

Similarly, administration of this virus (NDV(F3aa)-GFP) intraperitoneally was reported an effective anti-tumor therapy against peritoneal carcinomatosis from human gastric cancer in a severe combined immunodeficiency mouse xenograft model [58]. Sustained remissions were also reported of malignant pleural mesothelioma upon treatment with this virus in an orthotopic tumor model [59].

3.2 Clinical studies with NDV as single agent

In 1970, NDV (strain 73 T) was administered intravenously to 17 healthy subjects. It was noted to be a strong IFN inducer. Overall tolerability was good with side effects being a transient drop in blood counts and mild flu-like symptoms [60].

In 1993, in a Phase II placebo-controlled clinical study in Hungary, oncolytic NDV (strain *MTH-68/H*) was applied via inhalation to 33 advanced chemorefractory patients in order to affect their lung metastases. The high virus doses applied were well tolerated and the clinical results, although not randomized, suggested a decrease in cancer-related symptoms and better survival [61]. Eighteen out of 33 (55%) patients, primarily colorectal carcinoma (CRC), responded to treatment compared with 2 out of 26 (8%) who did not receive NDV. After 2 years, there were seven survivors in the treated group compared with none in the control group.

Another NDV strain (HUJ, Theravir, Jerusalem, Israel, lentogenic) was administered intravenously to 14 glioblastoma patients using intrapatient dose escalation. One patient achieved complete response, while the rest of the patients had progressive disease [62].

Three Phase I clinical trials with NDV (strain PV-701), developed by Wellstat Biologics, were conducted in patients with various types of advanced solid cancer. A total of 113 patients were treated with intravenous injection of the virus in various treatment schedules. Doses of 3×10^9 infectious particles of this replication competent, oncolytic strain were well tolerated. Dose-limiting toxicities included dyspnea, diarrhea and dehydration. In some patients a transient thrombocytopenia and diffuse vascular leak was observed. When patients were desensitized with a lower initial dose, the maximal tolerated dose was increased 10-fold [21,63].

The results of direct administration of NDV to cancer patients, as summarized before [11,20], resulted in minimal toxicity with suggestions of clinical benefit in some patients. What has not been investigated is whether oncolytic NDV, when applied to patients being resistant to chemotherapy or radiotherapy, might be able to break such resistance. Basic research findings have shown i) that NDV replication is independent of cell replication, ii) that infection and oncolysis takes place in tumor cells with defects in anti-viral signaling and apoptotic pathways and iii) that NDV can exert oncolytic activity against hypoxic cancer cells (see Section 2.5). Perhaps oncolytic NDV can also target cancer stem cells which are in a resting state of the cell cycle.

3.2.1 *Clinical studies of postoperative vaccination with viral oncolysate*

Cassel and Murray [9] recognized that post-oncolytic anti-tumor immunity was possibly more important than direct effects of viral oncolysis. They therefore concentrated on using viral oncolysates for immunization purposes. They established a protocol for the preparation of a viral oncolysate utilizing NDV (strain 73T) and primary explants of human tumor cells and selected cell lines from malignant melanoma based on immunologic responses [64]. In 1977, the first results from vaccination of stage II malignant melanoma by this viral oncolysate were published with updates in 1992 [65] and 1998 [66]. Long-term vaccination and long follow-up demonstrated over 60% 10-year survival and 55% 15-year survival, a significant increase compared with historic controls.

Table 2 summarizes results obtain upon vaccination with viral oncolysates (VOLs) in four different studies. No clinical benefit was reported in two studies testing this approach for management of malignant melanoma stage III [67,68]. A forth study demonstrated improved survival compared with historic controls of NDV (73T) generated VOL when applied to prevent relapses of surgically removed renal cell carcinomas in conjunction with recombinant IL-2 and IFN-α [69].

3.3 Autologous live tumor cell vaccine infected by NDV

A different strategy utilized live whole-cell autologous irradiated tumor cell vaccines modified by infection with non-lytic NDV (strain Ulster). This strategy was based on various observations indicating that live tumor cells retaining membrane integrity are superior to oncolysates with regard to immunogenicity [70,71].

3.3.1 *Pre-clinical studies in mice*

Augmented T-cell responses to TAAs were observed against the high metastatic murine lymphoma variant ESb when using as immunogen irradiated ESb tumor cells that had been modified by infection with a low dose of NDV. Immune spleen cells from mice immunized with ESb-NDV contained enhanced immune capacity in both the $CD4^+CD8^-$ and the $CD4^-CD8^+$ T-cell compartments. The virus-mediated augmentation of the tumor-specific T-cell response involved increased T-helper activity [72]. The potentiation of tumor-specific cytotoxic T lymphocytes (CTL) activity by NDV was mediated via induction of IFN-α and -β [73].

To test the therapeutic potential of irradiated NDV-infected ESb cells as vaccine, ESb tumor-bearing mice were operated and vaccinated postoperatively. Mice which were either operated only or operated and vaccinated with a non-infected ESb vaccine were all dying from metastases

Table 2. Examples of clinical vaccination trials employing NDV oncolysates.

Oncolysate (virus strain)	Disease	Study type	Clinical outcome	Ref.
1. VOL (73 T)	Melanoma (stage II)	Phase II (n = 83)	Improved OS (> 60% [10 years])	[65,66]
2. VOL (Italien)	Melanoma (stage III)	Phase II (n = 24)	No benefit	[67]
3. VOL (Ulster) + IL-2	Melanoma (stage III)	Phase II/III (n = 29)	No benefit	[68]
4. VOL (73 T) (+IL-2, +IFN-2a)	Advanced renal Ca	Phase II (n = 208)	Improved OS	[69]

All oncolysate vaccines were applied intradermally.
Ad 1. Unusual disease-free survival at 10 years; similar results obtained from head and neck cancer (n = 23) and from cerebral metastases (n = 23). The staging later changed to AJCC III.
Ad 2. The study pretended to try to reproduce Cassel's findings, however, there were many violations of the original oncolysate preparation protocol (WA Cassel, personal communication).
VOL: Viral oncolysate from autologous or homologous tumor; OS: Overall survival.

within 3 weeks. In contrast, 60% of mice operated and vaccinated with ESb-NDV vaccine survived long-term (> 2 months) and developed specific protective anti-tumor immunity based on anti-tumor memory T cells (MTCs) [74].

3.3.2 *Clinical studies*

ATV-NDV stands for **A**utologous **T**umor cell **V**accine modified by infection with a low dose of lentogenic NDV. The rationale for the design of this vaccine, its transfer from animal studies to human application and the results obtained have been reported and summarized in the past [75].

Pre-existing tumor-reactive MTCs from cancer patients could apparently be activated *in situ* by the tumor vaccine ATV-NDV as seen by augmentation of anti-tumor skin delayed-type hypersensitivity (DTH) memory responses [7]. The conclusion that ATV-NDV vaccine can present TAAs directly to MTCs and stimulate them is supported by the following results: i) in a coculture with a TAA-specific memory T cell clone, ATV-NDV-stimulated T-cell proliferation and IL-2 production, while ATV without NDV infection induced tolerance [50], ii) viability of the irradiated vaccine was important for CTL activation [71] and for clinical efficacy [7,76] and iii) antigen-presenting cells transfected with the viral HN cDNA showed increased CTL-stimulatory capacity [51].

