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Oncolytic Adenovirus Therapy for Solid Tumors Through Induction of Xenogeneic Rejection

*Preclinical Proof of Concept and Safety for
Adf35(OGN)*

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Abstract

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Cancer immunotherapy has improved the survival for a substantial proportion of cancer patients but for many cancers treatment is still lacking and, hence, there is a great need to further develop cancer immunotherapies for the future benefit of more patients.

Oncolytic adenoviruses with various immunostimulatory transgenes have previously been well tolerated in clinical trials of cancer treatment. They are used both for their oncolytic and immunostimulatory effect but also as delivery platform of transgenes in cancer gene therapy. We have constructed an oncolytic adenovirus, Adf35(OGN), with transgenic expression of alpha-1,3-galactosyltransferase (GGTA1) from *Sus scrofa*, synthesizing the immune stimulatory glycosylation Galactose- α -1,3-galactose (α -gal) and neutrophil activating protein (NAP) from *Helicobacter pylori*, an immunomodulatory protein. α -gal and NAP are potent activators and modulators of the human immune system and have not previously been combined in cancer immunotherapy.

In paper I, Adf35(OGN) was shown to effectively infect human pancreatic tumor cells which further led to expression of α -gal and NAP, antibody opsonization and complement deposition on infected cells, complement and antibody dependent cellular cytotoxicity and activation of various immune cells. Furthermore, when Adf35(OGN) was injected intratumorally in pancreatic tumors in mouse, tumor growth was inhibited and mouse survival improved.

In paper II, a simple qPCR-based assay is presented that can be used to quantify replication competent adenoviruses, accidentally formed during production, in batches of oncolytic adenoviruses intended for use in clinical trials to ensure levels below acceptable limits.

In paper III, to evaluate the safety of Adf35(OGN), biodistribution and toxicity was studied in Syrian hamster and mouse. The viral treatment was well tolerated without treatment-related toxicity or viral replication in tissues and the shedding of virus to the environment was sparse.

In paper IV, various enzymes and polycations were evaluated as vehicles for intratumoral injections of oncolytic adenoviruses. Hyaluronidase tripled viral transduction and may be considered to improve the treatment efficacy of oncolytic viruses.

In conclusion, the preclinical efficacy and safety results presented in the thesis encourage future clinical trials with Adf35(OGN).

Keywords: Oncolytic adenovirus, cancer immunotherapy, cancer gene therapy, solid tumors, xenogeneic rejection, α -gal, Galactose- α -1, 3-galactose, neutrophil activating protein, NAP, *Helicobacter pylori*, replication competent adenoviruses, RCA, Adf35(OGN), toxicity, biodistribution, Hyaluronidase

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Till de som lever på hoppet

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

- I. Arwa Ali, Erik Yngve, Menghan Gao, Paola Contreras, Olle Korsgren, Magnus Essand¥, Chuan Jin¥, Di Yu¥. Oncolytic adenovirus therapy for pancreatic cancer through induction of immunogenic rejection. Manuscript
- II. Menghan Gao*, Erik Yngve*, Di Yu, Chuan Jin. A qPCR-Based Method for Quantification of RCA Contaminants in Oncolytic Adenovirus Products. *Front Mol Biosci* (2022)
- III. Erik Yngve, Malin Eriksson, Anders Hedin, Arwa Ali, Olle Korsgren, Di Yu. Biodistribution and toxicity evaluation of oncolytic adenovirus Adf35(OGN) in Syrian hamster and mouse. *Cancer Gene Ther* (2025).
- IV. Erik Yngve, Sofie Ingvast, Olle Korsgren, Di Yu. Evaluation of drug delivery vehicles for improved transduction of oncolytic adenoviruses in solid tumor tissue. *Uppsala Journal of Medical Sciences* (2025).

* Equal contribution – first author

¥ Equal contribution – last author

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Abbreviations

41BB	Tumor necrosis factor receptor superfamily member 9
α -gal (Gal)	galactose- α -1,3-galactose
ADCC	Antibody dependent cellular cytotoxicity
ADCP	Antibody dependent cellular phagocytosis
ADL	Activities of daily living
AIRE	Autoimmune regulator
ATP	Adenosine triphosphate
CAR	Chimeric antigen receptor
CD	Cluster of differentiation
CDC	Complement dependents cellular cytotoxicity
CTLA4	Cytotoxic T lymphocyte associated protein 4
CTCAE	Common terminology criteria for adverse events
DAMP	Damage associated molecular pattern
DEAE	Diethylaminoethyl
DTH	Delayed type hypersensitivity
EGFR	Endothelial growth factor receptor
EMA	European medicines agency
FDA	U.S. Food and drug administration
GGTA	Glycoprotein alpha-galactosyltransferase
GM-CSF	Granulocyte-monocyte colony-stimulating factor
GMP	Good manufacturing practice
HER2	Human epidermal growth factor receptor 2
HLA	Human leucocyte antigen
ICAM	Intercellular adhesion molecule
IFN- γ	Interferon gamma
Ig	Immunoglobulin
IL	Interleukin
iRECIST	immune Response evaluation criteria in solid tumors
LAG3	Lymphocyte activation gene 3
MHC	Major histocompatibility complex
MALT	Mucosa associated lymphoid tissue
NAP	Neutrophil activating protein of Helicobacter pylori
NK	Natural killer
OX40	Tumor necrosis factor receptor superfamily member 4
PD1	Programmed Cell Death Protein 1

PDAC	Pancreatic ductal adenocarcinoma
PEI	Polyethyleneimine
qPCR	Quantitative polymerase chain reaction
RAG	Recombination activating gene
RCA	Replication competent adenovirus
TCR	T cell receptor
TGF- β	Tumor growth factor β
TIM3	T-cell immunoglobulin mucin 3
TNF- α	Tumor necrosis factor α
VP	Viral particle

Introduction

Cancer and the immune system

In the last decade, cancer immunotherapy has improved the prognosis for malignancies such as hematological cancers, malignant melanoma and renal cancer¹⁻³. Still, many patients with these diagnoses do not show long lasting responses³ and for many other types of cancer, efficient treatments are lacking. Hence, there is a great need to further develop cancer immunotherapies for the future benefit of more patients.

The idea that our immune system can identify and eliminate tumor cells have been debated since the 1950s when Macfarlane Burnet and Lewis Thomas presented the concept of immunosurveillance^{4,5}. They suggested that the adaptive immune system should be able to identify tumor cells due to their expression of new tumor specific antigens. An accumulation of tumor cells could thus provoke an effective immunologic reaction that should eliminate the tumor without any clinical hint of its existence. In a Darwinistic manner, tumor cell clones that evade immune destruction will be naturally selected and develop into clinically meaningful tumors.

Evidences of immunosurveillance have been retrieved from mouse studies in which mouse strains that lack critical functions of the adaptive immune system, such as knockout of genes encoding IFN- γ ⁶, recombination activating genes⁶ (RAG 1 and RAG2) or perforin⁷, show an increased incidence of tumors, both spontaneous occurrence and increased sensitivity to tumor induction by carcinogenic chemicals.

In humans, some populations with post transplantation immunosuppression or primary immunodeficiencies have had increased incidence of certain tumor types, such as lymphomas, gastric cancer and cancers of the oropharyngeal and uro-ani-genital tracts, indicating a tumor protective role of the immune system⁸. In addition, presence of tumor infiltrating lymphocytes in tumors is associated with longer survival⁹⁻¹³ indicating that the immune system prohibits tumor growth.

However, the increased tumor incidence in immunocompromised individuals may be explained by increased susceptibility for chronic infections, which also is known to drive tumor development⁸. Additionally, common tumor types such as colon-, lung-, breast- and prostate cancer, are not more common in immunocompromised populations¹⁴.

Regardless how many potential tumors our immune system manage to eliminate in an early stage, it is a fact that tumors do develop in immunocompetent individuals. Interestingly, it is shown that tumors formed in immunocompetent wild type mouse grow more rapid than tumors formed in RAG2^{-/-} mouse lacking adaptive immune system, if these tumors are taken out from their original host and transplanted to a secondary immunocompetent host⁶. This shows that tumors that develop in immunocompetent hosts acquire properties that allow them to avoid attack by the immune system.

This dynamic relationship between the tumor and the immune system was further described and developed by Dunn et al. 2002¹⁵. They described the course of tumor development with three phases: *elimination*, *equilibrium* and *escape*. *Elimination* is the functional antitumoral phase including recognition and elimination of tumor cells through specific and effective immune responses. The anti-tumor immune response will be discussed in detail bellow. *Equilibrium* occurs when some tumor cells gain new mutations that prevent immediate immune destruction. At this phase the immune system likely provides the tumor with both inhibitory and supportive signals which prohibit the tumor from further growth but yet allows its persistence. Over time, often many years, further mutations decrease tumor cell immunogenicity and long-term exposure to weakly immunogenic tumor antigens induces immune tolerance mechanisms. This leads to the final phase, *escape*, where the tumor cells are free to proliferate and form a clinically meaningful malignancy.

Thus, in established tumors, effective antitumoral immune responses are no longer in place. Some tumors almost totally lack infiltrating immune cells indicating very low immunogenicity. Others have large numbers of tumor reactive infiltrating lymphocytes that, however, are inactive due to immune checkpoint molecules expressed by the tumor cells. In other cases, the infiltrating immune cells are not tumor reactive but rather immunosuppressive and tumor supportive. The recent successes of immunotherapy, however, have shown that it possible to restore anti-tumoral immunity and that this can be an effective cancer treatment.

The cancer immunity cycle (Figure 1), presented by Chen and Mellman in 2013¹⁶, is a seven-step model for the establishment of affective anti-tumoral immunity. These steps are probably in place during the previously mentioned elimination phase but for various reasons become disturbed during the course of cancer development. Regaining efficacy in the cancer immunity cycle is the ultimate goal of immunotherapy and several ways to re-elicite effective immune responses have been proposed. So far, however, only a limited portion of patients benefit from cancer immunotherapies, highlighting the need for novel immunotherapeutic strategies.

Here, I review the most important steps involved in an anti-tumor immune response and how these steps can be boosted by a new oncolytic adenovirus, Adf35(OGN), expressing an immune stimulatory antigen and an immune modulatory protein which may boost all steps of the cancer immunity cycle.

The work aims to show that this virus is functional and safe, with the goal to proceed into a clinical trial.

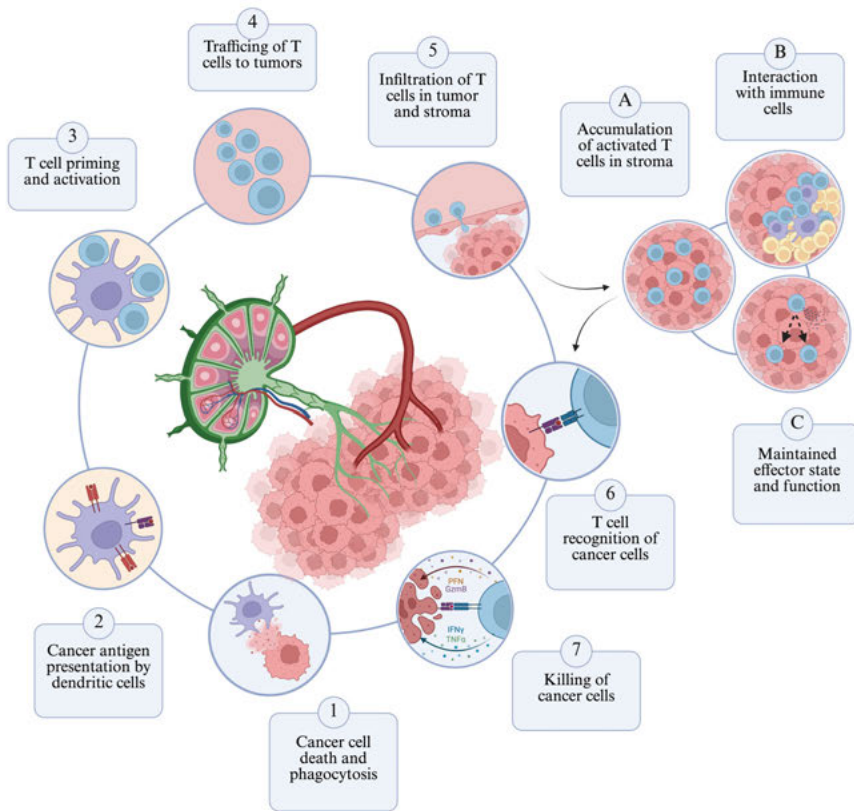


Figure 1. The cancer immunity cycle as presented by Mellman et al. 2013 (1-7) and updated with the cancer immunity sub-cycle in 2023 (A-C). The seven steps and three sub-cycle steps describe the naturally occurring immunity against tumor cells that form the basis for cancer immunosurveillance. In every case of clinically meaningful tumors one or several steps have become dysfunctional and need to be boosted in order to regain an effective antitumor immune response. The figure was created with Biorender.com.

