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ONCOLYTIC VIRUS THERAPY FOR CANCER TREATMENT

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ABBREVIATIONS

ABD	Albumin union domain
Ag	Antigen
ASR	Age-standardized rate
BCC	Basal cell carcinoma
BiTEs	Bi-specific T-cell engagers
CAFs	Fibroblasts
CAR	Chemical antigen receptor
c-Myc	Family of MYC proto-oncogenes
CpG	Cytosine-phosphodiester bond- guanine
CTLA4	Cytotoxic-lymphocyte antigen 4
DAF	Decay acceleration factor
DAMP	Damage-associated molecular patterns
DC	Dendritic cells
eIF2-α-P	Eukaryotic Initiation Factor 2
EGFR	Epidermal growth factor receptor
E1A	Early 1A
E2F	Family of transcription factor
FAP	Fibroblast activation protein
FDA	Food and Drug Administration
GBM	Glioblastoma
GLUT1	Glucose transporter 1
GM-CSF	Granulocyte-macrophage colony-stimulating factor
HER 2	Human epidermal growth factor receptor 2
HER 4	Human epidermal growth factor receptor 4
HIF-1α	Hypoxia Inducible Factor- 1α
ICAM-1	Intracellular adhesion molecule 1
ICB	Immune checkpoint blockage
INFα/β	interferon alpha/beta
IFN γ	Interferon gamma
IL-12	Interleukin 12
IRF3	Interferon regulatory factor 3
IRF7	Interferon regulatory factor 7
IT	Intratumoral
IV	Intravenous
JAK	Janus kinase
LAG-3	Lymphocyte-activation gene 3
MDSC	Myeloid-derived suppressor cells
MHC I	Major histocompatibility complex I
NMPA	National Medical Products Administration

NRP-1	Neuropilin-1
NK	Natural killer
NKG2D	Natural killer group 2D
NSCLC	Non-small cell lung carcinoma
oAd	Oncolytic adenovirus
PAMP	Pathogen-associated molecular patters
PD-1	Programmed cell death protein 1
PD-L1	programmed death-ligand 1
PH20	Recombinant human hyaluronidase
PKR	Protein kinase R
PRR	Pattern recognition receptors
PSA	Prostate-specific antigen
RAS	Rat sarcoma
Rb	Retinoblastoma
RIG-1	Retinoic acid-inducible gene 1
ROS	Reactive oxygen species
SCC	Squamous cell carcinoma
TAMs	Tumour-associated macrophages
TRAF 3	TNF-Receptor Associated Factor 3
STAT	Signal Transducers and Activators of Transcription
TAAs	Tumour associated antigens
TCR	T cell receptor
TK	Tyrosine Kinase
TLR	Toll-like receptors
TME	Tumour microenvironment
TNF	Tumour necrosis factor
TP53	Tumour protein 53
VAP	Viral attachment proteins
VEGF	Vascular endothelial growth factor
MHLW	Ministry of Health, Labor and Welfare
WHO	World Health Organization
eIF2-α-P	Phosphorylated Eukaryotic translation initiation factor 2 subunit alpha

ABSTRACT/ RESUM

In the last few years, oncolytic virus therapy has been increasingly recognised as a new class of immunotherapy for the treatment of cancer. To date, a variety of oncolytic viruses, intact and genetically modified, have been developed for the treatment of cancer, and many clinical studies have been or are being conducted. In fact, three oncolytic viruses have already been approved and commercialised in some countries. The therapy has multiple effects such as, direct lysis of cancer cells without damaging healthy cells, the activation of the immune system, the induction of chemokines to turn a cold tumour into a hot tumour and elimination of distant and uninfected tumour cells. In addition, biotechnology is increasingly being used in oncolytic viruses to improve the targeting and obtain better efficacy. Although it has good results, the use of this therapy must take into account the characteristics of the patient, the virus and the cancer which means that each case must be assessed individually. Furthermore, clinical guidelines for viral administration remain unclear and many limitations and features of the therapy need to be investigated. This work provides an overview of the basic concepts of cancer, viruses and immunology to describe and discuss the challenges in the development of oncolytic viruses as cancer therapeutics, either as monotherapy or in combination with other drugs, and to describe the future perspective of the therapy.

Key words: oncolytic virus, cancer treatment, immunotherapy, clinical studies, limitations.

En els últims anys, la teràpia amb virus oncolítics ha sigut reconeguda com una nova classe d'immunoteràpia pel tractament del càncer, és per aquest motiu que s'estan portant a terme molts estudis clínics en els que s'han desenvolupat una àmplia varietat de virus oncolítics, intactes o modificats genèticament, per ser utilitzats en la teràpia. De fet, actualment, es poden trobar tres virus oncolítics aprovats i comercialitzats en alguns països. Els efectes de la teràpia son diversos, com la lisi directa de les cèl· lules cancerígenes sense danyar les cèl·lules sanes, l'activació del sistema immunitari, la inducció de citocines per transformar un tumor fred a un tumor calent i l'eliminació de cèl·lules tumorals llunyanes i no infectades. Però, per fer-ne ús d'aquesta teràpia s'han de considerar característiques del pacient, del virus i del càncer, el que implica una valoració individual. I tot i que l'ús de la biotecnologia ha permès millorar la selectivitat i eficàcia de la teràpia, i sembla presentar bons resultats, falten estudis per resoldre algunes limitacions de la teràpia. Aquest treball aporta una visió sobre conceptes bàsics del càncer, els virus i el sistema immunitari per descriure i discutir sobre els reptes en el desenvolupament dels virus oncolítics com a agents terapèutics en la monoteràpia o teràpia combinada pel càncer i descriure la perspectiva de futur de la teràpia.

Paraules clau: virus oncolítics, tractament del càncer, immunoteràpia, estudis clínics, limitacions.

INTEGRATION OF THE DIFFERENT FIELDS

The main field is **Pharmacology and Therapeutics**. This is because the project is about cancer treatment. It also discusses the main treatments currently available for cancer and how these can be combined with oncolytic virus therapy. To understand how treatments work, there's also an introduction to cancer and its pathogenesis. In addition, the different pharmacological characteristics of the treatment are explained. These include route of administration, distribution, adverse effects and limitations, among others.

The second field is **Microbiology**, because they are used in this therapy. For this reason, these microorganisms, their main characteristics and their mechanism of action are described. It also outlines some of the genetic modifications that are being made to viruses to make therapy more targeted and effective. Finally, the main viruses used in the therapy and the approved ones for therapy are also described.

The third field is **Immunology**. One of the mechanisms of action of this therapy is the activation of the immune system, so it is important to understand how the immune system works and which cells are part of it. There is also a need to identify the impact and relationship between the immune system and cancer.

These three fields are found in all the blocks of the work and they are all complementary to each other. The two secondary areas help to understand and extend the main field of this work.

SUSTAINABLE DEVELOPMENT GOALS (SDG)

The United Nations 3030 Agenda sets out 17 universal, inclusive and ambitious Sustainable Development Goals (SDG)

In order to improve people's health and quality of life, investment in research into new treatments is essential. This paper presents a therapy that can be used today as an alternative treatment for people who do not have any other options or who do not respond fully to the treatment they are currently receiving. In the future, however, the ambitious goal would be to be able to include this therapy in the first line of cancer treatment. For these reasons, the third SDG, entitled **"Good health and well-being"**, is represented in this work. The aim of this SDG is to ensure healthy lives and promote well-being for all, at all ages. This SDG proposes, among others, to reduce premature mortality from noncommunicable diseases (3.4), achieve universal health coverage (3.8), reduce deaths and diseases from hazardous chemicals and air, water and soil pollution and contamination (3.9), support research and development of vaccines and medicines (3B), and increase health financing (3C).

It is essential to ensure equitable and universal healthcare. Oncolytic virus therapy can reach the entire population and all age groups, and many clinical trials are not only for adults but also for children. This is related to the tenth SDG "**Reduced inequalities"**.

Finally, the environment is a predisposing factor that can increase the risk of developing cancer. Therefore, one way to reduce cancer incidence is to take action to prevent climate change and pollution. Another important point is the ability to reduce waste generation in the pharmaceutical industry, as well as encouraging companies to adopt sustainable practices. The twelfth SDG is a representation of these ideas and is described as **"Responsible consumption and production"**. It talks about ensuring sustainable ways of consuming and producing to ensure the livelihoods of current and future generations.

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1. INTRODUCTION

1.1. What is cancer?

Neoplasia is defined as abnormal growth of cells in a tissue, either because cells grow faster than normal, don't die when they should, or a combination of both. As well as altering cell division, it can also affect differentiation. (1).

Neoplasms can be benign, or they can become aggressive and be called malignant. They generally form masses of solid tissue that are called tumours, which can also be non-cancerous (benign) or cancerous (malignant).
However, some neoplasms do not form solid masses. For example, in leukaemia, neoplastic cells circulate in the bloodstream without forming a tumour mass.
Differences between benign and malignant neoplasms can be observed in **Table 1**.

Benign neoplasia	Malignant neoplasia
Encapsulated (tumour is localized).	Generally, not encapsulated.
Similarity between cells.	Little or no similarity between cells (pleomorphism).
Specific function.	Without specific function (anaplasia).
Contact inhibition.	No contact inhibition.
Lower growth velocity.	Fast and decontrolled growth velocity (hyperplasia).
They don’t invade other tissue.	They invade other tissue through lymphatic system or circulatory stream (invasive power).
Easy to extirpate.	Difficult to extirpate.

Table 1. Main differences between benign and malignant neoplasms. *Adapted from* (1–3).

Therefore, cancer refers to a group of diseases that can occur in almost any organ or tissue when cells grow out of control and invade neighbouring parts of the body and even spread to other organs. (4).

1.1.1. Characteristics of cancer

For cancer to develop, there must be an accumulation of genetic alterations. This explains the direct relationship between cancer incidence and older age. Acquired mutations give the cell a higher rate of division and the conservation of mutations in its descendants (5).