Intradermal vaccinations of cancer patients with ATV-NDV were well tolerated and could be repeated many times without causing adverse events. Viability of the 2 – 10 million irradiated vaccine cells was important not only for CTL activation, but also for clinical efficacy [7]. The best DTH reaction was obtained using 10 million tumor cells and 32 hemagglutinating units of NDV (strain *Ulster*) (median induration of 8 mm) [7]. Histological examination of the vaccination site revealed a dense infiltration of predominantly helper T lymphocytes.

Vaccination in 2-week intervals for three times was often sufficient to obtain peak anti-tumor DTH reactivity. This means three rounds of expansion and retraction of tumor-reactive T cells involving three waves changing between effector memory T cell activation in peripheral tissues and central memory T cell resting states in the bone marrow [77,78].

Details about the immunological mechanisms of function of ATV-NDV in cancer patients have been reported [78]. They include the rationale for autologous and live tumor cell vaccines, for the choice of NDV to break the immunological tolerance barrier, BM niches for cancer-reactive memory T cells, the individuality of the pre-existing cancer-reactive memory T-cell repertoire from cancer patients as well as the activation and recruitment of this repertoire from the BM to the sites of tumor.

Table 3 lists examples of clinical vaccination trials employing NDV whole cell vaccines [7,76,79-86]. The studies 1 – 9 with ATV-NDV were performed in the years 1990 – 2008. Only some studies will be described below. Study 10 is a contribution from China.

In a postoperative active-specific immunotherapy (ASI) study of locally advanced primary breast cancer [7] (study 4), the follow-up revealed a 5-year survival rate that was 36% higher in group A (high-quality ATV-NDV vaccine; > 10^6 cells) than in group B (low-quality ATV-NDV vaccine; < 10^6 cells) [7,57]. In a postoperative ASI study of locally advanced CRC (study 1), there was an apparent 25% increase in the 2-year survival rate in a group of 20 patients who received a high-quality ATV-NDV vaccine [79]. The OS in ATV-NDV vaccinated head and neck squamous cell carcinoma (study 8) was improved by 23% in comparison to historic controls [84].

Twenty-three patients suffering from GBM were vaccinated with ATV-NDV from cell culture to assess feasibility, safety and clinical benefit [85] (study 9). The median progression-free survival of vaccinated patients was 40 weeks (vs 26 weeks in 87 non-vaccinated control subjects from the same time period and the same clinic) and the median OS was 100 weeks (vs 49 weeks in control subjects; $p < 0.001$). In the vaccinated group, immune monitoring revealed significant increases of skin DTH reactivity, the number of tumor-reactive MTCs in the blood and the numbers of $CD8^+$ tumor-infiltrating T lymphocytes in frozen tissue slices from GBM recurrencies. There was one complete remission of non-resectable remaining tumor [85].

Another study, a prospectively randomized Phase II/III trial investigated the efficiency of ATV-NDV after liver resection

Table 3. Examples of clinical vaccination trials employing NDV whole cell vaccines.

Vaccine (virus strain)	Disease	Study type	Clinical outcome	Ref.
1. ATV-NDV (Ulster)	CRC (locally advanced) (stage II & III)	Phase II (n = 57)	Improved OS and DFS	[79]
2. ATV-NDV (Ulster)	CRC (R0 res. liver mets) (stage IV)	Phase II (n = 23)	Improved OS and DFS	[80]
3. ATV-NDV (Ulster)	Colon Ca (R0 res liver mets)	Phase II/III	Improved OS (p = 0.01)	[81]
	Rectum Ca (R0 res liver mets)		Not significant	
	(*A prospective randomized-controlled study*; n = 51)			
4. ATV-NDV (Ulster)	Breast Ca (locally adv)	Phase II (n = 32)	Improved OS and DFS	[7]
5. ATV-NDV (Ulster)	Advanced Ovarian Ca	Phase II (n = 82)	Improved PFS	[82]
6. ATV-NDV (Ulster)	Pancreatic Ca (G3)	Phase II (n = 53)	Improved OS and DFS	[76]
7. ATV-NDV (+ IL-2,+ IFN-2a)	Renal cell Ca	Phase II (n = 40)	Improved OS	[83]
8. ATV-NDV (Ulster)	Head and neck Ca (stage III + IV)	Phase II (n = 18)	Improved OS and DFS	[84]
9. ATV-NDV (+ IL-2)	Glioblastoma multiforme	Phase II (n = 23)	Improved OS and PFS	[85]
10. ATV fixed + NDV (La Sota)	Gastrointestinal Ca (stage I-IV)	Phase III (n = 310)	Sign improved OS (7 vs 4.46 years)	[86]

The postoperative clinical studies with ATV-NDV received beforehand approval by local ethical committees. All vaccines were applied intradermally.
Ad 5. ATV-NDV vaccines derived from cell culture could be applied multiple times (up to 15 times) without causing adverse events.
Ad 6. The results were obtained by R Gerhards, Marienhospital Herne (Germany), who kindly provided them to the author for publication. The tumor cells were derived from cell culture and inactivated after virus infection by cytostatic drugs. Remarkably, none of the vaccinated patients developed bone or liver metastases.
Ad 7. Of the 40 patients, 23 showed some clinical benefit. Median OS was 31 months compared with 13 months for historical controls. It is unclear, how much benefit can be attributed to the vaccine and how much to the low dose IL-2 and IFN-α.
Ad 1 – 5, 7 – 9. In all these studies, the tumor cells were inactivated by X-irradiation (200 Gy); this treatment interferes with host cell DNA but not with viral RNA replication; viral RNA replication can be inhibited with UV light irradiation; studies 1 – 5 and 7: the tumor cells were dissociated from fresh tumor specimens to single cell suspensions; study 4: tumor-infiltrating lymphocytes (TILs) were depleted by immunomagnetic beads; studies 5, 6, 8 and 9: tumor cells, separated from fresh tumor specimens, were first adapted to cell culture and expanded before they were used for vaccine preparation.
Ad 10. Autologous tumor vaccine and NDV vaccine were applied separately; details about the tumor vaccine are lacking, most likely it consisted of formalin-fixed and/or paraffin-embedded tumor tissue, a type of vaccine developed in China and India.
CRC: Colorectal carcinoma; DFS: Disease-free survival; OS: Overall survival; PFS: Progression-free survival.

for hepatic metastases of CRC as a tertiary prevention method [81] (study 3). Twenty-five of such stage IV CRC patients were vaccinated and compared with a similar number of non-vaccinated comparable patients. After an exceptionally long follow-up period of 9 – 10 years, there was no significant difference between the vaccinated and the control arm. However, when stratified for tumor localization there were significant differences between colon and rectum. While there was no significant difference for rectal cancer, a significant benefit was seen in the colon cancer subgroup in terms of long-term metastasis-free survival and OS. In the control arm, 78.6% had died, in the vaccinated arm only 30.8% [81]. The trial provides clinical evidence for the value and potential of the cancer vaccine ATV-NDV.