Tumor specific antigens, innate immunity and antigen presentation

There is a long list of known antigens that are expressed in many malignant cells, since they are critical for the tumor development¹⁷. They are often the result of mutations in certain oncogenes and tumor suppressor genes. These antigens, known as tumor specific antigens provides a possibility for our

immune system to specifically recognize and eliminate tumor cells. The cancer immunity cycle describes this process.

The cancer immunity cycle is initiated by the recruitment of phagocytosing and antigen presenting cells to the tumor site. These are for example dendritic cells and macrophages that are part of the innate immune system and by so recognize general patterns of microbes and infected, damaged or dying cells. Notably, they do not recognize tumor specific antigens. The antigen presenting cells are attracted to the tumor by chemokines released from damaged or dying tumor cells or other surrounding immune cells from the innate immune system. When stimulated by general danger associated molecular patterns (DAMPs) which are also released from damaged or dying tumor cells, such as calreticulin, antigen presenting cells phagocytose tumor cells and undergo maturation^{18,19}. Mature dendritic cells migrate to draining lymph nodes and process and load tumor antigens on major histocompatibility complex (MHC), which is presented on the cell surface. MHC in humans is also referred to as human leucocyte antigen (HLA).

The presentation of antigens on MHC class II and presentation of internalized antigens on MHC class I, known as cross presentation, are unique features of antigen presenting cells, such as dendritic cells, and necessary to stimulate an adaptive immune response against specific antigens e.g. tumor specific antigens. Tumor specific antigens presented on MHC class II may be recognized by the T cell receptor (TCR) of CD4⁺ T cells, known as helper T cells. If the affinity is strong enough, this interaction together with co-stimulatory signals primes the helper T cell. The presentation of tumor antigens on MHC class I may be recognized by the TCR of CD8⁺ T cells, known as cytotoxic T cells. This interaction is critical for the priming of cytotoxic T cells and the formation of a T cell based antitumoral immune response, which will be described below.

Antigen presentation and cross presentation can be stimulated by various factors. Firstly by factors in the environment in which the antigen is encountered, such as DAMPs including for example calreticulin and ATP^{18,19}, released from dying tumor cells. Secondly the interaction with antigen reactive helper T cells stimulate or “license” the dendritic cell to increase especially cross presentation.

Apart from priming antigen-specific T cells in the lymph node, dendritic cells also have an important function in the tumor site where they activate primed T cells by secretion of IL-12. This secretion is in turn stimulated by IFN- γ secreted by the T cells, leading to a positive feedback loop, amplifying the immune response²⁰.

In summary, dendritic cells’ presentation of tumor antigens to the adaptive immune system is a fundamental step in the tumor immune response. The existence of tumor specific antigens is uncontroversial but they are often weakly immunogenic, scantily expressed and unstable molecules. Moreover, the human HLA repertoire is wide and individual and all types of peptides

cannot be loaded on all types of HLA, meaning that a particular individual will only be able to present a fraction of the potential tumor antigens to her/his adaptive immune system²¹. Therapies that improve tumor antigen presentation are important for a broader efficacy of immunotherapy, but today, no such therapies are yet used in clinical routine.

Early T cell development

T cells are small leucocytes with ability to recognize foreign antigens through their TCRs which are unique for each T cell clone. When activated, they are the most effective and accurate killers of the immune system due to their ability to induce apoptosis with single-cell precision. T cell development have been carefully described previously²² and is summarized in the following paragraphs. Aspects of importance for tumor immunology are highlighted.

T cells originate in the bone marrow but most of their early development take place in the thymus. After commitment to the T cell lineage the T cell undergo gene rearrangements encoding the variable parts of the TCR. This results in a great variation in TCR affinities and an individual's TCR repertoire ranges from 1×10^5 to 1×10^8 different possible receptors, with affinity for different antigens²³.

TCRs are supposed to recognize foreign antigens when those antigens are presented on MHC. To select appropriate T cell clones, there is a positive selection for T cells whose TCRs engage self-MHC. Those that do not have any affinity for MHC will not receive survival signals but will undergo apoptosis. This is followed by negative selection against T cells whose TCRs binds too strongly to MHC presenting self-peptides. This generates T cell clones which recognize peptides only when combined with self-MHC (MHC-restriction) but do not initiate a response to the self-peptides (self-tolerance). This self-tolerance mechanism, known as central tolerance depends on the presentation of self-peptides in the thymus. It is executed by medullary thymic epithelial cells expressing the protein autoimmune regulator (AIRE)²⁴. These cells has the unique ability to access and express the whole coding genome and is thus able to express proteins that normally is expressed only by certain specialized tissues, such as insulin or myelin basic protein²⁴.

The tiny proportion of T cell progenitors that survive positive and negative selection, about 2-5%, further commit to effector cell lineages of either CD4⁺ helper T cells or CD8⁺ cytotoxic T cells. In some cases, T cells with high affinity for MHC presenting self-peptides survive negative selection. These cells then differentiate into regulatory T cells, that exerts anti-inflammatory functions and contribute to maintained self-tolerance²⁵. These “educated” but still naive T cells then leave the thymus and migrate to lymph nodes.

Somatic mutations in tumors encoding tumor specific antigens cannot be expressed by medullary thymic epithelial cells, thus preventing tumor

tolerance. Unfortunately though, medullary thymic epithelial cells do express several antigens that also are expressed by tumor cells, for example various melanoma antigens^{26,27}. Therefore, tumor reactive T cells may, after strong affinity binding to antigens presented in the thymus, differentiate into regulatory T cells that migrate to the tumor and contribute to establishing an immunosuppressive tumor microenvironment. In various mouse models, negative regulation of AIRE have led to increased immune responses against melanoma and prostate cancer²⁷⁻²⁹.

Out of all T cell clones that are generated during the slow and gradual development of a tumor, certainly some are able to recognize aberrant tumor specific antigens. Further strictly regulated steps are, however, necessary to make these T cells ready to kill tumor cells. Those steps and the problems around them are discussed below.

Priming of helper T cells

Naïve T cells leave the thymus and migrate to peripheral lymph nodes where they may come in first contact with antigens. If a helper T cell has affinity for an antigen, presented on MHC class II of a dendritic cell, that stimulates an activating signal mediated by the TCR, co-receptor CD4 and intracellular CD3 domain. This signal, known as “signal one” in the T cell priming, initiates the formation of a synapse between the T cell and the dendritic cell. If sufficient amounts of antigen are presented the synapse will grow stronger. 8000 interactions are needed to prime the T cell only through the TCR stimulation. Co-stimulatory signals reduce this number to about 1500 interactions³⁰, and thus constitute a crucial contribution to the priming, called “signal two”. It can be mediated by various receptors on the T cell, CD28 binding to CD80 and CD86 on the dendritic cell, being the most typical.

Autocrine and paracrine signaling through IL-2, IL-4, IL-12, TGF- β and TNF- α constitute “signal three”. The activation of the three signals leads to proliferation and depending on various location- and context dependent factors the helper T cell differentiate into various phenotypes (Th1-Th22) with different function for the further immune response. Interferon gamma, IL-12, and IL-18 stimulate differentiation into Th1 phenotype that is pro-inflammatory and promote cytotoxic T cell responses which can combat intracellular pathogens such as viruses and intracellular bacteria. IL-4 stimulates differentiation to Th2 phenotype that mediate anti-inflammatory functions but also may stimulate a class switch to IgE antibodies that are important for the clearance of extracellular pathogens and play a central role in allergic disease. TGF-beta and IL-6 stimulates differentiation to Th17 phenotype which is important for fungal and extracellular bacterial infections, but also contributes to chronic inflammation and autoimmunity.

Since tumor antigens are mainly intracellular, Th1 is the preferred phenotype to orchestrate an effective tumor immune response. Th1-cells express CD40L which in turn stimulate dendritic cells to differentiate into a corresponding “type one” phenotype. They exert increased cross presentation of tumor antigens on MHC class I, expression of B7 family ligands, CD70 and secretion of IL-2, all of which can stimulate cytotoxic T cells and contribute to their priming, which will be discussed below.

Additionally, CD40L signaling from T cells to B cells leads to B cell differentiation, affinity maturation and class switch and thus enables a humoral antitumoral response. Lastly, Th1-cells can have direct anti-tumoral effect in the tumor site by secretion of TNF- α and IFN- γ . If stimulated by IL-12, secreted by dendritic cells, they could also gain cytotoxic properties²⁵.

In summary, the priming of helper T cells and Th1 differentiation are important to elicit effective antitumoral immune responses.

Priming of cytotoxic T cells.

The priming of cytotoxic T cells is similar to the priming of helper T cells. It takes place in lymph nodes when naïve cytotoxic T cells come in contact with licensed dendritic cells. A requirement for this contact is that the dendritic cell is licensed and, by so, presents tumor antigens on MHC class I, on which normally only self-antigens are presented. This mechanism ensures that two different TCRs, one from the helper T cell and one from the cytotoxic T cell, independently of each other have affinity for the antigen and thus defines it as “non-self” before a cytotoxic T cell response is initiated.

The initial signal from the TCR must be followed by two co-stimulatory signals in order for the cytotoxic T cell to be fully primed. The need for second signals protects the T cell from reacting on self-antigens and several other mechanisms have developed to limit T cell responses to its primary purpose, to defeat foreign invaders. As previously described for helper T cells, the second signal generally lowers the stimulation threshold of the naïve cytotoxic T cell and induces proliferation, promotes survival and activates cytokine synthesis, especially IL-2, and the cytotoxic function³¹. It can be mediated by CD28 on the T cell binding to CD80 or CD86 expressed by dendritic cells, macrophages, B cells or tumor cells. Other stimulatory signals can be mediated by CD40L, 4-1BB and OX40 on the cytotoxic T cell³², that may bind to CD40, 4-1BBL, and OX40L on licensed dendritic cells, T-helper cells or B cells³³. The third signal is mediated by autocrine and paracrine cytokine signaling, mainly IL-2 and IL-12.

In summary, the T cell priming, proliferation, and survival that follows stimulation of the T cell is controlled by several factors including the strength of TCR stimulation, the presence or absence of co-stimulatory signals and the availability of pro-survival cytokines. Thus, effective T cell priming can only

be acquired if the previous steps of the cancer immunity cycle are established in a favorable way, indicating that immune therapy development need to focus on the optimization of these early steps.

Cytotoxic T cell activation and inhibition

Following the three-step priming, effector T cells proliferate at a speed close to the physiological maximum of mammalian cells. They express various molecules for endothelial cell adhesion and extravasation. At the site of a local inflammation the endothelial cells of post-capillary venules express adhesion molecules in response to cytokines like TNF- α and IL-1 β . The T cell to endothelial cell interactions facilitate trans endothelial migration³⁴. In the tumor microenvironment, the primed T cell may come in contact with its target antigen and become activated. If a primed T cell experience TCR stimulation, a contact is formed to the target cell and perforins, granzymes and several other factors induce tumor cell apoptosis and lysis. Most effector cells are programmed to undergo apoptosis and thus terminate the immune response, leaving only around 5 % of the cells to develop into memory T cells³⁵.

In cases when all antigen cannot be cleared, which is common for tumor antigens, the initial T cell response is prolonged. However, then peripheral self-tolerance mechanisms are initiated to avoid self-reactivity. This involves upregulation of immune-checkpoint receptors such as PD1, CTLA4, LAG3 and TIM3 on T cells³⁶. PD1 and CTLA4 inhibit CD28-CD80/86 signaling, while LAG3 reduce TCR signaling and TIM3 antagonize T cell factor 1, a transcription factor that maintain stemness and T cell effector function³⁶. This result in T cell anergy, exhaustion and apoptosis. Long term, weakly immunogenic antigen exposure also tends to alter the phenotypes of other immune cells. Regulatory T cells, tumor associated macrophages and myeloid derived suppressor cells contribute to peripheral tolerance and tissue recovery, but unfortunately also tumor cell growth and protection.

The up regulation of immunological check point molecules and further T cell exhaustion eventually occur in almost all tumors experiencing a prolonged T cell driven attack. Antibodies that block these molecules, immune checkpoint inhibitors, have clearly revolutionized the treatment of some tumor types and is the first intervention in the cancer immunity cycle that actually is working as a cancer treatment. Their application and limitations are discussed below.