There are two mechanisms that neoplastic cells use to improve their conditions and to increase their survival.

Firstly, they modify energy metabolism by overexpressing the GLUT1 transporter to obtain glucose and cover the demand (5). They do this through various mechanisms such as hypoxia-induced stimulation of the HIF-1α factor (Hypoxia Inducible Factor- 1α), c-Myc protooncogenes (family of MYC proto-oncogenes) or through RAS (rat sarcoma) signalling modifications.
Overexpression of GLUT-1 can be used as a biomarker in some cancers as well as to obtain information about prognosis and survival of the patient (6). However, it seems that not all tumours have this overexpression (7).

The other is the expression of telomerase, an enzyme that prevents telomeres from shortening. Telomeres are located at the end of eukaryotic chromosomes and contain condensed DNA that gives chromosomes stability. Each time the cell multiplies, some of the DNA is lost, causing the telomeres to break down. At the end, chromosomes start to malfunction, and cells die (2). Telomerase adds the TTAGGG DNA sequence to keep cells alive.
Neoplastic cells often have increased telomerase activity, which allows these cells to replicate indefinitely and prevent cell ageing. In fact, increased telomerase activity can be seen in around 90% of cancers. Other types of neoplastic cells use other mechanisms to maintain telomeres, such as homologous recombination, in which telomeres fluctuate in length during the cell cycle. This mechanism can occur at the same time as telomerase is increased, particularly in neoplasms of mesenchymal tissue (8).

The neoplasm must be able to compete with healthy cells and the immune system (called tumoral immunosurveillance). When malignant cells overcome these barriers, a fixed primary tumour is created (9). This can usually be palpable or detected through diagnostic imaging tests, and in many cases, surgery is possible (10).

Cancer can be classified according to the type of tissue in which the malignant tumour originates (1,10).

There are three types of solid malignant tumours:

- **Carcinomas:** they have an epithelial origin and represent the 80-90% of adult cancers. Example: hepatocellular carcinoma.
- **Sarcomas:** have a mesenchymal origin (connective tissue, bone, muscle, cartilage and fat). They account for 1% of cancers and are usually found in young people, especially teenagers. Example: Ewing's sarcoma.
- **Blastomas:** these come from immature precursor cells or embryonic tissue. They comprise for 1% of cancer and are usually found in children. Example: Neuroblastoma.

The most common solid tumours are prostate cancer, breast cancer, cervical cancer, lung cancer, bowel cancer, stomach cancer and skin cancer.

Liquid tumours are those that originate in the haematopoietic system and account for 5% of adult cancers. The most common are leukaemia, lymphoma, multiple myeloma, myelodysplastic syndrome and myeloproliferative syndrome.

This classification facilitates the communication between health professionals and helps to design and establish guidelines to follow the course of the disease (10).

A distinction is made between non-cancerous neoplasms:

- **Hyperplasia:** a faster-than-normal increase in cells in a tissue, leading to an accumulation of cells. However, the cells appear normal when viewed under a microscope.
- **Dysplasia:** a more advanced condition than hyperplasia because there's also an accumulation of cells, but the cells look abnormal and the structure of the tissue changes.
- **Carcinoma in situ:** localised advanced malignancy. Although it is sometimes called stage 0 cancer, it is not, because the abnormal cells have not spread to adjacent tissue, as happens with cancer cells.

1.1.2. Incidence and triggering causes

Cancer is one of the major diseases affecting the world's population. It is the second leading cause of death in the United States and the first one in Spain (5,9). More than 14 million new cases are reported each year, and it's estimated that 1 in 4 people are at risk of developing cancer during their lifetime. 90% of mortality is due to metastases (2), and 80% of patients with metastases, develop cachexia (10). Cachexia is a cancer-related condition that negatively affects patients' quality of live and survival (11). It is characterised by a decrease in skeletal and muscle mass (12). Its identification and management are complicated because, unfortunately, there's no current treatment that can completely reverse this disorder (11,12).

Cancer is a multifactorial process caused by an accumulation of mutations that turn a healthy cell into a cancerous one. This process is called tumourigenesis and can be influenced by various parameters such as genetics, epigenetics, gender, age (the incidence increases exponentially after the age of 45), diet, physical activity, alcohol, tobacco and the environment (the main risk factor) (13). Some studies suggest that between 5-10% of cancers are linked to genetic factors, while others put the percentage at over 20% (14).

Epigenetic factors are DNA modifications that regulate the activation and deactivation of a gene and are influenced by an individual's lifestyle, bad habits, stress and individual's environment (15). Physical agents are external epigenetic factors. Examples include genotoxic chemicals, chemotherapy, radiotherapy, tobacco, some chronic infections and UV radiation (14).

Figure 1 shows data from the World Health Organization (WHO). It displays the incidence (blue) and mortality (red) estimated by age-standardized rate (ASR) in 2020 worldwide, including all sexes and ages. It can be seen that breast cancer has the highest incidence (47,8) and lung cancer has the highest mortality (18,0).

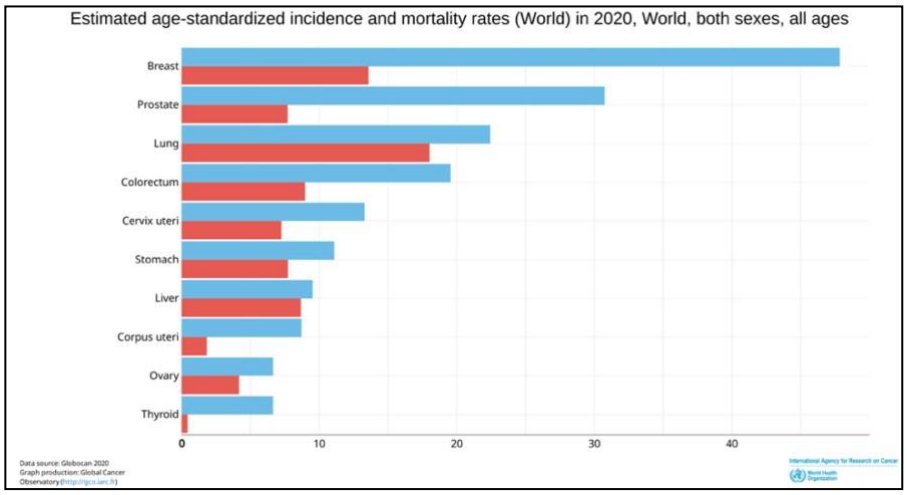

Figure 1. Graphic of global incidence and mortality estimated by ASR in 2020 worldwide. Information from WHO (16).

Analysing the data for Spain, there were 223.054 of new cases of cancer and 113.054 deaths in 2020 with prostate cancer being the most common cancer in men and breast cancer in women. In terms of incidence, Spain ranks sixth in Europe (16).

1.1.3. Aetiology and pathogenesis

The process can be divided into 4 phases: initiation, promotion, progression and expansion. **Figure 2** shows the different phases of cancer.

In the **initiation** phase, initiating or carcinogenic factors directly damage DNA or increase susceptibility to mutations that cause irreversible damage to DNA. As a result, genetic information is altered (genetic mutation), leading to neoplastic transformation. These genetic mutations may activate proto-oncogenes, inactivate anti-oncogenes or affect other genes such as DNA repairers or apoptosis regulators (3,10).

Proto-oncogenes are genes that encode proteins that regulate cell growth and cellular differentiation. When a proto-oncogene mutates, it becomes an oncogene which causes tumours and their uncontrolled growth.

Anti-oncogene inactivation refers to suppressor genes. They reduce cell division, repair DNA errors and control cell apoptosis. When they lose their function, control over cell division is lost and organisms becomes more susceptible to developing cancer. The inactivation of anti-oncogenes can occur through punctual mutations, deletions or methylation (17). The last one happens because cytosine can be methylated when it is in front of guanine, and about half of genes contain these sequences called CpG (cytosine-

phosphodiester bond-guanine) islands of promoter regions. Most of these regions are demethylated, but in silenced regions they are generally methylated. This causes the region to remain in a silenced state so that the genes are not expressed (15).

Finally, in the case of DNA repair genes, function is lost, and DNA is not be repaired. If apoptosis regulators are mutated, the ability to induce apoptosis is lost and the chances of new mutations increases.

Although initiation is necessary for neoplastic transformation, in most cases it's not enough to cause a tumour. It requires phase **promotion**, which usually lasts for years and is reversible when the promoter is removed (2). They don't damage DNA but induce a local chronic inflammatory response that causes the secretion of proteases and growth factors that stimulate cell proliferation (9). These two phases give rise to a primary malignant tumour.

The progression phase is defined by the evolution of neoplasia and aggressiveness (10). Mutations from mutated cells are passed on to the descendants and new mutations may appear with the capacity for invasion, angiogenesis, metastasis, drug resistance and immunodepression.

Expansion is the final phase and consists of local invasion and metastasis. A second tumour is formed at another site of the primary tumour. Cells from the second tumour are similar to the primary cells, which allows the location of the tumour to be known.

Metastases spread through the lymphatic system and blood vessels. The first lymph node or set of nodes to which the tumour drains, is called the sentinel lymph node.
During metastasis, the primary tumour secretes proteins and enzymes to destroy the surrounding tissue. They attach to blood components such as platelets and produce inflammatory effects on the compromised immune system, increasing invasion (3). They then use fixation factors to attach to the vessel wall and secrete proteolytic enzymes to degrade the basal membrane, facilitate migration and establish the secondary tumour (17).

In the case of vascular metastasis, the first organ affected is the venous flow coming from the digestive tract, pancreas and spleen, which are the ones that end up in the liver. For this reason, these organs are the most affected by this type of metastasis (2).