There are not many successful randomized-controlled studies of anti-tumor vaccination [87]. Two of those were performed with a vaccine composed of 10 million live autologous human colon carcinoma cells that were irradiated and injected intracutaneously along with BCG, an attenuated mycobacterium as an immunostimulant. Such ATV/BCG vaccine [88] was later termed OncoVAX [89]. Both Phase III trials [88,89] revealed that this vaccine, in an adjuvant setting significantly improved the 5-year recurrence-free survival and OS in stage II colon cancer patients. There was, however, no significant effect in stage III [89]. It is remarkable that the vaccine ATV-NDV improved survival in colon patients of stage II, III and also IV with operated liver metastases. A comparison between these two types of autologous live cell vaccines, their mechanism of function and their clinical significance has been reviewed and discussed [78].

3.4 Viral onco-lysate pulsed DC (VOL-DC)

While most of these clinical studies showed some promising results, there was still a majority of patients that had to be considered as non-responders. So efforts were undertaken to further increase the effectivity of the vaccine ATV-NDV. One strategy consisted of constructing NDV-specific single chain antibodies with dual specificity (bispecific antibodies) for attachment to ATV-NDV to augment T-cell stimulatory signals (see 3.5.4). The other strategy (see 3.4.2) was to combine ATV-NDV with dendritic cells (DCs) in order to improve the *de novo* generation of TAA-specific cells from naive T cells.

3.4.1 *Effect of NDV on human DCs*

Many studies have demonstrated that DCs can be pulsed with tumor lysate and that such cells can function as TAA-presenting cells. A safe and reproducible preparation protocol has recently been published [90].

The effect of NDV or of NDV-mediated VOL on DCs had not been studied. Meanwhile, a very sophisticated analysis of cell activation has been performed with NDV and human DCs. NDV was considered a prototype avian virus to study an uninhibited cellular response to virus infection in human DCs [91]. This study of systems biology involved among others microarray experiments, time-dependent promoter analysis,

transcription factor binding site analysis, electromobility shift assays and regulatory network construction. The analysis revealed that the genetic program underlying the anti-viral cell-state transition during the first 18 h post-infection can be explained by a single convergent regulatory network involving 24 critical transcription factors. These regulated 779 of the 1351 up-regulated genes. Many of these genes were associated with polarization of the DCs towards DC1 and for induction of Th1 T-cell-mediated immune responses.

Such polarization of human DCs by NDV to DC1 was confirmed in functional studies [92].

3.4.2 VOL-DCs potently stimulate T cells from cancer patients

Instead of viable ATV-NDV tumor cell vaccine, viral oncolysates (VOL) were prepared from ATV-NDV. Patient-derived DCs were then pulsed with these oncolysates to obtain the vaccine VOL-DC. In this way, the DCs receive relevant information about the patients TAAs which they express as peptide/MHC complexes for presentation to T cells. In addition, the DCs receive through NDV from the oncolysates information about potential immunological danger signals (PAMPs and DAMPs). This methodology has meanwhile been established and quality certified as an Advanced Therapeutic Medicinal Product at the Immunological and Oncological Center in Cologne (IOZK), Germany.

It was demonstrated that VOL-DCs can potently stimulate autologous T cells from cancer patients. They showed increased expression of costimulatory molecules and induced higher IFN-γ ELISPOT responses. Supernatants from co-cultures of MTCs and oncolysate-pulsed DCs contained increased titers of IFN-α and IL-15 [93].

3.5 Combinatorial treatments

3.5.1 Combining NDV with vaccination: helper effects of OVs

Before patients at the IOZK receive biological cancer therapy, their immune system is monitored and conditioned for active specific immunization. Conditioning of the immune system is done by systemic (intravenous) application of oncolytic NDV 1 week before vaccination. This has several positive effects: i) induction of type I IFNs which inhibit secretion of Th2 cytokines (IL-4 and IL-5) and stimulate Th1 cells [94], ii) induction of ICD (**Table 1**) and iii) priming of NDV oncolysate reactive Th cells. The latter can be monitored regularly by an *in vitro* ELISPOT assay [95]. Upon later vaccination with oncolysate pulsed DCs (see below), such Th cells are likely recruited to the vaccination site. There they might help the response via release of the chemokine CCL3, thus facilitating DC migration. In this way, the induction of an anti-viral immune T-cell response could be beneficial for the subsequent vaccination effect.

Such mechanism has recently been proven for Tetanustoxoid Th cells [96]. Not only chemokines, but also cytokines might enhance OV therapy in synergy with anti-viral Th cells. For instance, the therapeutic activity of some OVs was significantly enhanced in animals immunized against the same viruses and pre-administered with GM-CSF, but not IL-2 or G-CSF [97].

3.5.2 Combining NDV with therapeutic transgenes

Recombinant NDV strains with enhanced oncolytic potential were developed by incorporation of F gene mutations [98] or by addition of the NS1 [99] or the apoptin [100] gene. The immunostimulatory capacity could be augmented by incorporation of genes coding for cytokines, such as IL-2 [101], GM-CSF [102], IL-15 [103] or IFN-γ [104]. NDV could also be modified by incorporation of two transgenes, one coding for the light chain and the other for the heavy chain of a monoclonal antibody interfering with angiogenesis [105]. The transfer of a gene coding for a TAA created a vector with which the immune response could be targeted to a specific TAA in order to compete with the usually stronger response to viral antigens (VA) [106]. For more details regarding these genetically engineered NDVs, see [20].

3.5.3 Combining NDV with carrier cells for improving tumor targeting

Further enhancement of OV-mediated therapy may consist of combining it with adoptive T-cell immunotherapy. It was shown that NDV binds to activated T cells [107]. Upon co-culture with tumor cells, such NDV-loaded T cells released infectious virus to the tumor cells and produced oncolytic effects. In a tumor neutralization assay *in vitro*, monolayers of human tumor cells could be completely destroyed by the addition of polyclonally activated T cells loaded with oncolytic NDV. In this process, synergistic effects between CTLs and OVs were apparent in the tumor contact zone [107]. *In vivo* hitchhiking of NDV on immune T cells could be anticipated to improve tumor targeting of OVs.

Several groups have explored carrier cell systems for improving systemic delivery of viruses with promising results in pre-clinical models [108]. Mesenchymal stem cell carriers for paramyxovirus therapy protected the virus from neutralizing antibodies and effectively transferred the virus to tumors [109].

3.5.4 Combining NDV with bispecific antibodies

To augment the immune-stimulatory properties of NDV-infected tumor cells, another innovative approach was successful: the attachment of single-chain variable fragment bi-specific antibodies (bsAbs). These attach with one arm to a VA (e.g., HN of NDV) and with the other arm to a target on immune cells. In case of T cells, such targets were CD25 [110], CD3 [111] and CD28 [112]. The idea is that VAs of NDV can serve as universal anchor molecules for bsAbs. In this way, T-cell co-stimulatory molecules can be attached to any type of tumor cell infectable by NDV [113].