Activation of B cells and humoral response

B cells are largely overlooked in the development of cancer immunotherapy but may have a crucial role to broaden the anti-tumor immune response and increase antitumoral effector functions. Basic B cell development will be presented as well as their potential role in cancer immunotherapy.

B cells develop in the bone marrow where they undergo gene rearrangements that makes them express membrane bound immunoglobulins with affinities of great variation. Certain dendritic cells present self-antigens in the bone marrow, and B cells that produce immunoglobulins that bind to these antigens receive apoptotic signals, protecting the individual from self-reactive antibodies.

Immature B cells then move to secondary lymphoid structures where they encounter non-self-antigens presented by dendritic cells. This could be processed peptides presented on MHC class II but also unprocessed proteins bound to the dendritic cell surface. B-cells may also encounter free antigens in the circulation and various tissues they infiltrate.

When a B cell immunoglobulin binds an antigen with a certain affinity it results in a first activating signal that transfer the cell into active cell cycle. A second signal is needed, however, to exert proliferation of the B cell and class switch of the immunoglobulin. To get this signal, the B cell must internalize the encountered antigen and present it on MHC class II. If the same antigen also is recognized by a helper T cell a contact will be formed and signal two will be exerted through CD40-CD40L, IL-4 and IL-21. In some cases, the second signal may be independent of the helper T cell and instead originates from toll like receptors that recognize features of large extracellular antigens.

Following signal two, the B cell proliferate and undergo class switch and affinity maturation. Depending on the co-stimulatory signals the B cell will differentiate to produce different types of immunoglobulins. In the defense against microbes, IgG is important to neutralize viruses or bacteria by binding to them and prohibit them from infecting cells. The coating of tumor cells by IgG can stimulate various cytopathic mechanisms, such as phagocytosis through Fc receptor interaction with macrophages, activation of natural killer cells and complement factors.

As previously mentioned, tumor antigens are mainly intracellular and the role of antibody-mediated reactions in antitumoral immunity is not as prominent as T cell immunity. However, broadening the immune response to include humoral effector mechanisms is likely crucial for the success of immunotherapy in a wider range of tumor types. Monoclonal antibodies directed against various tumor antigens such as EGFR and HER2 are already established therapies. The mechanism of action have been debated³⁷ but is considered a combination of blocked receptor signaling on one hand and antibody dependent cellular cytotoxicity (ADCC), by NK cells and antibody dependent cellular phagocytosis (ADCP) by macrophages³⁷ on the other hand.

The success of these therapies in solid tumor types with upregulated EGFR and HER2 expression highlights the potential in developing other strategies to acquire substantial tumor specific opsonization by antibodies. This is one of the proposed mechanisms of action in treatment with our new oncolytic adenovirus Adf35(OGN) and will be discussed below.

Immunotherapies in clinical routine

Only one type of immunotherapy with intention to modulate the tumor immunity cycle have so far been implemented in routine cancer therapy. Checkpoint inhibitors, blocking the immune checkpoint interactions, have been a successful strategy to regain cytotoxic T cell function in tumors with high numbers of infiltrating lymphocytes³⁸. Such immune infiltration occurs in tumors with high mutational burden and thus a high number of tumor-specific antigens. Examples are malignant melanoma³⁹, renal adenocarcinoma, certain types of lung cancer, and some cases of gastrointestinal cancer with miss match repair mutations and microsatellite instability causing high mutational burden⁴⁰. Cases that respond are those where an appropriate anti-tumor immune response have been established but where the last effector step, T cell mediated tumor cell killing, have been inhibited by peripheral tolerance mechanisms. However, the need for a pre-existing antitumoral T cell response excludes many immunologically cold tumor types from responding to this therapy.

There are a few other types of established “immune”-therapies that circumvent the delicate fine tuning of tumor rejection and self-tolerance. These therapies do not affect the host immune system but instead use engineered immunological tools to target various tumor associated antigens, abundantly expressed on the tumor cells and on healthy cells from which the tumor originates.

Chimeric antigen receptor (CAR)-T cells targeting the B cell antigens CD19 or CD20 are effective in recurrent B cell lymphoma. The treatment leads to depletion of the whole B cell population and chronic B cell deficiency is common, with increased risk of infections. Overall, though, these side effects are manageable.

Bispecific antibodies are used to connect T cells to tumor cells. One part binds a tumor associated antigen e.g. CD19 and the other part binds the CD3 domain of the TCR. This signal can lead to activation of T cells close to tumor cells without need for tumor antigen recognition^{41,42}.

The success of CAR-T cells and bispecific antibodies is so far unfortunately limited to hematological malignancies and lymphomas in which targetable cell-lineage antigens are abundantly expressed on both tumor and healthy cells. For solid tumors, it is much more difficult to find tumor associated antigens that can be targeted with therapies. Either they are not

expressed in sufficient abundance on the tumor cells or they are expressed in too high abundance on normal cells. In both cases targeting such antigens will be ineffective and cause side effects.

In conclusion, the established immunotherapies of today have clear limitations and mainly target the last step of the anti-tumor immune response, the cytotoxic T cell effector function. Currently, efforts are made to boost earlier phases in the cancer immunity cycle.

Ongoing immunotherapy development

Cancer vaccines

The idea to identify tumor specific neoantigens and immunize the patient against them is appealing. As mentioned above, there are several known tumor specific antigens that are expressed in many different types of cancer cells. Additionally, individualized mapping of tumor specific neoantigens can be made by sequencing and comparing a patient's genome to the tumor cell genome and mRNA-expression. Different strategies have been proposed to present these antigens to the immune system. mRNA and DNA based cancer vaccines can elicit *in vivo* expression of selected tumor antigens in a similar manner as viral antigens when used for Covid-19 vaccination. Results from clinical trials utilizing single antigens have not shown clinical benefit⁴³. Consequently, ongoing studies include several antigens in the same product, in most cases tailored to the patient's own tumor antigen profile. Treatment with multiple antigens after resection of melanoma⁴⁴ and pancreatic ductal adenocarcinoma (PDAC)⁴⁵ have led to reduced recurrence rate/prolonged time to recurrence in "responders" in whom the treatment generated tumor neoantigen specific T cells. In reported clinical phase III studies, only one product have been tested, with favorable safety profile but without therapeutic efficacy in esophageal squamous cell carcinoma⁴⁶.

Ex-vivo loading of the patients own tumor antigens on autologous dendritic cells that, after stimulation, are transferred back to the patient is another type of tumor vaccination. The advantage is that the patient's own tumor can be used without need to identify its specific antigens because the whole tumor lysate is used to prepare the dendritic cells. In a recently published phase 3 study in Glioblastoma, this treatment strategy rendered an increased median overall survival from 16 to 19 months⁴⁷.

Oncolytic viruses

Viruses were tested as cancer therapy already in the 1950s and with modern biotechnologies they can be engineered to gain various desired properties. Viral genes affecting the host cell cycle control can be deleted, thus producing

viruses without ability to replicate in normal cells. The failed cell cycle control in tumor cells, however, allows these “oncolytic viruses” to replicate in tumor cells. The viral capsid proteins can be selected to target certain entry receptors with increased expression on tumor cells, thus increasing infectivity of tumor cells while reducing infectivity of normal cells. With these modifications, oncolytic viruses can infect and replicate in tumor cells, while to a large extent sparing healthy cell. Ideally, this leads to selective tumor cell lysis, immunogenic tumor cell death, release of tumor specific antigens, amplification of the initial inoculum, spread of virus to neighboring tumor cells, and stimulation of a cytotoxic T cell response against viral antigens and bystander immunity reaching also tumor antigens.

Despite this theoretically appealing multifaceted mechanism of action, the effect of oncolytic viruses has been marginal. This is due to limited viral transduction efficacy, limited spread within solid tumor tissue and effective immune responses neutralizing injected virus. Thus, to further increase the antitumoral immune response following the treatment, oncolytic viruses can be armed with various transgenes that are expressed in infected tumor cells. This treatment modality can also be seen as cancer gene therapy, in which the oncolytic virus serves as gene delivery platform.

Typically, immune stimulatory transgenes have been used, such as granulocyte-macrophage colony-stimulating factor (GM-CSF) which is the transgene of T-VEC, the only oncolytic virus currently approved by the American Food and Drug Agency (FDA) and European Medicines Agency (EMA), for use in treatment of grade III-IV melanoma⁴⁸. LOAd703 is another oncolytic virus currently in phase II clinical trials, armed with the transgenes CD40L and 4-1BBL. The membrane bound trimerized CD40L stimulate dendritic cell phagocytosis in the tumor site and cross presentation of tumor antigens and T cell priming in the lymph node. The receptor 4-1BBL activate cytotoxic T cells, memory T cell differentiation and inhibit regulatory T cells in the tumor site⁴⁹.

Different types of viruses have been used to generate oncolytic viruses. The most common is adenovirus, which is preferred due to its low pathogenicity and easily editable genome. It is also appreciated for its capacity to deliver and express transgenes in infected cells without integration in the host cell genome.

The clinical experience with oncolytic conditionally replicating adenoviruses is extensive. Since the first studies on ONYX-015 reported in 2000, no deaths related to oncolytic adenovirus treatment have been reported. Adverse events have generally been graded according to the common terminology criteria for adverse events (CTCAE)⁵⁰. Grade 1-2 indicates mild to moderate symptoms, requiring no treatment or only minimal, local or non-invasive intervention. These symptoms may transiently limit activities of daily living (ADL), such as personal hygiene or household activities. Grade 3 indicates severe or medically significant symptoms but not immediately life-

threatening. Hospitalization or prolongation of hospitalization is indicated and the symptoms are disabling and limiting self-care ADL. Grade 4 indicates life-threatening consequences where urgent intervention is required. Grade 5 indicate death related to adverse events.

Grade 3-4 toxicity related to the virus have been mainly fever, lymphopenia and injection site pain. In few cases asymptomatic decreases in blood pressure have been noticed within the first 24 h of intravenous or intraarterial injection⁵¹. Common symptoms, usually of grade 1-2 and resolving within two days, are fever, fatigue, chills, nausea and vomiting^{52,53}. Self-limiting and transient elevation of liver enzymes, alanine aminotransferase, alkaline phosphatase and aspartate aminotransferase, have been observed⁵². Usually the frequency of all treatment emergent adverse events have been highest within 24 h of the first virus infusion and decreased upon subsequent dosings⁵⁴. Since these studies have been performed on patients with advanced malignancies, adverse events have often occurred due to tumor progression⁵⁵ or as effect of concomitant oncological therapy⁵⁶ such as chemotherapy and radiotherapy.

Intratumoral (i.t), intraperitoneal (i.p.), intravenous (i.v.) and intra hepatic artery (i.h.a.) administration have been well tolerated as described above^{51,52,54-91}. Intracranial injections have been associated with neurological symptoms such as seizures, confusion, and increased intracranial pressure⁹²⁻⁹⁶.

In summary, the favorable safety profile of oncolytic adenoviruses makes this tumor selective, oncolytic gene therapy platform very attractive in cancer therapy. The clinical benefits are, however until today, too modest to make this therapy a substantial complement to established treatments. More effective combinations of transgenes and improved transduction efficacy are needed to make the most of the potential in this treatment modality.

Aspects of antimicrobial defense in tumor immunotherapy

As mentioned in the introduction, several factors suppress effective immune responses against tumors. Secretion of IL-35 inhibits initiation of the immune response by prohibiting dendritic cell maturation and make them unable to produce proper secondary signal stimulation to T cells⁹⁷. Cytokines such as IL-4, IL-10 and TGF- β secreted by the tumor cells also have an inhibitory effect on T cells, NK cells and dendritic cells⁹⁸. Over-expression of the endothelin B receptor by tumor endothelial cells inhibits intercellular adhesion molecule (ICAM)-I clustering which in turn reduce T cell infiltration in the tumor⁹⁹. Furthermore, weak immunogenicity of tumor antigens, down regulation of antigen presentation or alterations of the MHC molecules on

tumor cells¹⁰⁰ and upregulation of immune checkpoint ligands such as PD-L1 disable the initial anti-tumor T cell responses from eliminating all tumor cells. Instead, prolonged antigen exposure contributes to turning those T cells into an exhausted, anergic and dysfunctional state¹⁰¹. This leads to an immunosuppressive microenvironment that promote cell growth and angiogenesis in which tumors find their way into a sanctuary of self-tolerance and escape immunosurveillance.

Our immunological defense against microbes may in many cases seem much more effective than that against tumor cells. It typically includes massive expansion of antigen specific T-cells and B-cells, effective microbe clearance and development of functional immunological memory that effectively can clear any rechallenge of the antigen. It is therefore tempting to try to establish similar responses against tumors by using immunotherapy with microbial antigens.