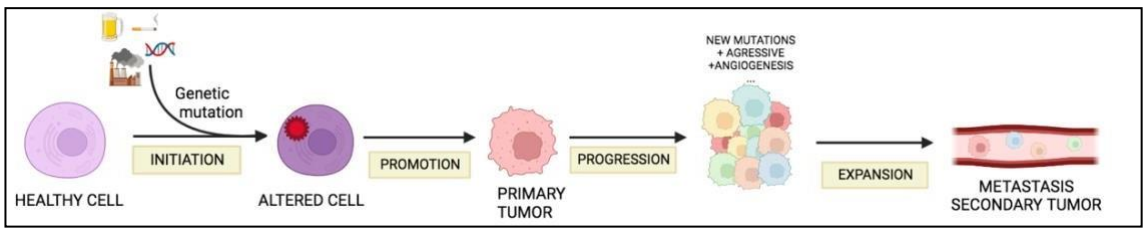

Figure 2: Representation of the different stages of cancer pathogenesis. Made by author with: https://www.biorender.com/ (18).

1.2. Current cancer treatments and therapies

Oncology is a multidisciplinary field that focuses on the study, treatment, prevention and diagnosis of neoplasms, especially malignant ones (17).

To diagnose cancer, in addition to reviewing the clinical history and performing a physical examination, a biopsy is usually performed to obtain a histopathological diagnosis. Therefore, imaging tests and serological marker studies can be performed. These two are related to the neoplasia origin, so they are important in the diagnosis (3).

The objective of the treatment is to eradicate the tumour, but if this isn't possible, the goal is to prolong the patient's life and give them a good quality of life. If this is not possible, the aim is to relieve pain. Statistics show that 1 in 3 cancer cases will be cured (17).
Stem cell transplants are often given at the same time as cancer treatments to increase the number of dead cells caused by the treatment.

There is now a wide range of treatments for neoplasms. However, research continues, and it has been reflected in the new drugs in 2022, as 14 of the 32 are from ATC L (antineoplastic therapy and immunomodulatory agents) (19).

1.2.1. Conventional treatments

1.2.1.1. Surgery

Consists of surgical removal or resection of the tumours. Whenever possible, the whole tumour is removed, especially if it is caught early and the cancer is well controlled. If this isn't possible because of tissue damage or nearby organs, partial removal might be considered.

Surgery is the most effective and efficient treatment, but it is also the way to prevent premalignant lesions from becoming malignant neoplasms. However, not always ensure that cancer will not develop (3,17). It can also have a palliative purpose when the aim is to reduce the number of tumour cells or control symptoms (3).
It is the first-line treatment for resectable cancer. However, in most cases, it's not enough to treat the cancer at all, so it's usually combined with other conventional treatments such as chemotherapy or radiotherapy (3). This combination increases the cure rate by about 9% (17).

1.2.1.2. Chemotherapy

It involves the use of chemicals, alone or in combination, to disrupt DNA replication, cell metabolism and microtubule binding (during mitosis) to block cell division and cause tumour cell death (3,20). These agents act in a non-specific manner, causing damage to both tumour and healthy cells, particularly rapidly reproducing cells (3).

Nowadays, chemotherapy can be used as an induction treatment, as an adjuvant treatment, in combination with radiotherapy or as palliative treatment. Induction chemotherapy is used as a first treatment or when the cancer is advanced and not resectable, as the use of chemotherapy in this case can transform the tumour into a resectable one (3,17).

In general, polychemotherapy is used, which consists of using more than one antineoplastic drug with different mechanisms of action at the same time. This type of treatment is highly recommended because it enhances the antineoplastic effect (17).

1.2.1.3. Radiotherapy

Is one of the main treatments for cancer, as one of the most effective. It is calculated that the 50% of patients receive radiotherapy during their treatment (17).

Consists of the propagation of energy in electromagnetic waves or particles that cause irreparable damage to DNA and prevent the replication and division of tumour cells (17). Recent studies explain that radiotherapy also stimulates the immune system by releasing TAAs (tumour-associated antigens) and DAMP (damage-associated molecular patterns). DAMP cause inflammation when they are released from damaged or dying cells (20).

The purpose is curative, adjuvant, neoadjuvant (use of radiotherapy to reduce tumour growth and then surgery), palliative and prophylactic (17). The aim is to achieve local control of the tumour and pain. In some cases, it is combined with surgery or chemotherapy (3).

Nowadays, treatments are combined, but in order to do this, the following requirements must happen: the drugs used must have individual efficacy, adverse effects must not overlap, and toxicity produced by doses and synergy must be considered when using the combination.

1.2.2. Alternative or complement therapies

1.2.2.1. Immunotherapy

It uses agents that activate the immune system to recognise and destroy tumour cells. Conventional mechanisms focus on destroying tumour cells, but immunotherapy is based on activating the immune system to destroy the cancer (3). To do this, the therapy fights against the tumour mechanisms used to evade the immune system (evading immunesurveillance), against the tumoural microenvironment (TME) or against some components of immunity (such as the lack of recognition of tumour cells by immune cells). It therefore consists of activating immune system cells or counteracting inhibitory mechanisms. Although is a revolutionary therapy, survival has only been observed in some patients. (21).

1.2.2.1.1. Immune checkpoint blockage (ICB)

Immune checkpoints prevent the immune system from overreacting and destroying the body's own cells. Immune checkpoint proteins recognise a cell host, bind to it and silence T cells so that they do not destroy it. In a neoplasm, immune checkpoint proteins do the same thing, but to cancer cells. This prevents tumour cells from being destroyed.
Blocking the binding of these immune checkpoint proteins allows the T cells to kill tumour cells (22).

The most commonly used mechanisms are the apoptosis of the T-cell protein PD-1 (programmed cell death protein 1) and its ligand PD-L1 (programmed death-ligand 1) located in the tumour, and the T-cell proteins CTLA4 (cytotoxic-lymphocyte antigen 4) located in the T cell. **Figure 3** shows the mechanism of action of an anti-PD-1.

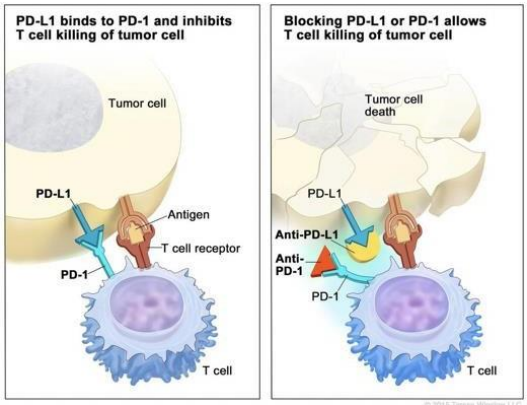

Figure 3. The mechanism of action of an anti-PD-1 (22)

Cemiplimab is a new drug for 2020 and is an anti-PD-1 that has been conditionally approved for the first-line treatment of locally advanced non-small cell lung cancer (NSCLC) when chemotherapy cannot be used. It is also approved as monotherapy for the treatment of metastatic or locally unresectable squamous cell carcinoma (SCC) and for advanced and metastatic basal cell carcinoma (BCC) (19,23).

ICB are used as monotherapy and in combination regimens. They are showing positive results in terms of response and survival in patient with solid tumours, particularly in hot tumours. However, some patients show resistant or poor responses.

1.2.2.1.2. Monoclonal antibodies

Consists of the use of laboratory- designed antibodies. They specifically bind to tumour antigens involved in tumour growth. Compared to chemotherapy, they attack neoplastic cells while sparing healthy cells (3,17).

There are different types of antibodies used in this therapy.

1) Recombinant: they bind to tumour cells and either stimulate the immune system or block tumour antigens responsible for tumour growth. These antibodies are genetically modified to overcome limitations and have good affinity.

2) Conjugated: used in conjunction with chemotherapy or radiotherapy and are used as vehicles to transport drugs.

3) Radiolabelled: they target tumour antigens and have radiation toxicity.

4) Chemomarkers: are combined/conjugated with chemotherapy drugs.

5) Bispecific: contains two antibodies capable of binding two different antigens simultaneously.

1.2.2.1.3. Inhibitors of the tyrosine kinase (TK) pathway

Molecules that bind to and inhibit the catalytic site of the epidermal growth factor receptor (EGFR). It is a TK receptor that, when is overexpressed, is a prognostic marker for certain cancers. It is also responsible for tumour growth and the proliferation of tumour cells (3). Neratinib is a new drug in 2022 and is a TK inhibitor against EGFR-positive carcinomas and human epidermal growth factor receptors 2 and 4 (HER 2, HER 4).

1.2.2.1.4. Therapy with oncolytic viruses

It consists of using intact or genetically modified viruses to directly lyse tumour cells, using their machinery to replicate and destroy them. The therapy also stimulates the immune system in order to attack the tumour.

1.2.3 Other therapies

Hormone therapy: slows or stops the growth of cancer cells that use hormones to grow (24). It is used for two main purposes, to treat cancer or to reduce cancer symptoms.

CAR-T cell therapy: T cells are taken from the patient's blood. The T cells are then genetically engineered to express a specific antigen receptor called a chemical antigen receptor (CAR) that recognises cancer cells. This receptor helps the T cells to attach to the specific antigen on the cancer cells (25). The T cells are then multiplied and infused into the patient. This is a way of getting the patient's own T cells to attack tumour cells more effectively. **Figure 4** shows the process of obtaining CAR-T cells.

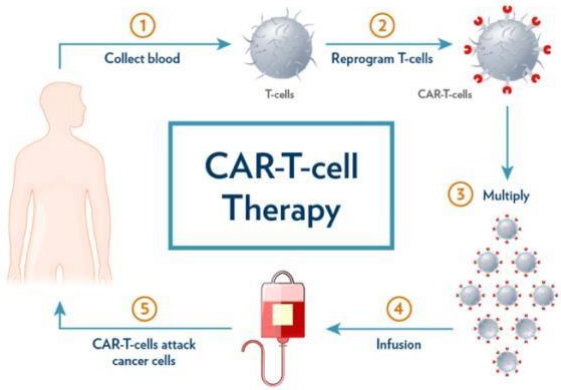

Figure 4. The process to obtain T cells for the CAR-T cell therapy (26).