With the modular approach of first infecting a tumor cell by NDV and then attaching one or two bsAbs, it is possible to create vaccines with increased T-cell-stimulatory capacity. Signal intensity mediated through CD3, CD25 or CD28 can be adapted to the clinical situation by varying the amounts of the bsAb fusion proteins [111]. In this way, T cells from healthy donors could be non-specifically activated by the attachment of suboptimal amounts of anti-HN-anti-CD3 (for signal 1) plus anti-HN-CD28 (for signal 2) [110]. The strongest non-specific anti-tumor activity could be generated from T cells stimulated with ATV-NDV modified by attachment of anti-HN-anti-CD3 plus the tri-specific fusion protein anti-HN-IL-2-anti-CD28. The latter molecules transmit simultaneously two independent co-stimulatory signals through CD25 and CD28 [112,113].

In a Phase I clinical study, 14 CRC patients with late-stage disease which could not be operated anymore with curative intent were treated with the vaccine ATV-NDV to which bsHN-CD28 was attached. No severe adverse events were seen. With the highest dose of 1 µg protein of purified bsHN-CD28, strong T-cell costimulation occurred which enabled re-activation of TAA-specific MTCs. The study suggests that the three-component vaccine is safe and can activate possibly anergized T cells from a chronic disease like advanced-stage cancer [114].

That T-cell costimulatory signals can have effects in late-stage cancer patients has been demonstrated in a clinical study using an agonistic monoclonal antibody (Mab) against OX40 [115].

3.5.5 *Combining NDV with immune checkpoint inhibitors*

The immune system contains not only T-cell costimulatory signalling pathways, but also T-cell inhibitory pathways. Tumors often use such inhibitory pathways for their own immune escape. To prevent such immune escape by tumors, Mabs have been developed with the goal of reversal of tumor-induced T-cell inhibition [116]. Mabs targeting cytotoxic T-lymphocyte antigen 4 (CTLA-4) [117] and programmed death receptor 1 [118] have already demonstrated significant promise in clinical trials. Clinical benefit of these systemically applied antibodies as single agents was however associated with severe side effects.

One could imagine that locoregional application in body cavities such as the pleura or the peritoneum would be associated with less side effects. This would be expected to be the case when lower amounts of antibodies could be applied and also when such antibody treatment would be combined with immunostimulatory agents like oncolytic NDV.

Clinical benefit of checkpoint blocking antibodies appears to be limited to subsets of patients with pre-existing lymphocytic infiltrations of their tumors. A successful rational combination strategy consisted of localized oncolytic virotherapy followed by systemic checkpoint blocking immunotherapy. Intra-tumoral application of NDV to B16 melanoma in mice induced lymphocytic infiltrates not only locally, but also in distant non-injected lesions. Combining localized NDV treatment with systemic CTLA-4 blockade led to rejection of pre-established distant tumors and protection from tumor re-challenge in poorly immunogenic tumor models. The therapeutic effect was associated with infiltration of distant tumors with activated $CD8^+$ and $CD4^+$ effector but not Treg and was dependent on $CD8^+$ cells, NK cells and type I IFN [119].

3.5.6 *Combining NDV with hyperthermia*

At the IOZK in Cologne, systemic NDV application is combined with local hyperthermia in cases where tumor tissue such as metastases are accessible to the Oncothermia EHY-2000 device. For further details, see a recent case report of long-term remission of prostate cancer with extensive bone metastases [120] and another case report on long-term survival of a breast cancer patient with extensive liver metastases [95].

4. Oncolytic NDV with potential to break therapy resistance

Many characteristics of oncolytic NDV, already mentioned in this review, suggest that this biological agent has the potential to break resistance of cancer cells to a variety of therapies. First of all, this agent, in contrast to chemotherapy (CT) and radiotherapy (RT), does not require cells to be in a proliferating state. Since the virus replicates in the cytoplasm, it is independent of DNA replication. The virus also replicates in X-irradiated cells (such as ATV-NDV vaccine cells) because X-irradiation damages DNA but not RNA. Many tumor cells in a resting state such as tumor stem cells or cells from tumor dormancy may not be affected by CT or RT, but could possibly be targeted by oncolytic NDV. The findings indicating that NDV selectively infects apoptosis-resistant, hypoxic or IFN-resistant tumor cells suggests that this OV may very well complement conventional cancer therapies which lack these properties. Finally, NDV may even break resistance mechanisms to immunotherapy. It may break T-cell tolerance to TAAs and it could overcome resistance to immune checkpoint blockade immunotherapy.

Thus, oncolytic NDV can function as cutting edge between tumor and host [10] by disrupting barriers of ignorance, tolerance or immune suppression by activating host immune mechanisms and destroying tumor cells by ICD.

Table 4 gives an overview of these potential activities of NDV which deserve further exploitation.

5. Summary and conclusion

This review deals with NDV, a biologic agent with potent oncolytic activity as well as with potent immunostimulatory capacity. Its tumor selectivity and its capacity to replicate distinguish it from chemical or physical modes of cancer treatment. Negative side effects of NDV are negligible compared with CT or RT. Many genetically engineered OVs try to

Table 4. NDV, a biological agent with potential to break therapy resistance.

1. In contrast to most cytostatic drugs and to RT, oncolysis of NDV does not depend on cell proliferation. It is even enhanced in X-irradiated cells, possibly due to a combination of cellular stress from X-irradiation and virus infection [121]
2. Perhaps oncolytic NDV can also target cancer stem cells which are in a resting state of the cell cycle
3. In contrast to most cytostatic drugs and RT, virus replication and cell lysis following NDV infection of non-permissive hosts shows selectivity for tumor cells. This is due to defects of tumor cells in various signaling pathways (see Section 2.5)
4. Tumor cells with resistance to CT or RT are often also resistant to apoptosis. Oncolytic NDV shows selectivity for apoptosis-resistant cells [33]
5. Hypoxic cancer cells in the center of a growing tumor are often also resistant to CT or RT. NDV can exert oncolytic activity against hypoxic cells [26]
6. Many tumor cells are resistant to treatment by type I IFNs because of defects in type I IFN receptor signaling pathways. Oncolysis by NDV is supported by such defects [13,27-29]
7. Glioblastoma multiforme tumor cells are characterized by resistance to many conventional therapies and recur in the brain via motility and invasion. This is due to Rac1 protein expression and aberrant signaling. Interestingly, Rac1 is targeted by NDV infection [35] (see Section 2.5)
8. A human T-helper clone with specificity for autologous malignant melanoma was resistant to *in vitro* stimulation with such tumor cells. Following infection by NDV, the melanoma cells could break this T-cell tolerance [50]
9. Systemic tumor resistance to immune checkpoint blockade immunotherapy could be overcome by localized oncolytic virotherapy with NDV [119,122]

CT: Chemotherapy; NDV: Newcastle disease virus; RT: Radiotherapy.

achieve maximal tumor cell killing by reducing anti-viral immunity. This review provides examples that in case of NDV anti-viral immunity and immunostimulatory capacities of an OV can be integrated to achieve maximal post-oncolytic T-cell-mediated anti-tumor immunity.

Oncolytic NDV induces ICD with multiple effects on the immune system. Innate and adaptive immune responses that are thus generated can help to destroy tumor cells so that direct maximal tumor cell killing by the virus is not necessary. Post-oncolytic cancer-reactive T-cell responses are equipped with a memory function which provides over long times protective activity against minimal residual disease. Without such support from the immune system, OVs would have to be applied similar to cytostatic anti-cancer drugs.