α -gal stimulates the human immune system

An evolutionary event that gave humans a particular advantage in the co-evolution with microbes occurred around 28 million years ago in a common ancestor of apes, old world primates and humans¹⁰². It was an inactivating point mutation in gene *GGTA1* which encodes alpha-1,3-galactosyltransferase, an enzyme with the function to synthesize galactose- α -1,3-galactose (α -gal) which is a disaccharide decorating cellular glycoproteins and glycolipids¹⁰². *GGTA1* had previously been well conserved and α -gal is expressed by almost all mammalian species¹⁰². The mutation made alpha-1,3-galactosyltransferase dysfunctional with a subsequent loss of α -gal expression and in turn loss of α -gal tolerance since carriers of the mutation developed anti- α -gal antibodies¹⁰³. At some point after the mutation occurred the parental GGTA1-WT population was affected by a lethal epidemic, either caused by a virus enveloped in α -gal decorated cell membranes or a bacteria naturally expressing α -gal. Transmission of this pathogen to the GGTA1-mutated population was however stopped by preexisting anti- α -gal-antibodies¹⁰⁴. New world monkeys, in which *GGTA1* is conserved, likely survived the epidemic due to the ocean barrier¹⁰².

In all humans today, anti- α -gal-antibodies are produced from early childhood¹⁰⁵, constitutes about one percent of circulating IgG and is an important part of the intestinal barrier against gut bacteria that express α -gal analogs¹⁰⁶. α -gal have been considered one of the major obstacles for transplantation of animal organs to humans, since the preexisting anti- α -gal-antibodies mediate a hyperacute rejection¹⁰⁷.

The idea to utilize α -gal in cancer immunotherapy have previously been tested with promising results¹⁰⁸⁻¹¹⁰. Tumor cell suspensions were prepared from biopsies and incubated with recombinant bovine α -gal carbohydrates. Adhesion of α -gal on cell membranes was verified by flow cytometry.

Subsequently, the tumor cells were sonicated and mixed with human immunoglobulins to allow formation of α -gal-IgM and α -gal-IgG complexes. Autologous (patient's own) dendritic cells were incubated *in vitro* over night with the tumor cell-lysate and then used to generate and expand tumor specific T cells *in vitro*. After 12 days of co-culture of dendritic cells and T cells the entire cell suspension (up to 6×10^9 cells) were administered intravenously to the patient. Importantly, no serious adverse effects were observed in any of the studies¹⁰⁸⁻¹¹⁰. In all three clinical trials with different types of malignancies a clear delayed type hyperreactivity (DTH) response evolved and in parallel, quality of life improved. Radiology and blood analyses showed stable disease or partial responses and most importantly overall survival increased from 10.1 to 17.1 months for patients with hepatocellular cancer¹⁰⁸ and from 9.4 to 24.7 months for patients with pancreatic ductal adenocarcinoma¹⁰⁹. Complete or partial remission occurred in seven out of 14 patients with refractory B cell lymphoma, whereas disease status remained unchanged in five patients and progression was observed in only two patients¹¹⁰. This type of immunotherapy is categorized as a combination of dendritic cell-based cancer vaccine and adoptive T cell transfer. α -gal is used to gain immune complex formation on tumor cells that in turn activate dendritic cell phagocytosis and priming and activation of tumor reactive T cells.

Intratumoral injections of α -gal-containing lipids have been evaluated in phase I trials in patients with various solid malignancies, including malignant melanoma, PDAC, and colorectal cancer, without observing severe toxicity, α -gal allergy or autoimmunity^{111,112}.

In a study published in February 2025, Liping Zhong et al. present Newcastle disease virus – alpha-1,3-galactosyltransferase (NDV-GT) a Newcastle disease virus (NDV) with oncolytic properties engineered with the porcine gene GGTA1, encoding alpha-1,3-galactosyltransferase (GT), synthesizing α -gal. NDV-GT selectively infects, replicates in and expresses α -gal in various human tumor cell lines. When infected tumor cells were co-cultured with human serum and PBMCs multiple immune effector functions were activated *in vitro*. Repeated intravenous injections of NDV-GT was well tolerated, increased intratumoral T cell infiltration and significantly reduced tumor size and prolonged survival in cynomolgus monkeys with primary hepatocellular carcinoma. In a clinical phase I study, 23 patients with refractory advanced solid malignancies were treated with up to twelve weekly intravenous injections of NDV-GT. Importantly, no cases of cytokine release syndrome (CRS), immune effector cell associated neurotoxicity syndrome (ICANS), autoimmune disorders, red meat allergy or grade 4-5 adverse events were reported. Only one patient experienced a grade 3 adverse event with fever at maximum 40,2°C. Most of the patients experienced grade 1-2 adverse events such as fatigue, fever, chills, diarrhea, vomiting, limb pain and itching. Disease control, i.e. complete response, partial response or stable disease was reported in 90 % of the patients¹¹³.

HP-NAP modulates the human immune system

Apart from α -gal, our long co-evolution with bacteria have also trained the innate immune system to react strongly on various bacterial and viral proteins. The innate response can quickly address invading pathogens but are poorly suited to identify tumor antigens. *Helicobacter pylori* neutrophil activating protein (NAP) is a strong modulator of immunity. This virulence factor from the common gut bacteria *Helicobacter pylori* activates a wide range of leucocytes that causes inflammation in the gastric mucosa, a favorable environment for the bacteria. Around 50 % of the human population is colonized by *Helicobacter pylori* and the chronic inflammations contribute to 5% of the global cancer burden due to induced gastric cancer and MALT lymphoma¹¹⁴. Active eradication treatment of *Helicobacter pylori* during the last decades have also shed light on some benefits of our co-existence with this pathogen, including reduced risk of obesity and autoimmune inflammatory intestinal disease¹¹⁵.

HP-NAP is known to attract neutrophils and monocytes and dendritic cells through chemoattractant properties and induces a conformational change of integrin β 2 on leucocytes to a high affinity state that adhere to endothelial cells¹¹⁶. Through binding to toll like receptor 2, HP-NAP stimulate degranulation¹¹⁷ and IL-12 and IL-23 secretion from neutrophils¹¹⁸. Monocytes differentiates into matured dendritic cells¹¹⁸, with increased expression of HLA class II, TNF- α , IL-6, IL-12 and IL-23¹¹⁴. These cytokines recruit lymphocytes to the mucosa and in draining lymph nodes helper T cells are activated and differentiates towards a Th1 phenotype^{118,119}.

HP-NAP have been proposed as immunotherapy for cancer. Recombinant HP-NAP have been administered intratumorally and systemically in mouse tumor models¹²⁰, and have been used as adjuvant along with tumor antigens in dendritic cell-based tumor vaccines¹²¹. Additionally, CAR-T cells expressing HP-NAP have been shown to trigger bystander immune responses¹²².

Proposed mechanism of action for Adf35(OGN)

By arming an oncolytic adenovirus with the transgenes *GGT1*, encoding alpha-1,3-galactosyltransferase, and *napA* encoding NAP we have developed the oncolytic virus Adf35(OGN).

Adf35(OGN) has the genetic backbone of adenovirus serotype five, but the coxsackievirus and adenovirus receptor binding fiber knob of adenovirus serotype five is replaced by the fiber knob of serotype 35, which allows viral entry through interaction with CD46, a complement inhibitor abundantly expressed on many tumor types¹²³. The virus is restricted to replicate in cancer cells with aberrant tumor protein p53 (p53) and Rb-E2F mediated cell cycle

control, through a partial deletion of the E1A gene and a full deletion of the E1B gene that prevent viral replication in normally functioning cells¹²⁴.

Adf35(OGN) exhibits multiple antitumoral mechanisms of action. In order to maximize the infection of tumor cells and minimize off target effects, the virus will primarily be administered through intratumoral injection in solid tumors. The fiber knob binds to cell surface receptor CD46 on tumor cells and viruses are internalized through endocytosis. Viruses replicate in the tumor cell and the transgenes *GGTA1* and *napA* are transcribed with subsequent expression of the transgene products, i.e. the α -gal epitope and the NAP protein. The accumulation of viral particles in the cell lead to immunogenic cell death with expression of DAMPs (Figure 2, step 1). In addition, the α -gal epitopes are targeted by pre-existing anti- α -gal antibodies leading to tumor cell lysis via complement-dependent cytotoxicity (CDC) and natural killer cell (NK cell) mediated antibody dependent cellular cytotoxicity (ADCC) (Figure 2, step 2). NAP induces maturation of dendritic cells and the secretion of Th1 immune polarizing cytokines from dendritic cells, neutrophils and monocytes. The enhanced antigen presentation activates helper T cells that subsequently accumulate in the target tissue and induce a vigorous delayed-type hypersensitivity-like response by activating monocytes and macrophages. NAP further increases neutrophil infiltration in the tumor bed, leading to necrosis in the tumor tissue. Through these mechanisms, the immune suppressive microenvironment in the tumor changes to a proinflammatory environment with boosted immunosurveillance and optimal conditions to identify tumor specific neoantigens and expand cytotoxic T cells and memory T cells against them (Figure 2, step 3). Ideally this leads to elimination of the injected tumor lesion but also systemic immunity with elimination of metastases and immunologic memory protecting against disease recurrence.

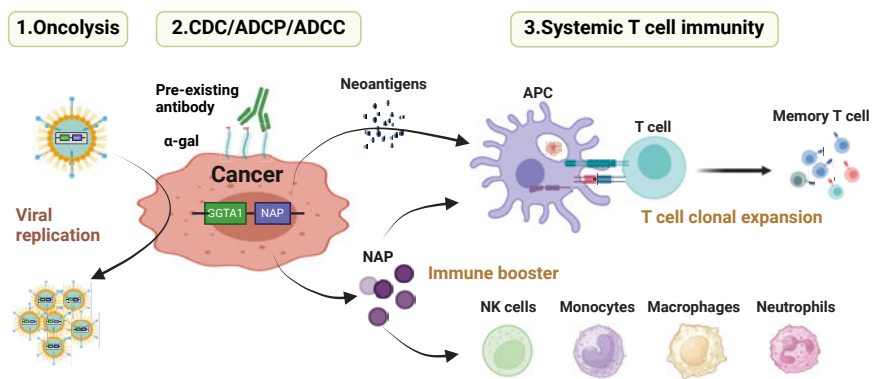


Figure 2. Proposed multi-modal mechanism of action for Adf35(OGN): 1. Acting locally at the injection site through viral replication and subsequent oncolysis. 2. Immune activation against the tumor through CDC, ADCP and ADCC stimulated by α -gal. 3. Systemic T cell immunity with the generation of memory T cells for durable anti-tumor response. The figure was created by Chuan Jin, PhD in Biorender.com.

The mechanisms of action mentioned above could potentially improve all steps in the cancer immunity cycle. Tumor cell death and antigen release is boosted both by viral lysis and CDC, ADCC and ADCP stimulated by α -gal expression (Figure 3, step 1). Antigen presentation by dendritic cells is increased by NAP that stimulate dendritic cell maturation and antigen presentation including cross presentation (Figure 3, step 2). The mature dendritic cells stimulate Th1 polarization of helper T cells and increased priming of cytotoxic T cells (Figure 3, step 3). The local inflammation stimulated by viral infection and transgene expression increase trafficking of T cells to the tumor site as well as infiltration and accumulation in the tumor stroma (Figure 3, step 4-5 and A). α -gal and NAP stimulate further Th1 polarization of T cells, induces reactive phenotype of other immune cells and inhibit immunosuppressive cells (Figure 3, step B). The immunogenic microenvironment contributes to maintained effector function of T cells (Figure 3, step C), which lead to increased recognition and killing of tumor cells (Figure 3, step 6 and 7).