2. OBJECTIVES

The aim of this work is to provide a deeper understanding of oncolytic virus therapy and to demonstrate the impact that the incorporation of this therapy can have on current guidelines for the treating of cancer. To achieve this, the basics of cancer and currently available therapies have been reviewed to provide a general overview and a deeper understanding of oncolytic virus therapy.

The objectives are:

1. To understand the process of pathogenesis and development of cancer.

2. To improve the knowledge of the main cancer treatments currently available.

3. To enhance awareness of oncolytic virus therapy for cancer treatment and its mechanism.

4. To learn about the main oncolytic viruses used in therapy and the criteria for virus selection.

5. To identify the main immunological factors and the influence they have on the therapy.

6. To understand the pharmacological characteristics of the therapy.

7. To review the most recent and relevant clinical trials of oncolytic virus therapy.

8. To provide an overview of the future perspective for this therapy.

3. MATERIALS AND METHODS

The information for this work has been searched in various bibliographic sources. The main ones are **PubMed** and **Scopus** databases. Boolean operators such as “and”, “or” and “not” are used to obtain more detailed information. In addition, in order to find the most recent documents, the publication date is taken into account, trying to limit most results to the last 5 years (2019-2023). When an interesting document is found, the “similar articles” section is also considered to increase the knowledge in that area.

CRAI's Cercabib has also been used to obtain books on different areas of work, particularly for the “Cancer”, “Cancer Treatments” and “Virus” sections.

Information about the therapy and current clinical trials, especially those involving adenoviruses, is mainly obtained from an interview with **Dr. Ramon Alemany**, who leads the Cancer Virotherapy Group at the Catalan Institute of Oncology (ICO) and the Institute of Biomedical Research of Bellvitge (IDIBELL).
Then, to build on the knowledge gained from the interview, the “author search” section of PubMed is used to find articles by Dr. Ramon Alemany.

In addition, the magazine "**Panorama Actual de los Medicamentos**" has been read to obtain the most recent information on new drugs in 2022 and new information on cancer.

Finally, some official websites related to cancer and clinical trials are used, such as **cancer.gov** and **clinicaltrials.gov**.

4. RESULTS

4.1. Oncolytic virus therapy

4.1.1. What is the oncolytic virus therapy?

It is a treatment based on the immunotherapy which involves treating cancer with viruses. The aim of this therapy is to directly lyse the tumour cells and stimulate the immune system in the cancer area (27).

Delivering viruses directly into a tumour was the first therapy developed in Egypt around 3000 years ago. They observed that when the virus was injected into the tumour with a needle, it caused infection and suppuration. The aim was to use the lytic effect of the virus to kill tumour cells. However, due to a lack of studies, they didn't control the effect of the virus, so in most cases they ended up killing the patient. (*Information from Dr. Ramon Alemany*).

Nowadays, the aim is to eliminate the tumour but in a controlled way. In fact, over the last few decades, several studies have shown very positive results, which is why certain therapies using oncolytic viruses have been approved by various regulatory authorities around the world (28).

In addition, the oncolytic virus therapy has good efficacy and safety. It also has limited side effects and is less painful than conventional treatments. However, it is a complex therapy as it requires considering various factors such as patient characteristics, the virus and the type of cancer (29).

This therapy is being studied in all types of cancer, from solid tumours to haematopoietic cell cancers and metastases. In haematopoietic cancers, it may be beneficial because the immune system is weakened by the disease. This may help to increase the lytic effect of viruses. Nevertheless, eliminating the deficient immune system can cause a tumour lysis syndrome (the tumour releases its contents into the bloodstream in response to therapy) and cytokine storm (the body releases too many cytokines) (*Information from Dr. Ramon Alemany*).

To make this therapy work, the tumour microenvironment (TME) needs to be studied. The TME is the whole of the tumour and its surroundings and it is a heterogeneous and complex environment consisting of the extracellular matrix and a variety of cells that provide the tumour with the necessary factors to grow and spread throughout the body. It also contributes to tumour growth, angiogenesis, drug resistance and suppression of the immune system (27,30).

The TME can determine the efficacy of the therapy, as there are immunologically cold and hot tumours (**Figure 5**) (31). Cold tumours are those with little T-cell infiltration, low

PD-L1 expression (a protein that acts as an inhibitor of immune responses), a small number of tumour mutations and the status of tumour-associated macrophages (TAMs) are immature or immunosuppressive. So, they have low inflammation, small histocompatibility complexes and are resistant to immunotherapy. In contrast, hot tumours have the opposite characteristics, high levels of infiltrating T cells, PD-L1 expression, high levels of tumour mutations and immunostimulatory TAMs. So, they have lots of signs of inflammation, histocompatibility complexes, increased inflammation and IFN-γ (gamma-interferon) and are sensitive to immunotherapy (31–34).

What is important to facilitate tumour destruction is to have a hot tumour, so that the immune system is enhanced and in optimal conditions to attack the tumour (31). Using biomarkers, it has been observed that oncolytic viruses have the ability to alter the tumour microenvironment towards a more immunostimulatory phenotype, transforming an immunologically cold tumour into a hot one. (13)

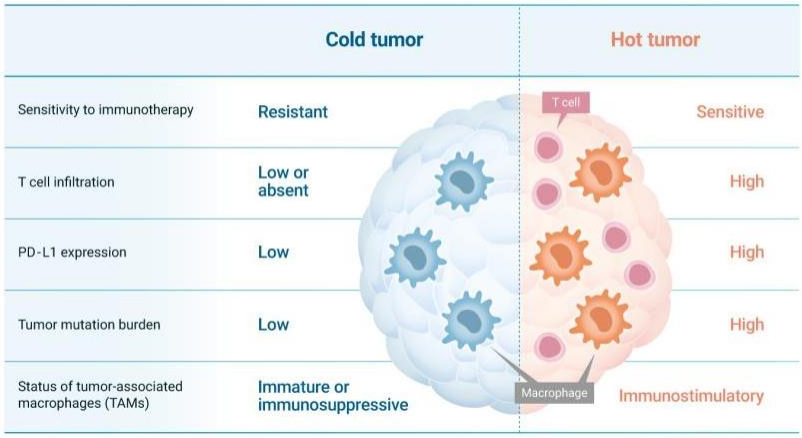

Figure 5. Characteristics of cold tumour and hot tumour (34).

4.1.2. Viruses

Viruses are the most common microorganisms on our planet. They are obligate intracellular parasites, which means that although they have their own genetic information, they need to enter a living cell to obtain energy and use the machinery necessary to replicate, make copies of themselves and transmit their properties to their descendants (35,36). In most cases, the virus kills the infected cell and can cause damage to the host (35).

In order to be able to interact and bind to the host cell, they contain viral antireceptors or viral attachment proteins (VAP) on their surface. These molecules also stimulate the synthesis of neutralising antibodies (37).

Viruses are the smallest infectious agents in nature, ranging in size from 20 nm to 400-800 nm in diameter (38). Its structure consists of a nucleic acid (a single or double strand of DNA or RNA), a capsid (a protective structure that protects the nucleic acid and is

made up of capsomers) and in some cases an envelope (a lipid bilayer, usually made up of the host cell glycoproteins. These help to recognise and enter the host cell) (35,36).

The viral genome replicates faster than the human genome. In order to replicate, the first phase is **approach** and **attachment**, where the virus must recognise the host cell by electrical currents on the surface of the virus. To attach to the cell, the virus binds to receptors on the surface of the host cell. The variety of receptors explains why some viruses show tropism for a certain tissue and why some cause localised infections.
Some viruses require binding to more than one receptor; other viruses have a wide range of organs and tissues affected by infection, making it difficult to identify which receptors are key to virus' attachment (35,37).

When the virus binds to the receptor, it induces intracellular signalling, cytokine secretion, apoptosis and stimulation or suppression of the immune system. Cytokines stimulate the Janus kinase (JAK) and the signal transducer and activator of transcription (STAT) pathways. Binding of the cytokine to its receptor in the host cell, induces receptor dimerization and activation of the associated JAK kinase, which in turn phosphorylates STAT proteins in the cytosol. After forming a homodimer, STAT proteins translocate to the nucleus to control gene expression (37).

Once the virus is attached, the genetic information enters the host cell. The capsid is then **disassembled** to release the genetic material. **Intracellular transport** of viral material using the host cytoskeleton and microtubules, is used to carry out **transcription and translation** to obtain mRNA and the synthesis of "early proteins". These proteins **replicate the genetic material.** "Late proteins" are then synthesised to **assemble** the new viral particles, which are then **released** (35,37).

The virus that is produced by the assembly process inside the host cell and has the ability to infect the cell is called a virion or mature/ complete viral particle (35–37).

4.1.2.1. Potential viruses used in therapy

Oncolytic viruses can be divided into two categories: intact viruses and genetically modified viruses. Although intact viruses such as reovirus or Newcastle virus (non-human virus) are still in use, genetically modified viruses are increasingly available. The motive is not only to reduce their pathogenicity, but also to improve the properties of the viruses in order to achieve better efficacy and fewer treatment limitations (39).

Although a wide range of viruses can be found, DNA viruses tend to be more widely used than RNA viruses (*Information from Dr. Ramon Alemany*). **Table 2** shows the classification of oncolytic viruses according to different characteristics. Currently, the most commonly used oncolytic viruses in studies are adenovirus, reovirus, vaccinia, Newcastle and herpes viruses (40,41).

GENETIC MATERIAL	CAPSIDE SYMMETRY	NAKED/ ENVELOPED	FAMILY	ONCOLYTIC VIRUS
DNA	ICOSAHEDRON	NAKED	Parvovirus	ParvOryx
			Adenoviridae	Oncorine
				VCN-01
				DNX-2401
				ColoAd
				Oncos102
				CG0070
				LOAd703
		ENVELOPE	Herpesviridae	T-Vec
				G47Δ
				RP1,2,3
				HF10
				HSV1716
				G207
	COMPLEX	ENVELOPE	Poxviridae	JNX-594
				GL-ONC1
RNA	ICOSAHEDRON	NAKED	Reoviridae	Reolysin
			Picornaviridae	Cavatak
				Rigvir
	HELICOIDAL	ENVELOPE	Rhabdoviridae	VSV-GP
				VSV-INF
			Paramyxoviridae	MV-CEA
				MV-NIS
				NDV
			Retrovirus	Toca511

Table 2. Classification of the most important oncolytic viruses. Adapted from personal communication *Dr. Ramon Alemany*.