Various types of anti-cancer therapy by NDV are summarized. They include intratumoral or locoregional virus application in animal model systems, systemic virus applications to cancer patients and clinical studies with tumor vaccines containing NDV. Many of the clinical studies, including one prospectively randomized controlled study, demonstrated clinical benefits.

Various combinatorial approaches are presented and discussed by which the effectivity of oncolytic NDV could be further augmented. One of this consists of systemic NDV pre-treatment before anti-tumor vaccination with tumor vaccines containing NDV. Such immune conditioning induces type I IFNs and Th cells against VOL, which can be measured and likely contribute to an improved response to vaccination.

An important message in the field of anti-tumor vaccination is the requirement of a quality product. Whether it concerns Cassel's oncolysate, Hanna's, Hoover's and Vermorkens's OncoVAX, or our vaccine types ATV-NDV and VOL-DC: each of these autologous vaccine products requires much skill and knowledge of detail without which the clinical effect can easily fail or become irreproducible.

6. Expert opinion

Cancer therapies based on biological principals and on involving the cancer-bearing host's immune system are expected to have a great future and will hopefully spare cancer patients the negative side effects of CT or RT. Oncolytic NDV, as a safe biological agent with anti-neoplastic as well as immune-stimulatory properties, gives an example of a prospective anti-cancer therapy. It may become a useful complement to CT or RT, especially in cases of therapy resistance. Even resistance to specific immunotherapy and to immune checkpoint blockade may be broken by the introduction of oncolytic NDV.

For the future, it is predicted that only multimodal approaches will be effective with a disease as complex as cancer. There are possibilities of pharmacological modulation of anti-tumor immunity induced by OVs and of combinations of oncolytic virotherapy with CT [3]. The combination with immunosuppressive agents is being discussed to suppress anti-viral immunity in order to augment viral replication in tumor tissue. The author is, however, skeptical about such an approach because this may reduce the subsequent immune response and therapeutic efficacy. Good future prospects are expected by combining oncolytic NDV with hyperthermia, with therapeutic transgenes, with DCs, T cells or bi-specific antibodies. While many of these combinations still require much work before they can be applied to patients, the combination of systemic NDV with anti-tumor vaccination is already a clinical reality, at least at one site in Germany.

Immune checkpoint modulation has already given a boost to the field of cancer immunotherapy. This will likely be a driving force also in the future, especially when combined with immune-stimulating agents such as OVs and anti-cancer vaccines.

Declaration of interest

V Schirrmacher has IP related to NDV and is affiliated with IOZK. He has no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending or royalties.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Kroemer G, Galluzzi I, Kepp O, Zitvogel I. Immunogenic cell death in cancer therapy. Ann Rev Immunol 2013;31:51-72

• **This review explains in molecular terms ICD and discusses its relevance to cancer therapy.**

2. Guo ZS, Liu Z, Bartlett DL. Oncolytic immunotherapy: dying the right way is a key to eliciting potent antitumor immunity. Front Oncol 2014;4:6-16

3. Schirrmacher V, Fournier P. Editorial: Harnessing oncolytic virus-mediated anti-tumor immunity. Front Oncol 2014.4:337-8. Available from: http://journal.frontiersin.org/researchtopic/1613

•• **In 12 articles, experts from Canada, Germany and the USA emphasize the potential importance of oncolytic viruses on systemic T-cell-mediated anti-tumor immunity.**

4. Liu TC, Galanis E, Kirn D. Clinical trial results with oncolytic virotherapy: a century of promise, a decade of progress. Nat Clin Pract Oncol 2007;4:101-17

5. Russel SJ, Peng KW, Bell JC. Oncolytic virotherapy. Nat Biotechnol 2012;30:658-70

6. Csatary LK, Gosztonyi G, Szeberenyi J, et al. MTH-68/H oncolytic viral treatment in human high-grade gliomas. J Neurooncol 2004;67:83-93

7. Ahlert T, Sauerbrei W, Bastert G, et al. 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;15:1354-66

8. Freeman AI, Zakay-Rones Z, Gomori JM, et al. Phase I/II trial of intravenous NDV-HUJ oncolytic virus in recurrent glioblastoma multiforme. Mol Ther 2006;13:221-8

9. Cassel WA, Murray DR. Newcastle disease virus as an antineoplastic agent. Cancer 1965;7:863-8

10. Fournier P, Schirrmacher V. Oncolytic Newcastle disease virus as cutting edge between tumor and host. Biology (Basel) 2013;2:936-75

11. Lam HY, Yeap SK, Rasoli M, et al. Safety and clinical usage of Newcastle disease virus in cancer therapy. J Biomed Biotechnol 2011;2011:718710

12. De Leeuw O, Peeters B. Complete nucleotide sequence of Newcastle disease virus: Evidence for the existence of a new genus within the subfamily Paramyxovirinae. J Gen Virol 1999;80:131-6

13. 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;40:287-98

14. Wilden H, Fournier P, Zawatzky R, Schirrmacher V. Expression of RIG-I, IRF3, IFN-beta and IRF7 determines resistance or susceptibility of cells to infection by Newcastle Disease Virus. Int J Oncol 2009;34:971-82

15. Hornung V, Ellegast J, Kim S, et al. 5'-Triphosphate RNA is the ligand for RIG-I. Science 2006;314:994-7

16. Melchjorsen J, Jensen SB, Malmgaard L, et al. Activation of innate defense against a paramyxovirus is mediated by RIG-I and TLR7 and TLR8 in a cell-type-specific manner. J Virol 2005;79:12944-51

17. Park MS, Garcia-Sastre A, Cros JF, et al. Newcastle disease virus V protein is a determinant of host range restriction. J Virol 2003;77:9522-32

• **This paper is important to understand the difference between a permissive and a non-permissive host for infection by NDV.**

18. Alexander DJ. Newcastle Disease. Kluwer Academic; 1988

19. Miller LT, Yates VJ. Reactions of human sera to avian adenoviruses and Newcastle disease virus. Avian Dis 1971;15(4):303-7

20. Zamarin D, Palese P. Oncolytic Newcastle disease virus for cancer therapy: old challenges and new directions. Future Microbiol 2012;7(3):347-67

• **This review is recommended for further reading about NDV, cancer therapy and genetically engineered NDV to increase its effectivity.**

21. Pecora AL, Rizvi N, Cohen GI, et al. Phase I trial of intravenous administration of PV701, an oncolytic virus, in patients with advanced solid cancers. J Clin Oncol 2002;20:2251-66

• **This clinical trial witnesses the tolerability of NDV even at high doses in cancer patients.**

22. Nagai Y, Hamaguchi M, Toyoda T. Molecular biology of Newcastle disease virus. Prog Vet Microbiol Immunol 1989;5:16-64

23. Elankumaran S, Rockemann D, Samal SK. Newcastle disease virus exerts oncolysis by both intrinsic and extrinsic caspase-dependent pathways of cell death. J Virol 2006;80:7522-34

24. Kumar R, Tiwari AK, Chaturvedi U, et al. Velogenic Newcastle disease virus as an oncolytic virotherapeutics: in vitro characterization. Appl Biochem Biotechnol 2012;167:2005-22