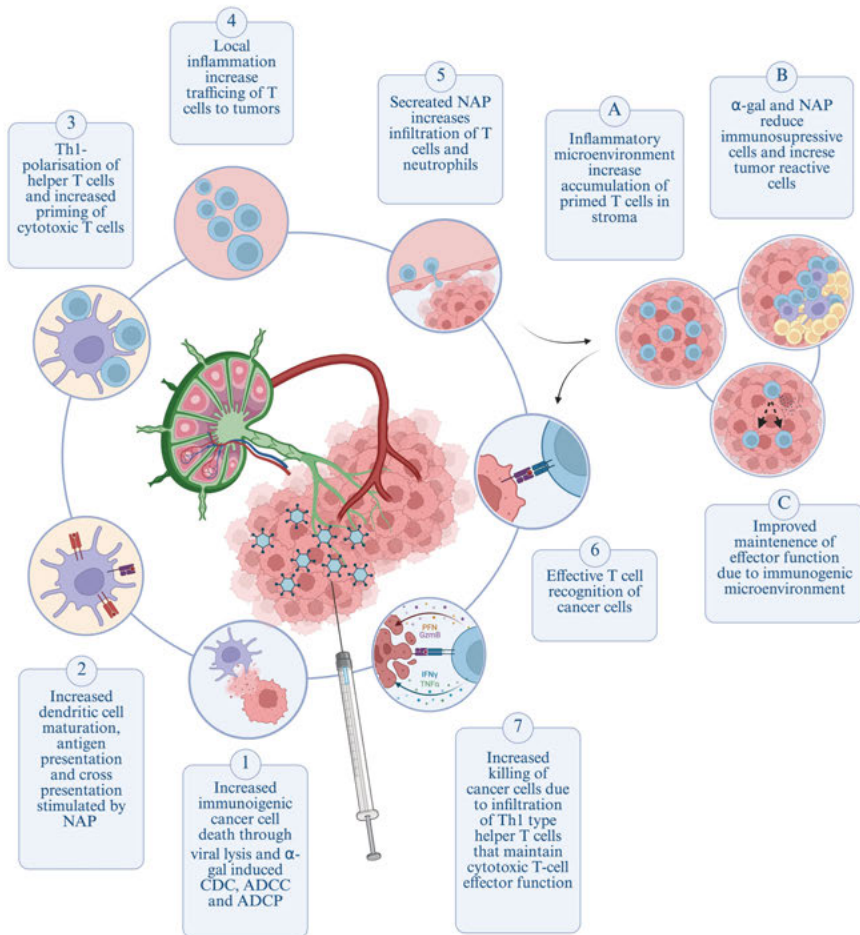


Figure 3. The cancer immunity cycle, adopted from Mellman et al. 2023, and how it can be affected by treatment with Adf35(OGN). The multimodal effect of Adf35(OGN) could stimulate all steps in the cycle. Especially important is the increased tumor antigen release and presentation that comprise the two first steps of the cycle, not currently targeted with immunotherapy in clinical routine. The figure was created with Biorender.com.

Conclusions

The immunological response against tumor cells have been characterized in detail in many different model systems. Additionally, advanced methods have been developed to finely edit and tune these biological processes. However, to date only few immunotherapies are effective and the responses are limited to certain tumor types. Therefore, we propose immunotherapy by α -gal and NAP, that may circumvent these obstacles since they generally activate the

immune system in a much broader way than for example immune checkpoint inhibitors, monoclonal antibodies, tumor vaccines and *ex vivo* activated or engineered immune cells.

Theoretically, Adf35(OGN) may induce a strong anti-tumor response, triggered by the preexisting immunity against α -gal and local activation of the innate immune system, resulting in bystander immunity with a broad T cell repertoire against tumor specific antigens. This therapy may tip the scales from immunological escape to tumor elimination. In addition, Adf35(OGN) is an “of the shelf” type of product that requires less time and labor in the lab, compared to for example personalized cancer vaccines and engineered immune cells. Therefore Adf35(OGN) may be an effective and accessible cancer immunotherapy of value for cancer patients currently without curative treatment options.

Aim of thesis

The overall aim of this thesis was to investigate and optimize the safety and therapeutic potential of Adf35(OGN) when used for cancer therapy, and to take the necessary steps for safe transition to clinical trials.

Specific aims

Paper I

To show pre-clinical therapeutic potential of Adf35(OGN) by investigating mechanistic functionality *in vitro* and antitumoral effect *in vivo*.

Paper II

To develop an accurate and simple method for quantifying possible re-mutated replication competent adenovirus contaminants in batches of oncolytic viruses intended for clinical use.

Paper III

To evaluate toxicity and biodistribution of Adf35(OGN) in the adenovirus-semi-permissive Syrian hamster model and to specifically evaluate the toxicity of transgenic α -gal expression in *GGTA1* knock out mouse.

Paper IV

To optimize the spread and transduction efficacy of oncolytic adenoviruses in solid tumor tissue. The potential of candidate vehicles, including enzymes and polycations, were compared to identify the best approach.

Results and discussion

Paper I

We present Adf35(OGN), a new oncolytic virus with enhanced infectivity in tumor cells, tumor cell selective replication and oncolysis and transgenic expression of the immune stimulatory molecules α -gal and NAP. In paper I, we show the mechanisms of action of Adf35(OGN) when used in cancer therapy.

Adf35(OGN) efficiently infected, replicated in and lysed cultured human tumor cells, inducing immunogenic cell death as noted by expression of calreticulin. Transgene expression of α -gal and NAP was confirmed in infected cells. Furthermore, infected tumor cells were cultured with human serum and various human immune cells. Increased binding of IgG was noted on the tumor cells infected with Adf35(OGN). This indicates that the pre-existing anti α -gal antibodies in the human serum bind to α -gal epitopes expressed on the transduced tumor cells. In addition, increased C3 deposition and complement dependent cytotoxicity were noted on infected cells, indicating that the antibodies binding to the tumor cells also lead to complement activation. When infected cells were co-cultured with human serum and isolated PBMCs, a significant increase in the activation markers CD80, CD40, CD86, CD70 and MHC-II was noted on dendritic cells. CD80 and MHC-II was increased on monocytes and B cells and CD69 was increased on NK-cells and T cells. This indicates that a broad cellular reaction is stimulated by the infected tumor cells.

To test Adf35(OGN) *in vivo*, we used an α -gal-knock out mouse vaccinated against α -gal to generate α -gal antibodies. The functionality of these antibodies was proved by measuring complement dependent cytotoxicity in a mouse pancreatic cell line (Panc02) culture that naturally express α -gal. α -gal knockout Panc02 cells were injected in the hind flank to establish tumors. Repeated intratumoral injections of Adf35(OGN) were tolerated without noticeable side effects and significantly slowed down the tumor growth and improved mouse survival.

In summary, the theoretically appealing approach to combine α -gal and NAP for immunotherapy of pancreatic cancer have been tested in the best available models. Adf35(OGN) effectively transduce human pancreatic tumor cells, induce immunogenic cell death and activate various immune cells. *In vivo* this also leads to therapeutic efficacy. We conclude that cancer

immunotherapy combining α -gal and NAP have promising potential and that Adf35(OGN) is an effective therapeutic agent to bring this potential to patients.

Paper II

Production of oncolytic adenoviruses may accidentally generate formation of re-mutated wild type-like replication-competent adenoviruses (RCA) which raises safety concerns in clinical usage of virus products. It is therefore a regulatory requirement to report the number of RCA in all batches of viral vectors and oncolytic viruses before clinical use. Cell-based assays, which traditionally have been used, can detect RCA in batches of nonreplicating adenoviral vectors but cannot distinguish RCA in batches of conditionally replicating oncolytic adenoviruses. Therefore, there is a need for comprehensive RCA-detection and quantification methods.

We developed a qPCR-based method for detection and quantification of RCA. It utilizes a primer pair detecting the viral gene E1B, which is deleted in oncolytic adenoviruses. If that region would be reacquired, causing an undesired RCA, it would be detected by the assay, which for that purpose needs to be sensitive and accurate. In order to obtain minimal loss of DNA, any DNA isolation step was avoided and instead DNA was released by enzymatic protein degradation followed by direct transfer to the qPCR reaction.

The assay had a low limit of detection at 10 viral particles (VP) per 40 μ L reaction volume and a limit of quantification at 70 VP/reaction. In addition, we showed that the method is working with the same accuracy even when viral contaminants are to be detected in highly concentrated oncolytic virus batches.

The assay was used to test the integrity of a batch of Adf35(OGN) which is produced under good manufacturing practice and is intended for use in clinical studies. No replication competent contaminants were detected in this batch.

In conclusion, we report a time-saving qPCR-based method for detecting and quantifying RCA in batches of conditionally replicating oncolytic adenoviruses, with high sensitivity and accuracy. The undetectable levels of replication competent contaminants in the clinical grade Adf35(OGN) support the use of the virus in a clinical setting.

Paper III

To study the toxicity and biodistribution of Adf35(OGN), the virus was repeatedly injected subcutaneously in treatment naïve Syrian hamster and

intratumorally in α -gal knock out mouse. Syrian hamster have become a standard model for toxicity studies of oncolytic adenoviruses since it is permissive for adenoviral infection and replication, which is largely compromised in other rodent species including mouse^{125,126}. The natural expression of α -gal in the wild type Syrian hamster, however, disables this model from evaluating the toxicity related transgenic α -gal expressed by Adf35(OGN). Therefore, results from the Syrian hamster were complemented by toxicity evaluation also in GGTA-1 knock out mouse.

At necropsy, viral genome copies were detected only in a small proportion of the analyzed tissue samples. The viral numbers were also low and the highest viral genome copy numbers detected were below 0,002 viral particles per hamster cell.

These results indicate that the viral particles are effectively cleared from the circulation, that viral accumulation in tissues is limited and that no biologically relevant replication occur. The same low viral numbers were seen in bio-fluid samples, which indicate that viral shedding is low and without risk of causing secondary infections.

No treatment related alterations were identified in blood hematology, biochemistry or tissue histopathology, indicating that Adf35(OGN) is well tolerated. In α -gal-knock out mouse, no treatment related histopathological alterations were identified.

Our results are in line with previous toxicity studies, in which oncolytic viruses have been well tolerated^{127,128}. Additionally, oncolytic viruses have been extensively used in clinical trials with favorable safety profile, as described above^{51,52,54-91}. Taken together our results support a clinical dose escalation study with repeated intratumoral injections of Adf35(OGN) as a cancer treatment.

Paper IV

Immune stimulatory gene therapy using oncolytic viruses as delivery platform is a promising strategy for cancer therapy, with one product already approved for clinical use¹²⁹ and several products in clinical trials¹³⁰. However, the full potential of this treatment may not have been reached, since spread of virus in the tumor tissue and virus-to-cell contact is limited by various factors. The enzymes *hyaluronidase* and *collagenase* and the polycations *DEAE-dextran*, *branched PEI* and *protamine sulfate* have previously shown potential to potentiate gene transfer in different forms of viral gene therapy, since they may help the virus to overcome these barriers. Therefore, these substances were evaluated as vehicles for adenoviral vectors and oncolytic viruses *in vitro* and *in vivo*. Beneficial effect on viral transduction was observed for hyaluronidase and DEAE dextran *in vitro*. In solid tumor tissue *in vivo*,

hyaluronidase tripled viral transduction, while no statistically significant effect was observed for the other tested vehicles.

The increase in transduction efficacy facilitated by hyaluronidase is likely due to the degradation of extracellular matrix, which lead to increased viral spread within the tumor tissue and increased access to tumor cells. This mechanism could potentially be even more important in a clinical setting where tumors may be larger and richer in extracellular matrix compared to the tumor model in mouse. The limited effect of collagenase is likely due to the low dose of the enzyme. However higher concentrations were not tolerated due to severe tissue damage. The lack of efficacy for polycations indicate that the viral spread in tumor tissue is the limiting factor in virus-based gene therapy, while repellent electrostatic forces between virus and cell may not be a rate limiting factor in viral transduction. Our findings indicate that hyaluronidase may be used as vehicle for oncolytic-virus-based gene therapy to improve its efficacy.

Conclusion and future perspectives

The work presented in this thesis show that Adf35(OGN) is functional and stimulate a broad immune response. It is also safe without significant replication or toxicity in normal tissue of used models. The GMP batch of Adf35(OGN) is confirmed without replication competent contaminants (below detectable levels).

Next, we propose a phase 1, single-center, open-label dose escalation study in patients with advanced solid malignancies. The overall purpose of the study is to assess the safety and tolerability of increasing doses of Adf35(OGN) administered intratumorally to patients with advanced solid malignancies. A standard 3+3 dose escalation design is planned with three dose levels, which means that the study will include minimum 9 and maximum 18 patients, depending on the toxicity observed. To each patient, up to six injections may be given, one every third week at doses of 1×10^{11} , 3×10^{11} or 1×10^{12} viral particles per injection.

The virus will be administered through intratumoral injections, usually ultrasound guided, of the most accessible tumor lesion, primary or metastasis. Cyclophosphamide will be given as conditioning therapy by intravenous injection every three weeks, one to three days before the virus injections and at a dose of 300 mg/m^2 .

Patients may receive up to six repeated injections of the virus. Radiological examinations are scheduled at baseline, after three treatment cycles and after six treatment cycles, and then every two to four months until progressive disease. The treatment will be discontinued if dose limiting toxicity occurs and/or progressive disease is evident.