Even though oncolytic viruses share a common mechanism of action, each virus may have different characteristics that are critical in choosing one or the other, depending on the target (28,29).

Size: large viruses are more suitable for gene insertion as they can carry larger transgenes (genetic material that has been transferred from one organism to another) but are less likely to cross physical and brain barriers. Small viruses, on the other hand, can penetrate easily but are less susceptible to genetic manipulation (42).

Genetic material: DNA viruses are easier to manipulate genetically and can be controlled with promoters, but they are more expensive and there is a greater chance of finding pre-existing antibodies that neutralise their effect. On the other hand, those with RNA material, replicate faster and more effectively, which has a greater local effect. There is less chance of finding pre-existing antibodies, which may be an advantage for intravenous administration. They also lyse more easily because they can kill tumour cells without reaching the nucleus. However, they are less selective for tumours than DNA viruses and are more difficult to manipulate genetically (28,42).

The presence of an envelope makes the virus easier to eliminate by the immune system (42). However, they can also facilitate the entry of the virus into tumour cells through the surface receptors they contain.

In addition to these characteristics, a virus with low pathogenicity must be selected or, if this is not possible, genetic modifications can be used to reduce it. Otherwise, it may cause damage to the host (*Information from Dr. Ramon Alemany*). There must also be a balance between the effect of the virus and its elimination by the immune system. For highly immunopathogenic viruses, intratumoral administration is usually used to avoid systemic effects and fast clearance. In addition, the stability of the virus must ensure that it can be prepared, administered and stored (42).

Finally, interactions between tumour cells and the TME must be considered. In many cases, viruses are modified to reduce TME from reducing effectiveness (33).

4.1.3. Immunological factors

The immune system makes it possible to distinguish between what is intrinsic to the organism and that what is not. Its function is to prevent pathogens from invading or, if they do invade, to stop and eliminate them so that they do not damage the organism. (21).

A distinction can be made between the innate and adaptive systems.

Innate immunity is non-specific, unaltered by repeated infections, fast and the first line of defence (40). It consists of epithelial barriers and various types of cells such as monocytes, granulocytes (neutrophils, basophils and eosinophils), mast cells and dendritic cells (DC) and Natural killer (NK) cells (21,43).
The innate system is also activated in the presence of PAMPs (pathogen-associated molecular patterns), which are recognised by PRRs (pattern recognition receptors). This binding promotes inflammation and activation of the immune system (44).

Adaptive immunity is specific. It has a slower response time and generates memory antibodies that can recognise the same pathogen more quickly and directly if it reappears (21). It is composed of T lymphocytes and B lymphocytes.

T lymphocytes are produced in the bone marrow and migrate to the thymus to mature and differentiate. There are different groups, cytotoxic T cells $CD8^+$, which neutralise and destroy pathogens, T helper cells $CD4^+$ that help cytotoxic T cells and B cells, and regulatory T cells $CD4^+$, $CD25^+$ which suppress and regulate the activation of immune system (21,45).
B lymphocytes are located in the bone marrow and they protect the body by secreting antibodies/immunoglobulins located in their membrane.

If a pathogen attempts to enter the body, innate immunity is activated and tries to eliminate the pathogen. If this is overcome, adaptive immunity is activated. So, in many cases innate and adaptative immunity work together to defend the organism (21,46).

Although the immune system controls and eliminates tumour cells, it can also promote tumour progression through immunosuppression. As the immune system exerts selective pressure on tumour cells to kill them, the tumour cells respond by modifying the immune system to escape. This process is called **immunoediting** and as shown in **Figure 6**, can be divided into three sequential phases (46).

The first is immunosurveillance, where the immune system has the ability to distinguish between what is part of the organism and what is not, and thus has the ability to attack cancer. Danger signals such as the natural killer group 2D (NKG2D) and calreticulin trigger the release of inflammatory signals such as interferon α/β (INFα/β), INF γ, tumour necrosis factor (TNF) and interleukin 12 (IL-12). In response, the innate system delivers NK, DC and macrophages to the tumour site and the adaptive system is activated to induce apoptosis. The result of this phase is the elimination of the tumour cells and the cessation of the immune response (21,46).

The second phase is equilibrium and consists of a constant balanced interaction between immune cells and tumour cells that have evaded the first phase (21).
Although the immune system prevents tumour growth and invasion, there are tumour cells that manage to evade immunosurveillance and enter a state of latency (46).

The last one is the scape phase. The constant pressure of tumour cells can lead to the formation of new variants with low immunogenicity that manage to escape from the immune system (21). Some of these mechanisms include downregulation or loss of major histocompatibility complex (MHC I) expression, increased expression of inhibitory molecules such as PD-L1, CTLA4 and lymphocyte-activation gene 3 (LAG-3), release of vascular endothelial growth factor (VEGF), the expression of TAMs that suppress the action of T lymphocytes and promote angiogenesis, and myeloid-derived suppressor cells (MDSC) that express mechanisms to induce immune suppression (21,46). Using these mechanisms, tumour cells modify the TME to evade from the immune system.

The majority of detectable cancers are found in the third phase, as this is when uncontrolled tumour growth occurs (46).

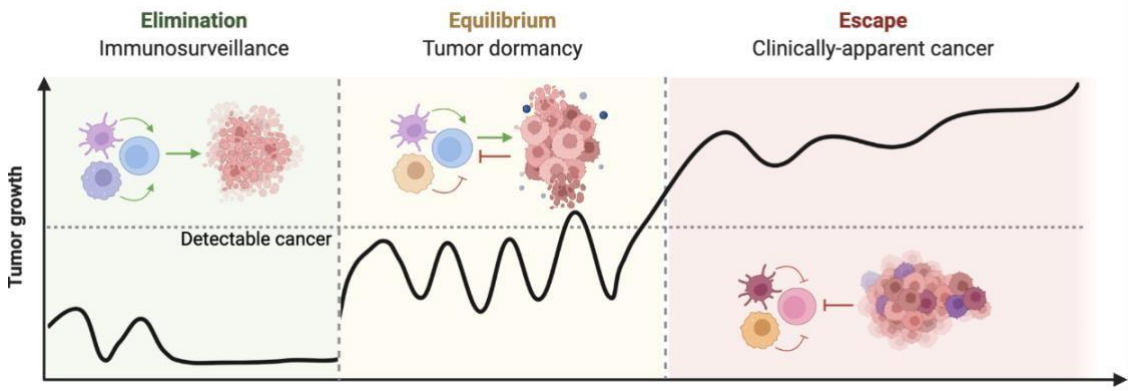

Figure 6. Representation of the three phases of immunoediting. Adapted from (47).

In order to treat cancer with immunotherapy, it is essential to understand the characteristics of the TME and its interactions, because the cells that make up the TME develop mechanisms to evade the immune system. By studying these behaviours, different strategies can be developed to attack the cancer and avoid resistance (21).

4.1.4. Mechanism of action of oncolytic virus therapy

Two main mechanisms of action can be distinguished (**Figure 7**): selective intracellular replication of the viruses, leading to the lysis of the tumour cells, and induction of the immune system (48).

These mechanisms may differ depending on the characteristics of the patient, the virus and the type of cancer. Therefore, the effectiveness of the treatment will also depend on all these factors (28,29,49).

When the virus is selectively delivered, it recognises and binds to tumour cells. The virus enters the cell and begins to replicate intracellularly. Infection triggers an antiviral response in the tumour cell, expressed as stress, leading to increased reactive oxygen species (ROS) and cytokines (especially IFNs), causing an inflammatory process and stimulating NK and DC (29).

NK, through the NKG2D receptor, releases stress-associated molecules to the affected tumour cells, causing toxicity (48).
The lysis of the tumour cell releases PAMP and DAMP, which activate DC by binding to their Toll-like receptors (TLRs) (29).

On the other hand, antigens (Ag) released by both viruses and tumours, known as tumour associated antigens (TAAs) are recognised by DC, which present the Ag to cytotoxic T cells via the MHC-1 and T cell receptor (TCR). Cytotoxic T cells are activated and bind to tumour cells to release perforins and granzymes that will cause tumour cells to burst, leading to apoptosis (29,48). In addition, T helper cells, help DC to activate cytotoxic T cells and produce cytokines that further activate NK (29).

When metastasis occurs, TAAs may contribute to the activation of new immune responses against cancer cells (28,29).

Therapy with oncolytic viruses aims to reverse the escape phase from immunosurveillance. Although there are certain mechanisms of this phase that cannot be reversed, such as the loss of MHC-I or the low Ag presentation, there are others that the virus can reverse inside the tumour, such as the non-entry of lymphocytes because there is no sign of danger or the increase or decrease of certain cells due to the presence of the virus. This last point refers to the ability of the virus to reduce regulatory cells that slow down the immune system such as regulatory T cells, M2 macrophages and MDSC, and to increase cells that activate the immune system such as M1 macrophages and DC.

So, a cold tumour that has no lymphocytes, activates lymphocytes and becomes a hot tumour. This increases the antiviral effect, but also the antitumour effect.
The therapy can work in one of two possible scenarios. In an irreversible immunoediting, the virus works by oncolysis, and in a reversible immunoediting, the virus acts by lysing and activating the immune system (*Information from Dr. Ramon Alemany*).