25. Elmore S. Apoptosis: A review of programmed cell death. Toxicol Pathol 2007;35:495-516

26. Chng WC, Stanbridge EJ, Yusoff K, Shafee N. The oncolytic activity of Newcastle disease virus in clear cell carcinoma cells in normoxic and hypoxic conditions: The interplay between VHL and interferon-beta signaling. J Interferon Cytokine Res 2013;33:346-54

27. Elankumaran S, Chavan V, Quiao D, et al. Type I interferon-sensitive recombinant Newcastle disease virus for

oncolytic virotherapy. J Virol 2010;84(8):3835-44

28. Fiola C, Peeters B, Fournier P, et al. Tumor selective replication of Newcastle disease virus: association with defects of tumor cells in antiviral defense. Int J Cancer 2006;119(2):328-38

29. Wilden H, Fournier P, Zawatzky R, Schirrmacher V. Expression of RIG-I, IRF3, IFN-beta and IRF7 determines resistance or susceptibility of cells to infection by Newcastle disease virus. Int J Oncol 2009;34(4):971-82

30. Wilden H, Schirrmacher V, Fournier P. Important role of interferon-regulatory factor (IRF)-3 in the interferon response of mouse macrophages upon infection by Newcastle disease virus. Int J Oncol 2011;39(2):493-504

31. Krishnamurthy S, Takimoto T, Scroggs RA, Portner A. Differentially regulated interferon response determines the outcome of Newcastle disease virus infection in normal and tumor cell lines. J Virol 2006;80(11):5145-55

32. 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(1):287-98

33. Mansour M, Palese P, Zamarin D. Oncolytic specificity of Newcastle disease virus is mediated by selectivity for apoptosis-resistant cells. J Virol 2011;85(12):6015-23

• **This paper is of clinical relevance, suggesting that cancer cells which are resistant to chemo- or radiotherapy can be attacked with oncolytic NDV.**

34. Lazar I, Yacoov B, Shiloach T, et al. The oncolytic activity of Newcastle disease virus NDV-HUJ on chemoresistant primary melanoma cells is dependent on the proapoptotic activity of the inhibitor of apoptosis Livin. J Virol 2010;84(1):639-46

35. Puhlmann J, Puehler F, Mumberg D, et al. Rac 1 is required for oncolytic NDV replication in human cancer cells and establishes a link between tumorigenesis and sensitivity to oncolytic virus. Oncogene 2010;29(15):2205-16

• **This paper suggests a link between tumorigenesis, cancer motility and invasion with susceptibility to oncolysis by NDV.**

36. Abdullah JM, Mustafa Z, Ideris A. Newcastle Disease Virus interaction in targeted therapy against proliferation and invasion pathways of Glioblastoma multiforme. Biomed Res Int 2014;2014:386470

37. Koks CAE, De Vleeschouwer S, Graf N, Van Gool SW. Immune suppression during oncolytic virotherapy for high-grade glioma; yes or no? J of Cancer 2015;6:203-17

38. Fábián Z, Csatary CM, Szeberényi J, Csatary LK. p53-independent endoplasmic reticulum stress-mediated cytotoxicity of a Newcastle disease virus strain in tumor cell lines. J Virol 2007;81:2817-30

39. Tesniere A, Panaretakis T, Kepp O, et al. Molecular characteristics of immunogenic cancer cell death. Cell Death Diff 2008;15:3-12

40. Meng C, Zhou Z, Yu S, et al. Newcastle disease virus triggers autophagy in U251 glioma cells to enhance virus replication. Arch Virol 2012;157:1011-18

41. Meng G, Xia M, Wang D, et al. Mitophagy promotes replication of oncolytic Newcastle disease virus by blocking intrinsic apoptosis in lung cancer cells. Oncotarget 2014;5:6365-74

42. Li Y, Wang LX, Yang G, et al. Efficient cross-presentation depends on autophagy in tumor cells. Cancer Res 2008;68:6889-95

43. Fournier P, Arnold A, Wilden H, Schirrmacher V. Newcastle disease virus induces pro-inflammatory conditions and type I interferon for counter-acting Treg activity. Int J Oncol 2012;40:840-50

44. Jarahian M, Watzl C, Fournier P, et al. Activation of natural killer cells by Newcastle disease virus hemagglutinin-neuraminidase. J Virol 2009;83:8108-21

45. 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

46. Molouki A, Hsu YT, Jahanshiri F, et al. The matrix (M) protein of Newcastle disease virus binds to human Bax trough its BH3 domain. Virol J 2011;8(1):385

47. Washburn B, Weigand MA, Grosse-Wilde A, et al. TNF-related apoptosis-inducing ligand mediates tumoricidal activity of human monocytes stimulated by Newcastle disease virus. J Immunol 2003;170:1814-21

48. Umansky V, Beckhove P, Rocha M, et al. Induction of NO synthesis in macrophages by Newcastle disease virus is associated with activation of nuclear factor-kappa B. Int Immunol 1996;8:491-8

49. Jurianz K, Haas C, Hubbe M, et al. Adhesive function of Newcastle-disease virus hemagglutinin in tumor-host interaction. Int J Oncol 1995;7:539-45

50. Termeer CC, Schirrmacher V, Bröcker EB, Becker JC. Newcastle disease virus infection induces B7-1/B7-2-independent T-cell costimulatory activity in human melanoma cells. Cancer Gene Ther 2000;7:316-23

• **This study suggests that tumor cell infection by NDV can break tolerance of human tumor-specific T-helper cells.**

51. Ertel C, Millar NS, Emmerson PT, et al. Viral hemagglutinin augments peptide-specific cytotoxic T cell responses. Eur J Immunol 1993;23:2592-6

52. Washburn B, Schirrmacher V. Human tumor cell infection by Newcastle Disease Virus leads to upregulation of HLA and cell adhesion molecules and to induction of interferons, chemokines and finally apoptosis. Int J Oncol 2002;21:85-93

• **This study characterizes changes in human tumor cells upon infection by NDV. Such pre-apoptotic changes increase the tumor cells immunogenicity.**

53. Lorence RM, Katubig BB, Reichard KW, et al. Complete regression of human fibrosarcoma xenografts after local Newcastle disease virus therapy. Cancer Res 1994;54:6017-21

54. Wei D, Sun N, Nan G, et al. Construction of recombinant Newcastle disease virus Italien strain for oncolytic virotherapy of tumors. Hum Gene Ther 2012;23(7):700-10

55. Koks CA, Garg AD, Ehrhardt M, et al. Newcastle disease virotherapy induces long-term survival and tumor-specific immune memory in orthotopic glioma through the induction of immunogenic

cell death. Int J Cancer 2015;136:E313-25

•• **This study demonstrates induction of ICD *in situ* in a relevant tumor model with subsequent establishment of anti-tumor immunity, memory and long-term survival.**

56. Apostolidis L, Schirrmacher V, Fournier P. Host mediated anti-tumor effect of oncolytic Newcastle disease virus after locoregional application. Int J Oncol 2007;31:1009-19

57. Altomonte J, Marozin S, Schmidt RM, Ebert O. Engineered Newcastle disease virus as an improved oncolytic agent against hepatocellular carcinoma. Mol Ther 2010;18(2):275-84