If no dose limiting toxicity is observed in three patients at a specific dose level, enrollment continues at the next dose level. If two out of the three patients experience dose limiting toxicity at a specific dose level, that dose level is considered as maximum tolerated dose. If one of the three patients experience dose limiting toxicity at a specific dose level, three additional patients will be enrolled at this dose level, and if in total only one of the six patients experience dose limiting toxicity, enrollment continues to the next higher dose level. If more than one of the six patients experience dose limiting toxicity, the dose level is considered as maximum tolerated dose.

The primary objective is to determine the maximum tolerated dose and/or the recommended phase II dose. Primary endpoint for safety and tolerability

assessments will be adverse events related or possibly related to Adf35(OGN), and dose-limiting toxicities. Toxicity will be patient reported adverse events, physical examination and standard laboratory screening.

Secondary endpoints will be clinical efficacy, i.e. anti-tumor activity, of Adf35(OGN), assessed radiologically by response evaluation criteria in solid malignancies for immune-based therapeutics (iRECIST) and overall survival.

Exploratory endpoints include changes in tumor metabolism, tumor cell lysis, tumor immune cell infiltration and systemic immune responses. This will be assessed using both biopsy material, when available, and blood, analyzing cell-free DNA, flow cytometry of immune cells, cytokines and potentially also other metabolic and immune activity parameters.

If Adf35(OGN) is well tolerated, various clinical studies can follow in order to optimize the treatment efficacy. Our primary concern is that virus will be trapped locally at the injection site and that treatment will lack efficacy due to low transduction of tumor cells. Combination of Adf35(OGN) and hyaluronidase may be tested to improve the transduction, but a tripled transduction efficacy, as seen in paper IV, may still be far too low to acquire the proper immune stimulation. Therefore, in order to circumvent the limitation of viral transduction *in vivo*, we are currently developing a technique to generate patient derived primary tumor cells, collected at surgery, transduce these cells *ex vivo* with Adf35(OGN). The resulting advanced therapy medicinal product (ATMP)-classed cell product would then be transferred back to the patient through intranodal injection. This would be a conceptually new form of cancer immunotherapy that places autologous tumor cells, containing an ideal tumor antigen repertoire, in the center of the adaptive immune system together with the activating and modulatory features of α -gal and NAP. This would then be the most specific and yet potent form of immunotherapy developed so far. It remains to be investigated whether this treatment approach, including transfer of live tumor cells to patients will be safe and thus accepted by regulatory authorities. However, therapies using genetically engineered primary human cells are currently in clinical trials, for other indications.

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References

1. Xu W, Atkins MB, McDermott DF. Checkpoint inhibitor immunotherapy in kidney cancer. *Nat Rev Urol.* 2020;17(3):137-150. doi:10.1038/s41585-020-0282-3
2. Schvartsman G, Taranto P, Glitza IC, Agarwala SS, Atkins MB, Buzaid AC. Management of metastatic cutaneous melanoma: updates in clinical practice. *Ther Adv Med Oncol.* 2019;11:1758835919851663. doi:10.1177/1758835919851663
3. Sequeira T, Almodovar MT. Immunotherapy in Non-small Cell Lung Cancer: a Review. *Port J Card Thorac Vasc Surg.* 2023;30(3):55-65. doi:10.48729/pjctvs.371
4. Burnet FM. The Concept of Immunological Surveillance. Published online September 24, 1970. doi:10.1159/000386035
5. Thomas L. On immunosurveillance in human cancer. *Yale J Biol Med.* 1982;55(3-4):329-333.
6. Shankaran V, Ikeda H, Bruce AT, et al. IFN γ and lymphocytes prevent primary tumour development and shape tumour immunogenicity. *Nature.* 2001;410(6832):1107-1111. doi:10.1038/35074122
7. van den Broek ME, Kägi D, Ossendorp F, et al. Decreased tumor surveillance in perforin-deficient mice. *J Exp Med.* 1996;184(5):1781-1790. doi:10.1084/jem.184.5.1781
8. Mortaz E, Tabarsi P, Mansouri D, et al. Cancers Related to Immunodeficiencies: Update and Perspectives. *Frontiers in Immunology.* 2016;7. doi:10.3389/fimmu.2016.00365
9. Clark WH, Elder DE, Guerry D, et al. Model predicting survival in stage I melanoma based on tumor progression. *J Natl Cancer Inst.* 1989;81(24):1893-1904. doi:10.1093/jnci/81.24.1893
10. Gooden MJM, de Bock GH, Leffers N, Daemen T, Nijman HW. The prognostic influence of tumour-infiltrating lymphocytes in cancer: a systematic review with meta-analysis. *Br J Cancer.* 2011;105(1):93-103. doi:10.1038/bjc.2011.189
11. Denkert C, von Minckwitz G, Darb-Esfahani S, et al. Tumour-infiltrating lymphocytes and prognosis in different subtypes of breast cancer: a pooled analysis of 3771 patients treated with neoadjuvant therapy. *The Lancet Oncology.* 2018;19(1):40-50. doi:10.1016/S1470-2045(17)30904-X
12. Schalper KA, Brown J, Carvajal-Hausdorf D, et al. Objective measurement and clinical significance of TILs in non-small cell lung cancer. *J Natl Cancer Inst.* 2015;107(3):dju435. doi:10.1093/jnci/dju435
13. Ding W, Xu X, Qian Y, et al. Prognostic value of tumor-infiltrating lymphocytes in hepatocellular carcinoma: A meta-analysis. *Medicine.* 2018;97(50):e13301. doi:10.1097/MD.000000000013301

14. Mayor PC, Eng KH, Singel KL, et al. Cancer in primary immunodeficiency diseases: Cancer incidence in the United States Immune Deficiency Network Registry. *J Allergy Clin Immunol*. 2018;141(3):1028-1035. doi:10.1016/j.jaci.2017.05.024
15. Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoediting: from immunosurveillance to tumor escape. *Nat Immunol*. 2002;3(11):991-998. doi:10.1038/ni1102-991
16. Chen DS, Mellman I. Oncology meets immunology: the cancer-immunity cycle. *Immunity*. 2013;39(1):1-10. doi:10.1016/j.immuni.2013.07.012
17. Leko V, Rosenberg SA. Identifying and Targeting Human Tumor Antigens for T Cell-Based Immunotherapy of Solid Tumors. *Cancer Cell*. 2020;38(4):454-472. doi:10.1016/j.ccell.2020.07.013
18. Kroemer G, Galassi C, Zitvogel L, Galluzzi L. Immunogenic cell stress and death. *Nat Immunol*. 2022;23(4):487-500. doi:10.1038/s41590-022-01132-2
19. Galluzzi L, Vitale I, Warren S, et al. Consensus guidelines for the definition, detection and interpretation of immunogenic cell death. *J Immunother Cancer*. 2020;8(1):e000337. doi:10.1136/jitc-2019-000337
20. Jhunjhunwala S, Hammer C, Delamarre L. Antigen presentation in cancer: insights into tumour immunogenicity and immune evasion. *Nat Rev Cancer*. 2021;21(5):298-312. doi:10.1038/s41568-021-00339-z
21. Feola S, Chiaro J, Martins B, Cerullo V. Uncovering the Tumor Antigen Landscape: What to Know about the Discovery Process. *Cancers*. 2020;12(6). doi:10.3390/cancers12061660
22. Owen JA. *Kuby Immunology*. Eighth edition. W.H. Freeman, Macmillan Learning; 2018.
23. Weng N ping. Numbers and odds: TCR repertoire size and its age changes impacting on T cell functions. *Seminars in Immunology*. 2023;69:101810. doi:10.1016/j.smim.2023.101810
24. Anderson MS, Su MA. AIRE expands: new roles in immune tolerance and beyond. *Nat Rev Immunol*. 2016;16(4):247-258. doi:10.1038/nri.2016.9
25. Tay RE, Richardson EK, Toh HC. Revisiting the role of CD4+ T cells in cancer immunotherapy-new insights into old paradigms. *Cancer Gene Ther*. 2021;28(1-2):5-17. doi:10.1038/s41417-020-0183-x
26. Träger U, Sierro S, Djordjevic G, et al. The Immune Response to Melanoma Is Limited by Thymic Selection of Self-Antigens. *PLOS ONE*. 2012;7(4):e35005. doi:10.1371/journal.pone.0035005
27. Zhu ML, Nagavalli A, Su MA. Aire Deficiency Promotes TRP-1-Specific Immune Rejection of Melanoma. *Cancer Research*. 2013;73(7):2104-2116. doi:10.1158/0008-5472.CAN-12-3781
28. Conteduca G, Ferrera F, Pastorino L, et al. The role of AIRE polymorphisms in melanoma. *Clinical Immunology*. 2010;136(1):96-104. doi:10.1016/j.clim.2010.03.002
29. Malchow S, Leventhal DS, Nishi S, et al. Aire-Dependent Thymic Development of Tumor-Associated Regulatory T Cells. *Science*. 2013;339(6124):1219-1224. doi:10.1126/science.1233913
30. Bernard A, Lamy L, Alberti I. THE TWO-SIGNAL MODEL OF T-CELL ACTIVATION AFTER 30 YEARS. *Transplantation*. 2002;73(1):S31.
31. Raskov H, Orhan A, Christensen JP, Gögenur I. Cytotoxic CD8+ T cells in cancer and cancer immunotherapy. *Br J Cancer*. 2021;124(2):359-367. doi:10.1038/s41416-020-01048-4

32. Redmond WL, Ruby CE, Weinberg AD. The role of OX40-mediated co-stimulation in T cell activation and survival. *Crit Rev Immunol.* 2009;29(3):187-201.
33. Chen L, Flies DB. Molecular mechanisms of T cell co-stimulation and co-inhibition. *Nat Rev Immunol.* 2013;13(4):227-242. doi:10.1038/nri3405
34. Alcaide P. Mechanisms Regulating T Cell–Endothelial Cell Interactions. *Cold Spring Harb Perspect Med.* 2022;12(7):a041170. doi:10.1101/cshperspect.a041170
35. Kretschmer L, Flossdorf M, Mir J, et al. Differential expansion of T central memory precursor and effector subsets is regulated by division speed. *Nat Commun.* 2020;11(1):113. doi:10.1038/s41467-019-13788-w
36. Kamali AN, Bautista JM, Eisenhut M, Hamedifar H. Immune checkpoints and cancer immunotherapies: insights into newly potential receptors and ligands. *Ther Adv Vaccines Immunother.* 2023;11:25151355231192043. doi:10.1177/25151355231192043
37. Mandó P, Rivero SG, Rizzo MM, Pinkasz M, Levy EM. Targeting ADCC: A different approach to HER2 breast cancer in the immunotherapy era. *Breast.* 2021;60:15-25. doi:10.1016/j.breast.2021.08.007
38. Hudry D, Le Guellec S, Meignan S, et al. Tumor-Infiltrating Lymphocytes (TILs) in Epithelial Ovarian Cancer: Heterogeneity, Prognostic Impact, and Relationship with Immune Checkpoints. *Cancers (Basel).* 2022;14(21):5332. doi:10.3390/cancers14215332
39. Wong PF, Wei W, Smithy JW, et al. Multiplex Quantitative Analysis of Tumor-Infiltrating Lymphocytes and Immunotherapy Outcome in Metastatic Melanoma. *Clinical Cancer Research.* 2019;25(8):2442-2449. doi:10.1158/1078-0432.CCR-18-2652
40. Chan TA, Yarchoan M, Jaffee E, et al. Development of tumor mutation burden as an immunotherapy biomarker: utility for the oncology clinic. *Annals of Oncology.* 2019;30(1):44-56. doi:10.1093/annonc/mdy495
41. Salvaris R, Ong J, Gregory GP. Bispecific Antibodies: A Review of Development, Clinical Efficacy and Toxicity in B-Cell Lymphomas. *Journal of Personalized Medicine.* 2021;11(5):355. doi:10.3390/jpm11050355
42. Kamakura D, Asano R, Yasunaga M. T Cell Bispecific Antibodies: An Antibody-Based Delivery System for Inducing Antitumor Immunity. *Pharmaceuticals (Basel).* 2021;14(11):1172. doi:10.3390/ph14111172
43. Chi WY, Hu Y, Huang HC, et al. Molecular targets and strategies in the development of nucleic acid cancer vaccines: from shared to personalized antigens. *Journal of Biomedical Science.* 2024;31(1):94. doi:10.1186/s12929-024-01082-x
44. Weber JS, Carlino MS, Khattak A, et al. Individualised neoantigen therapy mRNA-4157 (V940) plus pembrolizumab versus pembrolizumab monotherapy in resected melanoma (KEYNOTE-942): a randomised, phase 2b study. *The Lancet.* 2024;403(10427):632-644. doi:10.1016/S0140-6736(23)02268-7
45. Rojas LA, Sethna Z, Soares KC, et al. Personalized RNA neoantigen vaccines stimulate T cells in pancreatic cancer. *Nature.* 2023;618(7963):144-150. doi:10.1038/s41586-023-06063-y
46. Makino T, Miyata H, Yasuda T, et al. A phase 3, randomized, double-blind, multicenter, placebo-controlled study of S-588410, a five-peptide cancer vaccine as an adjuvant therapy after curative resection in patients with esophageal squamous cell carcinoma. *Esophagus.* 2024;21(4):447. doi:10.1007/s10388-024-01072-w