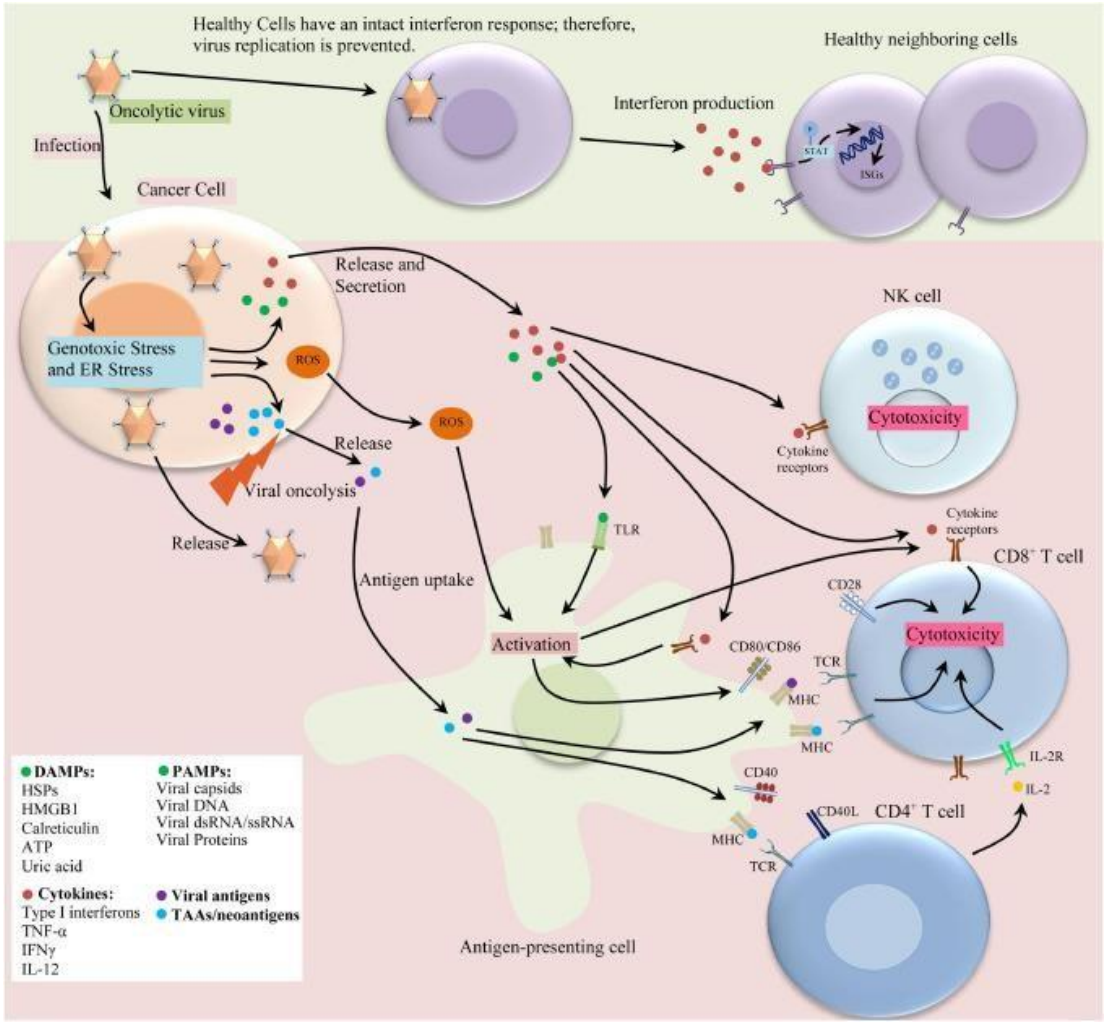

Figure 7. The induction of local and systemic anti-tumour immunity by oncolytic viruses (50).

Viruses need to **be selective** for tumour cells but not for healthy cells, so they only lyse tumour cells. There are several features that enhance selectivity.

1) One difference is that in healthy cells, the cell cycle is regulated by factors such as p16, retinoblastoma (Rb), the tumour protein P53 (TP53), known as the p53 suppressor gene, and protein kinase R (PKR). However, the tumour cells undergo changes that cause irregularities in the cell cycle, leading to increased cell proliferation. These mutations are associated with tumours growth and resistance to current therapies such as chemotherapy. Nevertheless, this scenario may be favourable for oncolytic viruses to selectively carry out their mechanism of action (29,48,51).
One example is the rat sarcoma (RAS) gene, a proto-oncogene that can become an oncogene through mutations and induce the RAS protein (**Figure 8**). This reduces the

activity of PKR, a protein that is activated during viral infections and induces apoptosis. So, when PKR is reduced, apoptosis is also be reduced, so eIF2-α-P (Phosphorylated Eukaryotic translation initiation factor 2 subunit alpha) activates translation, which leads to viral replication.
The same happens with the reduction of suppressor genes such as p53 (**Figure 8**), where apoptosis is reduced and therefore the action of oncolytic viruses is favoured (49,51,52).

Certain virulence factors and other genes that are not essential for infection but are for viral replication in normal cells can also be removed from the virus in order to promote selectivity for tumour cells (52).

2) Another factor leading to selectivity is the recognition of tumour receptors. In general, tumour cells have overexpressed receptors that are recognised by the virus (**Figure 8**). However, not all tumour cells express all or the same receptors (28,51). For example, the measles virus, enters through the surface receptor CD 46. Other viruses can enter through more than one receptor, such as coxsackie, which can enter via the intracellular adhesion molecule 1 (ICAM-1) receptor and the decay acceleration factor (DAF) receptor.
Others may have tissue tropism because their receptors are expressed in specific organs. For example, echovirus is an enterovirus that penetrates through domain 1 of the α2β1 integrin, which is overexpressed in neoplastic ovarian cells (29).

Genetic modification can be used to enhance this affinity and facilitate penetration. An example of this strategy is ICOVIR 15-iRGD, an oncolytic adenovirus that has incorporated a peptide to increase specificity. iRGD is a cyclic peptide that has an affinity for neuropilin-1 (NRP-1) integrins, they are receptors that are overexpressed in some tumours. This binding trigger NRP-1-dependent endocytosis, leading to enhanced penetration into the cell. The iRGD has other advantages such as low toxicity to normal cells and low synthesis costs (53). This oncolytic virus was developed by the research group of Dr. Ramon Alemany, in which they generated ICOVIR15 and incorporated the iRGD into its capside. They tested it *in vitro* on cancer cells and human glioblastoma cells (GBM). The result was an increase in tumour selectivity (54).

3) Other selective criteria include the IFN type I signalling pathway, which is involved in the activation of the immune system and the antiviral response of RNA viruses (as they replicate in the cytoplasm) and modified IFN-interferon-sensitive DNA viruses.

Tumour cells lack this signalling pathway in order to be able to evade the immune system (**Figure 8**). Silencing this pathway also helps oncolytic viruses to replicate inside tumour cells without being detected by the immune system (29,51).

In normal cells, antiviral pathways are activated during viral infection by IFN or by TLR recognition of PAMP. These activate the factors TNF- Receptor Associated Factor 3 (TRAF 3), IRF3 and IRF7 (Interferon regulatory factor 3 and 7) and RIG-1 (retinoic acid-

inducible gene 1), which activate the JAK-STAT pathway. This leads to further IFN release and activation of PKR to induce viral apoptosis.
In tumour cells, this pathway is compromised because IRF3, IRF7 and RIG-1 are reduced, which means that the JAK-STAT pathway is altered so that PKR is also reduced. This leads to reduced apoptosis of the virus. So, the virus replicates more selectively in these cells (29).

Although viruses have many proteins to stop IFN, if their genes are modified and removed, they will become IFN-sensitive, losing the ability to grow in a normal cell but not in a cancer cell, making the virus even more selective for cancer cells (*Information from Dr. Ramon Alemany*).

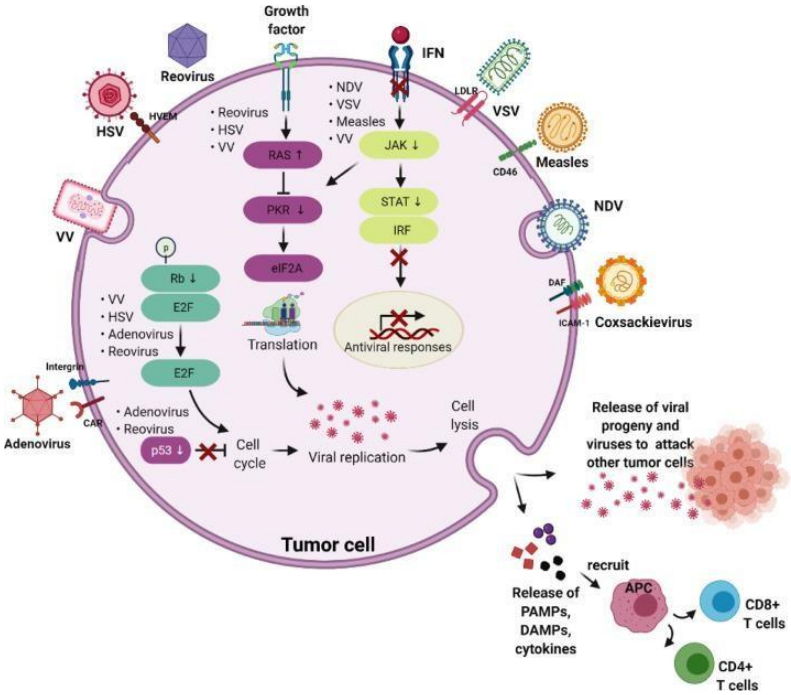

Figure 8. Tumour cell selectivity of oncolytic viruses. Firstly, the entry of oncolytic viruses is shown through unique cell surface receptors that are highly expressed is represented. In purple and white, p53 is reduced so apoptosis is also reduced, and the virus has selectivity. In dark green, Rb is phosphorylated and E2F will activate the cell cycle leading to virus replication. In purple and black, RAS is overexpressed, so PKR is reduced and so is apoptosis, so translation is activated, and virus replication is increased. Finally, in green, the IFN type I is altered, so JAK-STAT is also reduced and so is IRF, resulting in an inhibition of the antiviral response (51).