58. Song KY, Wong J, Gonzalez L, et al. Antitumor efficacy of viral therapy using genetically engineered Newcastle disease virus [NDV(F3aa)-GFP] for peritoneally disseminated gastric cancer. J Mol Med 2010;88(6):589-96

59. Silberhumer GR, Brader P, Wong J, et al. Genetically engineered oncolytic Newcastle disease virus effectively induces sustained remission of malignant pleural mesothelioma. Mol Cancer Ther 2010;9(10):2761-9

60. Merigan TCDCE. Finkelstein MS, Clever L, et al. Clinical studies employing interferon inducers in man and animals. Ann N Y Acad Sci 1970;173:746-59

61. Csatary LK, Eckhardt S, Bukosza I, et al. Attenuated veterinary virus vaccine for the treatment of cancer. Cancer Detec Prev 1993;17:619-27

• **An interesting clinical study in which NDV is applied to cancer patients by inhalation to achieve effects, in particular against lung metastases.**

62. Freeman AI, Zakay-Rones Z, Gomori JM, et al. Phase I/II trial of intravenous NDV-HUJ oncolytic virus in recurrent glioblastoma multiforme. Mol Ther 2006;13(1):221-8

63. Hotte SJ, Lorence RM, Hirte HW, et al. An optimized clinical regime for the oncolytic virus PV701. Clin Cancer Res 2007;13(3):977-85

64. Cassel WA, Murray DR, Torbin AH. Viral oncolysate in the management of malignant melanoma. I. Preparation of the oncolysate and measurement of immunological responses. Cancer 1977;40(2):672-9

65. Cassel WA, Murray DR. A ten-year follow-up on stage II malignant melanoma patients treated post-surgically with Newcastle disease virus oncolysate. Med Oncol Tumor Pharmacother 1992;9(4):169-71

66. Batliwalla FM, Bateman BA, Serrano D, et al. A 15-year follow-up of AJCC stage III malignant melanoma patients treated postsurgically with Newcastle disease virus (NDV) oncolysate and determination of alterations in the CD8+ T cell repertoire. Mol Med 1998;4(12):783-94

67. Plager C, Bowen JM, Fenoglio C, et al. Adjuvant immunotherapy with Newcastle disease virus oncolysate of M.D. Anderson (MDAH) stage III-B malignant melanoma. Proc Am Soc Clin Oncol 1986;5:137a

68. Voit C, Kron M, Schwurzer-Voit M, Sterry W. Intradermal injection of Newcastle disease virus-modified autologous melanoma cell lysate and interleukin-2 for adjuvant treatment of melanoma patients with resectable stage III disease. J Dtsch Dermatol Ges 2003;1(2):120-5

69. Kirchner HH, Anton P, Atzpodien J. Adjuvant treatment of locally advanced renal cancer with autologous virus-modified tumor vaccines. World J Urol 1995;13(3):171-3

70. Khazaie K, Prifti S, Beckhove P, et al. Persistence of dormant tumor cells in the bone marrow of tumor-cell vaccinated mice correlates with long-term immunological protection. Proc Natl Acad Sci USA 1994;91(16):7430-4

• **This study demonstrates the importance of tumor cell viability for immunogenicity. In addition, it directs the attention to the ear pinna as an optimal site for immunization and to the bone marrow as a site for tumor dormancy and immune control by memory T cells.**

71. Schirrmacher V, Schild HJ, Gückel B, von Hoegen P. Tumour-specific CTL response requiring interactions of four different cell types and recognition of MHC class I and class II restricted tumour antigens. Immunol Cell Biol 1993;71:311-26

72. Schild H, von Hoegen P, Schirrmacher V. Modification of tumor cells by a low dose of Newcastle disease virus. II. Augmented tumor-specific T cell response as a result of CD4+ and CD8+ immune T cell cooperation. Cancer Immunol Immunother 1989;28:22-8

73. von Hoegen P, Zawatzky R, Schirrmacher V. Modification of tumor cells by a low dose of Newcastle disease virus. III. Potentiation of tumor specific cytolytic T cell activity via induction of interferon-alpha/beta. Cell Immunol 1990;126(1):80-90

• **This study demonstrates the important role of type I IFNs for adaptive anti-tumor immune responses.**

74. Heicappell R, Schirrmacher V, von Hoegen P, et al. Prevention of metastatic spread by postoperative immunotherapy with virally modified autologous tumor cells. I. Parameters for optimal therapeutic effects. Int J Cancer 1986;37:569-77

• **This early study investigated the efficiency of the vaccine ATV-NDV as a postoperative secondary prevention method.**

75. Schirrmacher V, Haas C, Bonifer R, et al. 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

76. Schirrmacher V, Ahlert T, Pröbstle T, et al. Immunization with virus-modified tumor cells. Semin Oncol 1998;25:677-96

77. Sallusto F, Geginat J, Lanzavecchia A. Central memory and effector memory T cell subsets: function, generation, and maintenance. Ann Rev Immunol 2004;22:745-63

78. 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;13:117-30

79. Ockert D, Schirrmacher V, Beck N, et al. Newcastle disease virus-infected intact autologous tumor cell vaccine for adjuvant active specific immunotherapy of resected colorectal carcinoma. Clin Cancer Res 1996;2(1):21-8

80. Schlag P, Manasterski M, Gerneth T, et al. Active specific immunotherapy with Newcastle-disease-virus-modified autologous tumor cells following

resection of liver metastases in colorectal cancer. First evaluation of clinical response of a phase II-trial. Cancer Immunol Immunother 1992;35(5):325-30

81. Schulze A, Kemmner W, Weitz J, et al. Efficiency of adjuvant active specific immunization with Newcastle disease virus modified tumor cells in colorectal cancer patients following resection of liver metastases: results of a prospective randomized trial. Cancer Immunol Immunother 2009;58(1):61-9

• **This paper reports from the only prospective-randomized controlled clinical study involving NDV. Long-term clinical benefit is seen for stage IV colon but not rectum carcinoma patients.**

82. Möbus V, Horn S, Stöck M, Schirrmacher V. Tumor cell vaccination for gynecological tumors. Hybridoma 1993;12(5):543-7

83. Pomer S, Thiele R, Staehler G, et al. Tumor vaccination with and without adjuvant interleukin 2 in renal cell carcinoma. A clinical contribution to the development of effective specific immunization. Urologe 1995;34(3):215-20

84. Karcher J, Dyckhoff G, Beckhove P, et al. Antitumor vaccination in patients with head and neck squamous cell carcinomas with autologous virus-modified tumor cells. Cancer Res 2004;64:8057-61

85. Steiner HH, Bonsanto MM, Beckhove P, et al. Antitumor vaccination of patients with glioblastoma multiforme: a pilot study to assess feasibility, safety, and clinical benefit. J Clin Oncol 2004;22:4272-81

• **This study is exceptional with regard to sophistication in preparation and characterization of the vaccine and with regard to the immunological response monitoring.**

86. Liang W, Wang H, Sun TM, et al. Application of autologous tumor cell vaccine and NDV vaccine in treatment of tumors of digestive tract. World J Gastroenterology 2003;9(3):495-8

87. Fournier P, Schirrmacher V. Randomized clinical studies of anti-tumor vaccination: state of the art in 2008. Exp Rev Vaccines 2009;8(1):1-16