47. Liao LM, Ashkan K, Brem S, et al. Association of Autologous Tumor Lysate-Loaded Dendritic Cell Vaccination With Extension of Survival Among Patients With Newly Diagnosed and Recurrent Glioblastoma: A Phase 3 Prospective Externally Controlled Cohort Trial. *JAMA Oncol.* 2023;9(1):112-121. doi:10.1001/jamaoncol.2022.5370
48. Ferrucci PF, Pala L, Conforti F, Cocorocchio E. Talimogene Laherparepvec (T-VEC): An Intralesional Cancer Immunotherapy for Advanced Melanoma. *Cancers (Basel).* 2021;13(6):1383. doi:10.3390/cancers13061383
49. Eriksson E, Milenova I, Wenthe J, et al. Shaping the Tumor Stroma and Sparking Immune Activation by CD40 and 4-1BB Signaling Induced by an Armed Oncolytic Virus. *Clin Cancer Res.* 2017;23(19):5846-5857. doi:10.1158/1078-0432.CCR-17-0285
50. Common Terminology Criteria for Adverse Events (CTCAE).
51. Small EJ, Carducci MA, Burke JM, et al. A phase I trial of intravenous CG7870, a replication-selective, prostate-specific antigen-targeted oncolytic adenovirus, for the treatment of hormone-refractory, metastatic prostate cancer. *Mol Ther.* 2006;14(1):107-117. doi:10.1016/j.ymthe.2006.02.011
52. Musher BL, Rowinsky EK, Smaglo BG, et al. LOAd703, an oncolytic virus-based immunostimulatory gene therapy, combined with chemotherapy for unresectable or metastatic pancreatic cancer (LOKON001): results from arm 1 of a non-randomised, single-centre, phase 1/2 study. *Lancet Oncol.* 2024;25(4):488-500. doi:10.1016/S1470-2045(24)00079-2
53. Taylor IP, Lopez JA. Oncolytic adenoviruses and the treatment of pancreatic cancer: a review of clinical trials. *J Cancer Res Clin Oncol.* 2023;149(10):8117-8129. doi:10.1007/s00432-023-04735-w
54. Machiels JP, Salazar R, Rottey S, et al. A phase 1 dose escalation study of the oncolytic adenovirus enadenotucirev, administered intravenously to patients with epithelial solid tumors (EVOLVE). *Journal for Immunotherapy of Cancer.* 2019;7. doi:10.1186/s40425-019-0510-7
55. Makower D, Rozenblit A, Kaufman H, et al. Phase II clinical trial of intralesional administration of the oncolytic adenovirus ONYX-015 in patients with hepatobiliary tumors with correlative p53 studies. *Clin Cancer Res.* 2003;9(2):693-702.
56. Xu RH, Yuan ZY, Guan ZZ, et al. [Phase II clinical study of intratumoral H101, an E1B deleted adenovirus, in combination with chemotherapy in patients with cancer]. *Ai Zheng.* 2003;22(12):1307-1310.
57. Burke JM, Lamm DL, Meng MV, et al. A first in human phase 1 study of CG0070, a GM-CSF expressing oncolytic adenovirus, for the treatment of nonmuscle invasive bladder cancer. *J Urol.* 2012;188(6):2391-2397. doi:10.1016/j.juro.2012.07.097
58. Chiocca EA, Abbed KM, Tatter S, et al. A phase I open-label, dose-escalation, multi-institutional trial of injection with an E1B-Attenuated adenovirus, ONYX-015, into the peritumoral region of recurrent malignant gliomas, in the adjuvant setting. *Mol Ther.* 2004;10(5):958-966. doi:10.1016/j.ymthe.2004.07.021
59. Conley AP, Roland CL, Bessudo A, et al. BETA prime: a first-in-man phase 1 study of AdAPT-001, an armed oncolytic adenovirus for solid tumors. *Cancer Gene Ther.* 2024;31(4):517-526. doi:10.1038/s41417-023-00720-0
60. DeWeese TL, van der Poel H, Li S, et al. A phase I trial of CV706, a replication-competent, PSA selective oncolytic adenovirus, for the treatment of locally recurrent prostate cancer following radiation therapy. *Cancer Res.* 2001;61(20):7464-7472.

61. Fakih M, Harb W, Mahadevan D, et al. Safety and efficacy of the tumor-selective adenovirus enadenotucirev, in combination with nivolumab, in patients with advanced/metastatic epithelial cancer: a phase I clinical trial (SPICE). *J Immunother Cancer*. 2023;11(4):e006561. doi:10.1136/jitc-2022-006561
62. Freytag SO, Stricker H, Pegg J, et al. Phase I study of replication-competent adenovirus-mediated double-suicide gene therapy in combination with conventional-dose three-dimensional conformal radiation therapy for the treatment of newly diagnosed, intermediate- to high-risk prostate cancer. *Cancer Res*. 2003;63(21):7497-7506.
63. Freytag SO, Movsas B, Aref I, et al. Phase I trial of replication-competent adenovirus-mediated suicide gene therapy combined with IMRT for prostate cancer. *Mol Ther*. 2007;15(5):1016-1023. doi:10.1038/mt.sj.6300120
64. Freytag SO, Stricker H, Lu M, et al. Prospective randomized phase 2 trial of intensity modulated radiation therapy with or without oncolytic adenovirus-mediated cytotoxic gene therapy in intermediate-risk prostate cancer. *Int J Radiat Oncol Biol Phys*. 2014;89(2):268-276. doi:10.1016/j.ijrobp.2014.02.034
65. Galanis E, Okuno SH, Nascimento AG, et al. Phase I-II trial of ONYX-015 in combination with MAP chemotherapy in patients with advanced sarcomas. *Gene Ther*. 2005;12(5):437-445. doi:10.1038/sj.gt.3302436
66. Garcia-Carbonero R, Bazan-Peregrino M, Gil-Martín M, et al. Phase I, multicenter, open-label study of intravenous VCN-01 oncolytic adenovirus with or without nab-paclitaxel plus gemcitabine in patients with advanced solid tumors. *J Immunother Cancer*. 2022;10(3):e003255. doi:10.1136/jitc-2021-003255
67. García M, Moreno R, Gil-Martin M, et al. A Phase I Trial of Oncolytic Adenovirus ICOVIR-5 Administered Intravenously to Cutaneous and Uveal Melanoma Patients. *Hum Gene Ther*. 2019;30(3):352-364. doi:10.1089/hum.2018.107
68. He Y, Huang X, Li X, et al. Preliminary efficacy and safety of YSCH-01 in patients with advanced solid tumors: an investigator-initiated trial. *J Immunother Cancer*. 2024;12(5):e008999. doi:10.1136/jitc-2024-008999
69. Heo J, Liang JD, Kim CW, et al. Safety and dose escalation of the targeted oncolytic adenovirus OBP-301 for refractory advanced liver cancer: Phase I clinical trial. *Mol Ther*. 2023;31(7):2077-2088. doi:10.1016/j.ymthe.2023.04.006
70. Jirovec E, Quixabeira DCA, Clubb JHA, et al. Single intravenous administration of oncolytic adenovirus TILT-123 results in systemic tumor transduction and immune response in patients with advanced solid tumors. *J Exp Clin Cancer Res*. 2024;43(1):297. doi:10.1186/s13046-024-03219-0
71. Khuri FR, Nemunaitis J, Ganly I, et al. a controlled trial of intratumoral ONYX-015, a selectively-replicating adenovirus, in combination with cisplatin and 5-fluorouracil in patients with recurrent head and neck cancer. *Nat Med*. 2000;6(8):879-885. doi:10.1038/78638
72. Kim KH, Dmitriev IP, Saddekni S, et al. A phase I clinical trial of Ad5/3-Δ24, a novel serotype-chimeric, infectivity-enhanced, conditionally-replicative adenovirus (CRAd), in patients with recurrent ovarian cancer. *Gynecol Oncol*. 2013;130(3):518-524. doi:10.1016/j.ygyno.2013.06.003

73. Kimball KJ, Preuss MA, Barnes MN, et al. A phase I study of a tropism-modified conditionally replicative adenovirus for recurrent malignant gynecologic diseases. *Clin Cancer Res.* 2010;16(21):5277-5287. doi:10.1158/1078-0432.CCR-10-0791
74. Kirn D. Oncolytic virotherapy for cancer with the adenovirus dl1520 (Onyx-015): results of phase I and II trials. *Expert Opin Biol Ther.* 2001;1(3):525-538. doi:10.1517/14712598.1.3.525
75. Koski A, Kangasniemi L, Escutenaire S, et al. Treatment of cancer patients with a serotype 5/3 chimeric oncolytic adenovirus expressing GMCSF. *Mol Ther.* 2010;18(10):1874-1884. doi:10.1038/mt.2010.161
76. Lamont JP, Nemunaitis J, Kuhn JA, Landers SA, McCarty TM. A prospective phase II trial of ONYX-015 adenovirus and chemotherapy in recurrent squamous cell carcinoma of the head and neck (the Baylor experience). *Ann Surg Oncol.* 2000;7(8):588-592. doi:10.1007/BF02725338
77. Li JL, Liu HL, Zhang XR, et al. A phase I trial of intratumoral administration of recombinant oncolytic adenovirus overexpressing HSP70 in advanced solid tumor patients. *Gene Ther.* 2009;16(3):376-382. doi:10.1038/gt.2008.179
78. Li R, Shah PH, Stewart TF, et al. Oncolytic adenoviral therapy plus pembrolizumab in BCG-unresponsive non-muscle-invasive bladder cancer: the phase 2 CORE-001 trial. *Nat Med.* 2024;30(8):2216-2223. doi:10.1038/s41591-024-03025-3
79. Moreno V, Barretina-Ginesta MP, García-Donas J, et al. Safety and efficacy of the tumor-selective adenovirus enadenotucirev with or without paclitaxel in platinum-resistant ovarian cancer: a phase 1 clinical trial. *J Immunother Cancer.* 2021;9(12):e003645. doi:10.1136/jitc-2021-003645
80. Naing A, Khalil D, Rosen O, et al. First-in-human clinical outcomes with NG-350A, an anti-CD40 expressing tumor-selective vector designed to remodel immunosuppressive tumor microenvironments. *J Immunother Cancer.* 2024;12(10):e010016. doi:10.1136/jitc-2024-010016
81. Nemunaitis J, Senzer N, Sarmiento S, et al. A phase I trial of intravenous infusion of ONYX-015 and enbrel in solid tumor patients. *Cancer Gene Ther.* 2007;14(11):885-893. doi:10.1038/sj.cgt.7701080
82. Nemunaitis J, Tong AW, Nemunaitis M, et al. A phase I study of telomerase-specific replication competent oncolytic adenovirus (telomelysin) for various solid tumors. *Mol Ther.* 2010;18(2):429-434. doi:10.1038/mt.2009.262
83. Nokisalmi P, Pesonen S, Escutenaire S, et al. Oncolytic adenovirus ICOVIR-7 in patients with advanced and refractory solid tumors. *Clin Cancer Res.* 2010;16(11):3035-3043. doi:10.1158/1078-0432.CCR-09-3167
84. Nyati S, Stricker H, Barton KN, et al. A phase I clinical trial of oncolytic adenovirus mediated suicide and interleukin-12 gene therapy in patients with recurrent localized prostate adenocarcinoma. *PLoS One.* 2023;18(9):e0291315. doi:10.1371/journal.pone.0291315
85. O’Cathail SM, Davis S, Holmes J, et al. A phase I trial of the safety, tolerability and biological effects of intravenous Enadenotucirev, a novel oncolytic virus, in combination with chemoradiotherapy in locally advanced rectal cancer (CEDAR). *Radiat Oncol.* 2020;15(1):151. doi:10.1186/s13014-020-01593-5
86. Pakola SA, Peltola KJ, Clubb JHA, et al. Safety, Efficacy, and Biological Data of T-Cell-Enabling Oncolytic Adenovirus TILT-123 in Advanced Solid Cancers from the TUNIMO Monotherapy Phase I Trial. *Clinical Cancer Research.* 2024;30(17):3715-3725. doi:10.1158/1078-0432.CCR-23-3874