4) Although DNA viruses can be modified to be IFN-sensitive because replication process takes place in the nucleus, the strategy focuses on making genetic modifications within the nucleus.

One of the viruses that is most commonly used in this strategy is the adenovirus, because it acts on the E2F (family of transcription factors), a group of genes that encode a family of transcription genes, so they also regulate the cell cycle.

The mechanism of action of the adenovirus (**Figure 9**), starts when it reaches the cell membrane, then it injects DNA and expresses early genes causing the E1A (early 1A) protein to be produced. It enters the nucleus and binds to Rb, which forms a complex with E2F. The binding of E1A causes the release of E2F and its binding to E2 (viral promoter). This causes the expression of E2 genes that replicate DNA. A second promoter (late promoter) is then activated, which translates the late RNA into new virus capsids to form. The genetic material is packaged and accumulated inside the virus. The newly assembled virus is then released. (*Information from Dr. Ramon Alemany*, (55).

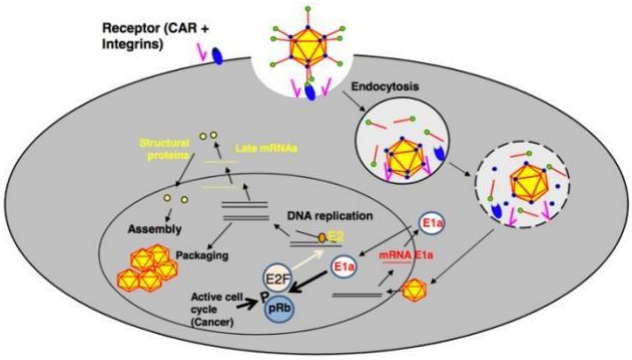

Figure 9. The mechanism of action of the adenovirus. *Information from Dr. Ramon Alemany.*

The idea to prevent the virus from replicating in healthy cells is to remove nucleotide 24 from the virus (E1AΔ24), so that E1A does not break the Rb-E2F complex and the virus does not replicate. In tumour cells, Rb is phosphorylated and therefore the complex is already broken. With this modification, it is possible to avoid replication in normal cells, further increasing selectivity (56).

5) Finally, regulating the expression of essential early viral genes with tissue- or tumour-specific promoters can enhance selectivity. This strategy restricts viral replication to those cells that express the necessary factors to initiate transcription. An example of this strategy is an oncolytic adenovirus (oAd) with a PSA (prostate-specific antigen) promoter that drives EA1 only in prostate cells expressing PSA (57).

Another mechanism of action is provided by oncolytic viruses armed with immunomodulatory transgenes. Many oncolytic viruses can incorporate exogenous sequences, so this can be useful to accommodate therapeutic immunomodulatory agent to obtain an effective boost anti-tumour immunity. Thus, they can be armed with components that enhance immunological responses. Some examples include cytokines (granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-12, IL-2 or type I IFNs), chemokines, TAAs… (57).

4.1.5. Application of oncolytic virus therapy

Routes of administration

There are different ways of administering the therapy and although more research is needed to determine which is the best option, it seems that the most common routes of administration are intratumoral (some studies suggest it's the most clinically effective)

and intravenous. However, the choice also depends on the patient's condition and the type of tumour, among other factors (28,42).

The intratumoral (IT) route is the most commonly used in preclinical and clinical trials due to its safety. It consists of direct injection into the tumour and therefore has a direct effect without affecting other organs and is less likely to produce antiviral antibodies (28,58). However, the tumour must be accessible and palpable, the dose must be adapted to the patient depending on the location and growth of the tumour, and all the cells need to be reached to avoid resistance and recurrence of cancer cells (28,42).

The intravenous (IV) route is also one of the most highly evaluated and is a good option formetastases (58) and haematopoietic cancers (*Information from Dr. Ramon Alemany*). It allows a fixed dose to be given to all patients and doesn't require direct access to the tumour. However, it has limited distribution due to the rapid clearance of the virus and dilution when administered in the blood (42).

One method being studied in animal models to improve the intravenous route is the use of "cell carriers". Mesenchymal cells are infected with the virus ex vivo and then administered intravenously to direct them to the tumour and release the virus (13,42).

Other less important routes include arterial injections, intraperitoneal, intrapleural and intravesical routes, the last 3 being useful because they are large compartments (42). The intrathecal and subcutaneous routes are currently under experimental investigation (58). Finally, the oral route is probably not an option because the virus would have many barriers, such as gastrointestinal pH or enzymes, which would reduce the effect (49).

Adverse effects and contraindications

The main side effects of this therapy are mild flu-like syndrome and local reactions such as pain, peripheral oedema, erythema, skin rash, nausea and diarrhoea (28,29). Flu-like syndrome and local reactions at the site of infection, can be avoided by administering paracetamol before starting treatment (28,40).

Other adverse effects such as anaemia, leukopenia, lymphopenia and liver dysfunction may also be observed. However, in most cases these are controlled and don’t cause serious health problems. Moreover, most viruses are genetically modified to reduce potential risks (28).

Oncolytic viruses can trigger a strong immune response, causing excessive inflammation that can damage organs (40,41). In some cases, the patient may need long-term treatment for the infection caused by the virus.

In addition, the patient must be isolated from other people when being treated with the virus. Care should also be taken by healthcare workers to avoid transmission of the virus

(41). The effect of this antiviral treatment on the effectiveness of the oncolytic therapy, and the possible interactions that may occur, have not yet been assessed (41).

In the case of contraindications, there would not be any initially. Each case is assessed in terms of the characteristics of the patient and the cancer and whether the therapy could be beneficial, so there is a benefit-risk assessment and selection criteria (28). Especially for specific populations such as pregnancy woman or immunocompromised people because studies on safety are currently insufficient (58).

Limitations/Constraints

Although oncolytic virus therapy has positive features, it also has some limitations. The most important of these are the limited delivery of the virus to the tumour with systemic administration, the poor distribution via the intratumoral route and the antiviral immune response (41).

- One of the limitations is the **low specificity of the immune system** and its activation against the virus. Inevitably, there will be an immune response against the virus itself, especially if it is a previously exposed virus, because there will be a memory response. However, this activation helps to unbalance the immune escape phase and reactivates the immune system against the virus (*Information from Dr. Ramon Alemany*).

To redirect the immune system towards the tumour and away from the virus, several studies have been carried out using BiTEs (bi-specific T-cell engagers), a type of bi-specific antibody that binds to a tumour antigen and an immune system molecule (29,59,60). A research group lead by Ramon Alemany has developed ICO15K-cBiTE, a modified OAd containing a BiTE that binds on one side to a factor overexpressed in many cancers, EGFR, and on the other side, to T-cell lymphocytes (61,62).

They then combined the therapy with CAR-T cell therapy against α- folate receptor (overexpressed in many solid tumours of epithelial origin) (63). The results were an increase in CAR-T proliferation and activity *in vitro* and a synergistic antitumour effect *in vivo* in mice (63,64).

- The use of a virus will inevitably trigger an immune response, especially if the person has previously been exposed to the virus, as **memory antibodies** can recognise, neutralise and eliminate the virus more quickly.

To avoid this, some strategies have been proposed, such as genetic modification of the viral epitopes (part of the virus that is recognised by the immune system) to obtain different serotypes that are not recognised by the immune system (65,66), and the use of nanocomplexes to encapsulate the oncolytic viruses are also being considerate (66). However, the positive aspect of having pre-existing antibodies is that if the virus is

recognised by the immune system, this can alter the escape phase and redirect the immune system against the tumour. So, these considerations need to be taken into account.

The use of immunomodulatory agents and the manipulation of viral signalling factors are also being studied in order to improve the control of the immune system (28).

- **Physical barriers** limit the spread of oncolytic viruses. This limitation can result in necrosis, calcification, hypoxia, acidosis, increased interstitial pressure and increased proteolytic activity (29).

Sometimes the intratumoral route is used to partially avoid these barriers, but this is not always possible. Moreover, this limitation is even more significant in the presence of a brain tumour or metastases, as the intratumoral route is not considered (28).

In addition, solid tumours are dense and have a **stroma**. The stroma is involved in tumour progression, invasion and immunosuppression. The main components of the stroma are cancer-associated fibroblasts (CAFs) and extracellular matrix, of which hyaluronic acid is one of the major components. In many cancers, such as melanoma, head and neck cancer, pancreatic cancer, colorectal cancer and others, hyaluronic acid and its receptor, CD44, are overexpressed. High levels of hyaluronic acid are associated with invasive and metastatic behaviour and poor cancer prognosis (67).

Incorporation of degradative enzymes such as hyaluronidase has been proposed as a strategy to overcome this limitation. The idea is to get hyaluronidase to express when the late major promoter is active, so that hyaluronidase is expressed after the virus has replicated and, in the tumour, rather than in the hepatocyte (*Information from Dr. Ramon Alemany*). The introduction of hyaluronidase not only breaks down hyaluronic acid, but also helps the delivery of chemotherapy (68).

Finally, an important and recent study carried out in 2021 shows four different strategies (**Figure 10)** incorporated into the oncolytic adenovirus VCN-11 to overcome some limitations.

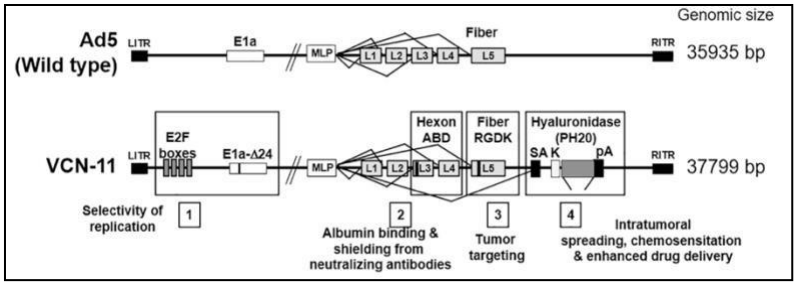

Figure 10. Schematic representation of VCN-11 adenovirus genome organization compared to human adenovirus serotype 5 (67).