88. Hoover HC Jr, Brandhorst JS, Peters LC, et al. Adjuvant active-specific immunotherapy for human colorectal cancer: 6,5-year median follow-up of a phase III prospectively randomized trial. J Clin Oncol 1993;11(3):390-9

89. Vermorken JB, Claessen AM, van Tinteren H, et al. Active specific immunotherapy for stage II and III colon cancer: a randomized trial. Lancet 1999;353(9150):345-50

90. Nava S, Lisini D, Pogliani S, et al. Safe and reproducible preparation of functional dendritic cells for immunotherapy in glioblastoma patients. Stem Cells Transl Med 2015. [Epub ahead of print]

91. Zaslavsky E, Hershberg U, Seto J, et al. Antiviral response dictated by choreographed cascade of transcription factors. J Immunol 2010;184:2908-17

• **This study from systems biology is recommended because it explains reprogramming of DC function following infection by NDV.**

92. 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(Suppl 1):S111-22

93. Bai L, Koopmann J, Fiola C, et al. Dendritic cells pulsed with viral oncolysates potently stimulate autologous T cells from cancer patients. Int J Oncol 2002;21:685-94

94. Wenner CA, Guler ML, Macatonia SE, et al. Roles of IFN-a and IFN-ß in IL-12-induced T helper-1 development. J Immunol 1996;156:1442-7

95. Schirrmacher V, Stücker W, Lulei M, et al. Long-term survival of a breast cancer patient upon immune and virotherapy: a case report. Immunother 2015; Epub ahead of print

96. Mitchell DA, Batich KA, Gunn MD, et al. Tetanus toxoid and CCL3 improve dendritic cell vaccines in mice and glioblastoma patients. Nature 2015;519:366-9

• **This study describes an interesting conditioning effect of Th cells upon recruitment to a DC vaccination site.**

97. Donnelly O, Ilett E, Kottke T, et al. Cytokine-enhanced intravenous oncolytic virotherapy. Lancet 2014;383:S42

98. Altomonte J, Marozin S, Schmidt RM, Ebert O. Engineered Newcastle disease virus as an improved oncolytic agent against hepatocellular carcinoma. Mol Ther 2010;18:275-84

99. Zamarin D, Martinez-Sobrido L, Kelly K, et al. Enhancement of oncolytic properties of recombinant Newcastle disease virus through antagonism of cellular innate immune responses. Mol Ther 2009;17:697-706

100. Wu Y, Zhang X, Wang X, et al. Apoptin enhances the oncolytic properties of Newcastle disease virus. Intervirology 2012;55:276-86

101. Janke M, Peeters B, Zhao H, et al. Activation of human T cells by a tumor vaccine infected with recombinant Newcastle disease virus producing IL-2. Int J Oncol 2008;33:823-32

102. Janke M, Peeters B, deLeeuw O, et al. Recombinant Newcastle disease virus (NDV) with inserted gene coding for GM-CSF as a new vector for cancer immunogene therapy. Gene Ther 2007;14:1639-49

103. Niu Z, Bai F, Sun T, et al. Recombinant Newcastle disease virus expressing IL-15 demonstrates promising antitumor efficiency in melanoma model. Technol Cancer Res Treat 2014; Epub ahead of print

104. Susta L, Cornax I, Diel DG, et al. Expression of interferon gamma by a highly virulent strain of Newcastle disease virus decreases its pathogenicity in chickens. Microb Pathog 2013;6:73-83

105. Pühler F, Willuda J, Puhlmann J, et al. Generation of a recombinant oncolytic Newcastle disease virus and expression of a full IgG antibody from two transgenes. Gene Ther 2008;15:371-83

• **This study is remarkable because it shows that NDV can be engineered to produce therapeutic antibodies at the infection site.**

106. Shobana R, Samal SK, Elankumaran S. Prostate-specific antigen-retargeted recombinant Newcastle disease virus for prostate cancer virotherapy. J Virol 2013;87:3792-800

107. Pfirschke C, Schirrmacher V. Cross-infection of tumor cells by contact with T lymphocytes loaded with

Newcastle disease virus. Int J Oncol 2009;34:951-62

- **This study suggests that oncolytic NDV can hitchhike on immune T cells to better reach tumor tissue.**

108. Mader EK, Maeyama Y, Lin Y, et al. Mesenchymal stem cell carriers protect oncolytic measles viruses from antibody neutralization in an orthotopic ovarian cancer therapy model. Clin Cancer Res 2009;15(23):7246-55

109. Castleton A, Dey A, Beaton B, et al. Human mesenchymal stromal cells deliver systemic oncolytic measles virus to treat acute lymphoblastic leukemia in the presence of humoral immunity. Blood 2014;123:1327-35

110. Aigner M, Janke M, Lulei M, et al. An effective tumor vaccine optimized for costimulation via bispecific and trispecific fusion proteins. Int J Oncol 2008;32:777-89

- **This study shows a new way of generating human activated T cells with selective anti-tumor activity without the need of recognizing a tumor-associated antigen. It could be used in case of TAA immune escape variants.**

111. Fournier P, Aigner M, Schirrmacher V. Optimization studies for the coupling of bispecific antibodies to viral anchor molecules of a tumor vaccine. Int J Oncol 2010;37:1203-17

112. Fournier P, Aigner M, Schirrmacher V. Transcriptome analysis and cytokine profiling of naïve T cells stimulated by a tumor vaccine via CD3 and CD25. Int J Oncol 2010;37:1439-52

113. 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

114. 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;46:71-7

- **This clinical study tests the recombinant bispecific fusion protein anti-HN-anti-CD28 upon attachment to the vaccine ATV-NDV. The results suggest that anergized memory T cells from late-stage cancer patients can be reactivated upon strong T-cell costimulation.**

115. Curti BD, Kovacsovics-Bankowski M, Morris N, et al. OX40 is a potent immune-stimulating target in late-stage cancer patients. Cancer Res 2013;73:7189-98

116. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer 2012;12:252-64

117. Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 2010;363:711-23

118. Hamid O, Robert C, Daud A, et al. Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma. N Engl J Med 2013;369:134-44

119. Zamarin D, Holmgaard RB, Subudhi SK, et al. Localized oncolytic virotherapy overcomes systemic tumor resistance to immune checkpoint blockade immunotherapy. Sci Transl Med 2014;6:226-32

- **This is a further example how NDV can overcome immunoresistance of tumor cells.**

120. Schirrmacher V, Bihari AS, Stücker W, Sprenger T. Long-term remission of prostate cancer with extensive bone metastases upon immuno- and virotherapy: A case report. Oncol Letters 2014;8:2403-6

121. Schirrmacher V, Jurianz K, Roth C, et al. Tumor stimulator cell modification by infection with Newcastle Disease Virus: analysis of effects and mechanism in MLT-CML cultures. Int J Oncol 1999;14(2):205-15

122. Zamarin D, Postow MA. Immune checkpoint modulation: rational design of combination strategies. Pharmacol Ther 2015;150:23-32

Affiliation

Volker Schirrmacher
Immunological and Oncological Center (IOZK), Tumor Immunology, Hohenstaufenring 30-32, D-50674 Köln, Cologne, Germany
E-mail: V.Schirrmacher@web.de