87. Ponce S, Cedrés S, Ricordel C, et al. ONCOS-102 plus pemetrexed and platinum chemotherapy in malignant pleural mesothelioma: a randomized phase 2 study investigating clinical outcomes and the tumor microenvironment. *J Immunother Cancer*. 2023;11(9):e007552. doi:10.1136/jitc-2023-007552
88. Reid TR, Freeman S, Post L, McCormick F, Sze DY. Effects of Onyx-015 among metastatic colorectal cancer patients that have failed prior treatment with 5-FU/leucovorin. *Cancer Gene Ther*. 2005;12(8):673-681. doi:10.1038/sj.cgt.7700819
89. Reid T, Galanis E, Abbruzzese J, et al. Hepatic arterial infusion of a replication-selective oncolytic adenovirus (dl1520): phase II viral, immunologic, and clinical endpoints. *Cancer Res*. 2002;62(21):6070-6079.
90. Shirakawa Y, Tazawa H, Tanabe S, et al. Phase I dose-escalation study of endoscopic intratumoral injection of OBP-301 (Telomelysin) with radiotherapy in oesophageal cancer patients unfit for standard treatments. *Eur J Cancer*. 2021;153:98-108. doi:10.1016/j.ejca.2021.04.043
91. Zhang Y, Qian L, Chen K, et al. Oncolytic adenovirus in treating malignant ascites: A phase II trial and longitudinal single-cell study. *Mol Ther*. 2024;32(6):2000-2020. doi:10.1016/j.ymthe.2024.04.029
92. Fares J, Ahmed AU, Ulasov IV, et al. Neural stem cell delivery of an oncolytic adenovirus in newly diagnosed malignant glioma: a first-in-human, phase I, dose-escalation trial. *Lancet Oncol*. 2021;22(8):1103-1114. doi:10.1016/S1470-2045(21)00245-X
93. Kieran MW, Goumnerova L, Manley P, et al. Phase I study of gene-mediated cytotoxic immunotherapy with AdV-tk as adjuvant to surgery and radiation for pediatric malignant glioma and recurrent ependymoma. *Neuro Oncol*. 2019;21(4):537-546. doi:10.1093/neuonc/nyy202
94. Lang FF, Conrad C, Gomez-Manzano C, et al. Phase I Study of DNX-2401 (Delta-24-RGD) Oncolytic Adenovirus: Replication and Immunotherapeutic Effects in Recurrent Malignant Glioma. *J Clin Oncol*. 2018;36(14):1419-1427. doi:10.1200/JCO.2017.75.8219
95. Ning W, Qian X, Dunmall LC, et al. Non-secreting IL12 expressing oncolytic adenovirus Ad-TD-nsIL12 in recurrent high-grade glioma: a phase I trial. *Nat Commun*. 2024;15(1):9299. doi:10.1038/s41467-024-53041-7
96. van Putten EHP, Kleijn A, van Beusechem VW, et al. Convection Enhanced Delivery of the Oncolytic Adenovirus Delta24-RGD in Patients with Recurrent GBM: A Phase I Clinical Trial Including Correlative Studies. *Clin Cancer Res*. 2022;28(8):1572-1585. doi:10.1158/1078-0432.CCR-21-3324
97. Chen X, Hao S, Zhao Z, et al. Interleukin 35: Inhibitory regulator in monocyte-derived dendritic cell maturation and activation. *Cytokine*. 2018;108:43-52. doi:10.1016/j.cyto.2018.03.008
98. Mirlekar B. Tumor promoting roles of IL-10, TGF- β , IL-4, and IL-35: Its implications in cancer immunotherapy. *SAGE Open Med*. 2022;10:20503121211069012. doi:10.1177/20503121211069012
99. Tang S, Ning Q, Yang L, Mo Z, Tang S. Mechanisms of immune escape in the cancer immune cycle. *Int Immunopharmacol*. 2020;86:106700. doi:10.1016/j.intimp.2020.106700
100. Cornel AM, Mimpfen IL, Nierkens S. MHC Class I Downregulation in Cancer: Underlying Mechanisms and Potential Targets for Cancer Immunotherapy. *Cancers (Basel)*. 2020;12(7):1760. doi:10.3390/cancers12071760

101. Chow A, Perica K, Klebanoff CA, Wolchok JD. Clinical implications of T cell exhaustion for cancer immunotherapy. *Nat Rev Clin Oncol*. 2022;19(12):775-790. doi:10.1038/s41571-022-00689-z
102. Macher BA, Galili U. The Gal α 1,3Gal β 1,4GlcNAc-R (alpha-Gal) epitope: a carbohydrate of unique evolution and clinical relevance. *Biochim Biophys Acta*. 2008;1780(2):75-88. doi:10.1016/j.bbagen.2007.11.003
103. Galili U. Anti-Gal: an abundant human natural antibody of multiple pathogenesis and clinical benefits. *Immunology*. 2013;140(1):1-11. doi:10.1111/imm.12110
104. Singh S, Thompson JA, Yilmaz B, et al. Loss of α -gal during primate evolution enhanced antibody-effector function and resistance to bacterial sepsis. *Cell Host & Microbe*. 2021;29(3):347-361.e12. doi:10.1016/j.chom.2020.12.017
105. Hamanova M, Chmelikova M, Nentwich I, Thon V, Lokaj J. Anti-Gal IgM, IgA and IgG natural antibodies in childhood. *Immunol Lett*. 2015;164(1):40-43. doi:10.1016/j.imlet.2015.02.001
106. Galili U. Anti-Gal: an abundant human natural antibody of multiple pathogenesis and clinical benefits. *Immunology*. 2013;140(1):1-11. doi:10.1111/imm.12110
107. Galili U. Interaction of the natural anti-Gal antibody with alpha-galactosyl epitopes: a major obstacle for xenotransplantation in humans. *Immunol Today*. 1993;14(10):480-482. doi:10.1016/0167-5699(93)90261-i
108. Qiu Y, Xu MB, Yun MM, et al. Hepatocellular carcinoma-specific immunotherapy with synthesized α 1,3- galactosyl epitope-pulsed dendritic cells and cytokine-induced killer cells. *World J Gastroenterol*. 2011;17(48):5260-5266. doi:10.3748/wjg.v17.i48.5260
109. Qiu Y, Yun MM, Xu MB, Wang YZ, Yun S. Pancreatic carcinoma-specific immunotherapy using synthesised alpha-galactosyl epitope-activated immune responders: findings from a pilot study. *Int J Clin Oncol*. 2013;18(4):657-665. doi:10.1007/s10147-012-0434-4
110. Qiu Y, Yun MM, Dong X, et al. Combination of cytokine-induced killer and dendritic cells pulsed with antigenic α -1,3-galactosyl epitope-enhanced lymphoma cell membrane for effective B-cell lymphoma immunotherapy. *Cytotherapy*. 2016;18(1):91-98. doi:10.1016/j.jcyt.2015.09.012
111. Whalen GF, Sullivan M, Piperdi B, Wasseff W, Galili U. Cancer Immunotherapy by Intratumoral Injection of α -gal Glycolipids. *Anticancer Research*. 2012;32(9):3861-3868.
112. Albertini MR, Ranheim EA, Zuleger CL, et al. Phase I study to evaluate toxicity and feasibility of intratumoral injection of α -gal glycolipids in patients with advanced melanoma. *Cancer Immunol Immunother*. 2016;65(8):897-907. doi:10.1007/s00262-016-1846-1
113. Zhong L, Gan L, Wang B, et al. Hyperacute rejection-engineered oncolytic virus for interventional clinical trial in refractory cancer patients. *Cell*. 2025;188(4):1119-1136.e23. doi:10.1016/j.cell.2024.12.010
114. Fu HW, Lai YC. The Role of Helicobacter pylori Neutrophil-Activating Protein in the Pathogenesis of H. pylori and Beyond: From a Virulence Factor to Therapeutic Targets and Therapeutic Agents. *Int J Mol Sci*. 2022;24(1):91. doi:10.3390/ijms24010091
115. Schubert JP, Rayner CK, Costello SP, Roberts-Thomson IC, Forster SC, Bryant RV. Helicobacter pylori: Have potential benefits been overlooked? *JGH Open*. 2022;6(11):735-737. doi:10.1002/jgh3.12842

116. Polenghi A, Bossi F, Fischetti F, et al. The Neutrophil-Activating Protein of *Helicobacter pylori* Crosses Endothelia to Promote Neutrophil Adhesion In Vivo. *The Journal of Immunology*. 2007;178(3):1312-1320. doi:10.4049/jimmunol.178.3.1312
117. Wang CA, Liu YC, Du SY, Lin CW, Fu HW. *Helicobacter pylori* neutrophil-activating protein promotes myeloperoxidase release from human neutrophils. *Biochem Biophys Res Commun*. 2008;377(1):52-56. doi:10.1016/j.bbrc.2008.09.072
118. Amedei A, Cappon A, Codolo G, et al. The neutrophil-activating protein of *Helicobacter pylori* promotes Th1 immune responses. *J Clin Invest*. 2006;116(4):1092-1101. doi:10.1172/JCI27177
119. D'Elia MM, Amedei A, Cappon A, Del Prete G, de Bernard M. The neutrophil-activating protein of *Helicobacter pylori* (HP-NAP) as an immune modulating agent. *FEMS Immunology & Medical Microbiology*. 2007;50(2):157-164. doi:10.1111/j.1574-695X.2007.00258.x
120. Codolo G, Fassan M, Munari F, et al. HP-NAP inhibits the growth of bladder cancer in mice by activating a cytotoxic Th1 response. *Cancer Immunol Immunother*. 2012;61(1):31-40. doi:10.1007/s00262-011-1087-2
121. Hou M, Wang X, Lu J, et al. TLR Agonist rHP-NAP as an Adjuvant of Dendritic Cell-Based Vaccine to Enhance Anti-Melanoma Response. *Iran J Immunol*. 2020;17(1):14-25. doi:10.22034/iji.2020.80291
122. Jin C, Ma J, Ramachandran M, Yu D, Essand M. CAR T cells expressing a bacterial virulence factor trigger potent bystander antitumour responses in solid cancers. *Nat Biomed Eng*. 2022;6(7):830-841. doi:10.1038/s41551-022-00875-5
123. Uhlen M, Zhang C, Lee S, et al. A pathology atlas of the human cancer transcriptome. *Science*. 2017;357(6352):eaan2507. doi:10.1126/science.aan2507
124. Fueyo J, Gomez-Manzano C, Alemany R, et al. A mutant oncolytic adenovirus targeting the Rb pathway produces anti-glioma effect in vivo.
125. Thomas MA, Spencer JF, La Regina MC, et al. Syrian hamster as a permissive immunocompetent animal model for the study of oncolytic adenovirus vectors. *Cancer Res*. 2006;66(3):1270-1276. doi:10.1158/0008-5472.CAN-05-3497
126. Bortolanza S, Alzuguren P, Buñuales M, Qian C, Prieto J, Hernandez-Alcoceba R. Human adenovirus replicates in immunocompetent models of pancreatic cancer in Syrian hamsters. *Hum Gene Ther*. 2007;18(8):681-690. doi:10.1089/hum.2007.017
127. Havunen R, Kalliokoski R, Siurala M, et al. Cytokine-Coding Oncolytic Adenovirus TILT-123 Is Safe, Selective, and Effective as a Single Agent and in Combination with Immune Checkpoint Inhibitor Anti-PD-1. *Cells*. 2021;10(2):246. doi:10.3390/cells10020246
128. Sonabend AM, Ulasov IV, Han Y, et al. Biodistribution of an oncolytic adenovirus after intracranial injection in permissive animals: a comparative study of Syrian hamsters and cotton rats. *Cancer gene therapy*. 2009;16(4):362. doi:10.1038/cgt.2008.80
129. Andtbacka RHI, Kaufman HL, Collichio F, et al. Talimogene Laherparepvec Improves Durable Response Rate in Patients With Advanced Melanoma. *JCO*. 2015;33(25):2780-2788. doi:10.1200/JCO.2014.58.3377
130. Lin D, Shen Y, Liang T. Oncolytic virotherapy: basic principles, recent advances and future directions. *Sig Transduct Target Ther*. 2023;8(1):1-29. doi:10.1038/s41392-023-01407-6

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