It has been genetically modified by removing nucleotide number 24 (E1a-Δ24) to make it selective for tumour cells. An albumin binding domain (ABD) has been inserted into the viral hexon so that ABD binds to serum albumin and the virus is recovered by albumin avoiding virus clearance by neutralising antibodies. The KKTK binding site has been replaced by RGDK to reduce liver uptake of the virus and increase the lifetime. And lastly, hyaluronidase PH20 (recombinant human hyaluronidase) has been incorporated to degrade hyaluronic acid (67).

Monotherapy and combined therapies

Several clinical trials have been conducted to evaluate the effect of oncolytic viruses as monotherapy, and although they show some significant results, they have little chance of achieving optimal therapeutic benefit (28,69). Current research tends to focus on the use of oncolytic viruses in combination therapies (13). In fact, they are considered good candidates for combination with other lines of therapy, especially immunotherapy (70).

Combining oncolytic viruses with another **immunotherapy** seems to be one of the most interesting combinations, especially with ICB and CAR-T cell therapies, as oncolytic viruses increase selectivity(63).

In the case of combining oncolytic viruses with **chemotherapy**, it seems to improve the cytotoxic mechanisms, the persistence of the virus and improves the control of immunomodulation (42,71).

Radiotherapy induces tumour cell sensibility, which facilitates lysis by oncolytic viruses. It has been proposed to use oncolytic viruses as radiosensitizers to achieve improved efficacy and less toxic doses of radiation (57).

Finally, the administration of **two different doses of oncolytic viruses** has been proposed to increase T cell activation and virus persistence (13,42).
In a study carried out in B16 murine melanoma cell lines, it was concluded that intratumoral administration of reovirus followed by intravenous administration of vesicular stomatitis virus was more effective in controlling the tumour than if they were administered separately (72).

These are the main combination therapies being studied, but there are currently several combinations that may become first-line cancer treatments in the future.

4.1.6. Clinical studies, products on market and future perspectives

Oncolytic viruses are promising agents because they offer a unique therapeutic approach to cancer treatment. That’s why a large number of clinical studies have been or are being carried out (54).

Looking at the 408 clinical trials up to October 2021, the 80% of the total studies are in phase I and II, and only the 6% of the total are in phase III. In addition, most of the phase III clinical studies are still in their early stages. However, as can be seen in **Table 3**, 3 oncolytic viruses are currently on the market, Oncorine, Imylgic and Delytact (41).

Product	Oncorine (Recombinant human adenovirus type 5)	Imlygic (T-VEC)	Delytact (G47Δ)
Virus type	Human adenovirus type 5	Herpes simplex virus type I	Herpes simplex virus type I
Gene construction	Deleted E1b-55kd, E3 gene fragment (78.3-85.8 mu)	Deleted the ICP34.5 and ICP47 regions of herpes simplex virus type I, inserted GM-CSF gene	Deleted the γ34.5 gene and the 312 bp base of α47 gene; Insert ECOL-I LacZ gene into ICP6 (UL39) region
Goal of gene construction	Deletion of E1B-55KD: the virus could selectively infect and multiply tumor cells with dysfunction of RB/P53 pathway Deletion of part of E3 gene: enhanced immune induction ability of virus, induced lymphocyte infiltration of lesions, and virus entering vascular system could be more easily cleared by immune recognition, enhancing safety	Deleted the γ34.5 gene of HSV-1: increased tumor cell infection specificity Insertion of GM-CSF gene: enhanced antigen delivery capacity	Knockout of the γ34.5 gene and the 312 bp base of α47 gene to eliminate the neurotoxicity of the virus, improve the replication and reproduction capacity of the virus Insertion of ECOL-I LacZ gene into ICP6 (UL39) region leads to inactivation of nucleotide reductase (RR), allowing the virus to reproduce only in tumor cells, improve the oncolytic effect
Clinical application	Approved indications: advanced head and neck cancer, nasopharyngeal carcinoma Related research: liver cancer, MPE, pancreatic cancer, cervical cancer, gastric cancer, etc.	Approved indications: advanced melanoma Related research: liver cancer, MPE	Approved indication: glioma Related research: breast cancer
Examination and approval	NMPA	FDA	MHLW
Approval time	2005.11.04	2015.10.27	2021.06.11

MPE, malignant pleural effusion; HSV-1, herpes simplex virus type 1; GM-CSF, granulocyte-macrophage colony-stimulating factor; NMPA, National Medical Products Administration; FDA, U.S. Food and Drug Administration; MHLW, Ministry of Health, Labor, and Welfare.

Table 3. List of oncolytic virus products on the market (41)

H101 (Oncorine) is an oncolytic adenovirus approved by the National Medical Products Administration (NMPA) in Xina in 2005 for nasopharyngeal carcinoma in combination with chemotherapy (cisplatin and 5-fluorouracil) (73). Its mechanism of action is based on selective replication in tumour cells with Rb/p53 pathway dysfunction and enhanced immune induction due to partial gene deletions.

T-VEC (Imlygic) is an oncolytic herpes simplex virus type I approved by the US Food and Drug Administration (FDA) in 2015 for unresectable advanced melanoma. It's a modified virus with deletions and the incorporation of GM-CSF, resulting in an inhibition of neurovirulence and the enhancement of anti-tumour activity.
It is the first oncolytic virus to be commercialised and is now available in Australia, the UK, Germany, Sweden and France (13,29,41). In Spain, it's been approved but not funded, so it's not being commercialised (74).

Finally, G47Δ (Delytact) is an oncolytic herpes simplex virus type I approved by the Ministry of Health, Labor and Welfare (MHLW) in Japan for the treatment of glioma in 2021. This oncolytic virus has some genes deleted to eliminate neurotoxicity and improve replication, and the ECO-I LacZ gene has been incorporated to selectively replicate in tumour cells (41).

A fourth oncolytic virus called Rigvir has also been mentioned as another approved oncolytic virus. However, there's almost no information about it, and the evidence for its

effectiveness is poor. Because of its controversy, many articles don't include it. (*Information from Dr. Ramon Alemany*).

Analysing data from clinical studies until October 2021, most of the oncolytic viruses are DNA, being adenovirus, herpes simplex virus and vaccinia virus the third most commonly used. For RNA viruses, reovirus, coxsackievirus and poliovirus were the most used (**Figure 11A**). IT is the most common route of administration, followed by IV (**Figure 11B**).

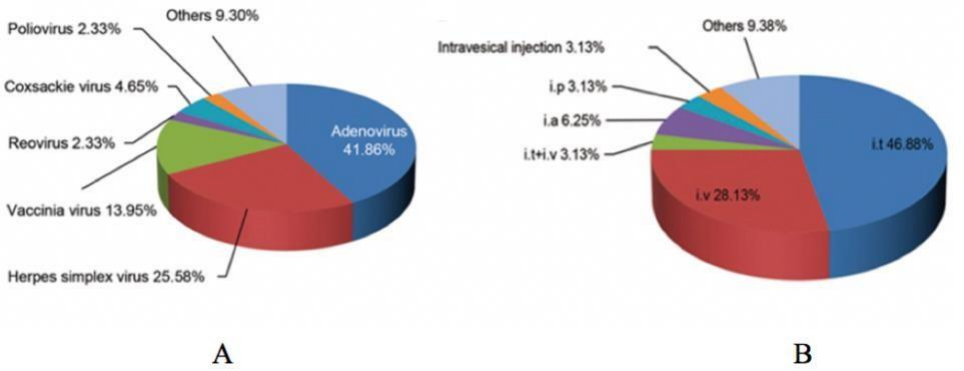

Figure 11. A. Proportion of oncolytic virus types in clinical studies. B. Proportion of drug administration routes in clinical studies of oncolytic virus products (i.t= intratumoral, i.v=intravenous, i.a= intra-arterial, i.p=intraperitoneal) (41).

There are several clinical trials underway in the paediatric population. In particular in brain tumours, as one third of all cancer diagnoses in children are malignant brain tumours. Five viruses are being tested in paediatric brain tumours: herpes simplex virus, reovirus, measles virus, poliovirus and adenovirus. Clinical trials have shown a favourable safety profile, especially compared to chemotherapy and radiotherapy. The only limitation is that oncolytic viruses can cause inflammation in the brain tumour area, but if this can be overcome, oncolytic viruses may be a potential therapy (75).

Looking at clinicaltrials.gov, 18 new trials using oncolytic viruses started this year. 5 of them are focused on children. So, the future perspective seems to be focused on carrying out new clinical trials and investigations to improve this therapy and become one of the most useful cancer treatments (76).

5. CONCLUSIONS

This therapy has shown good oncolysis and immune system activation against many types of tumours. In addition, side effects appear to be limited and contraindications may be few. However, many studies are still outgoing, and many tasks need to be established and improved before new oncolytic viruses can be brought to the market. Through this review, several conclusions have been confirmed.

- Even though there is so much knowledge about cancer, more research needs to be carried out to improve the treatments that are actually available.

- Many factors of the patient as well as the characteristics of the tumour and virus must be considered when using the therapy.

- This therapy has many effects that may provide different strategies from those of the conventional therapies to fight cancer.

- Strategies to improve the effect of oncolytic virus remains to be explored in future studies.

- The therapy has many limitations, such as route of administration, biodistribution, neutralising antibodies and physical barriers, which suppose interesting challenges that remain to be investigated.

- Although lots of studies have tested oncolytic viruses as monotherapy, for now, combined therapy seems to provide better results.

- Further studies on safety and effectiveness must be carried out to make it a safe therapy for the patient, including the family and healthcare staff.

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ANNEX I

To obtain more information about this therapy was contacted Dr Ramon Alemany, who leads the Cancer Virotherapy Group at the Catalan Institute of Oncology (ICO) and the Institute of Biomedical Research of Bellvitge (IDIBELL) to interview him about the therapy. It was really helpful, so an interview transcription has been done.

Mireia: Good morning, Dr. Ramon Alemany, thank you for accepting this interview, is a pleasure for me to be today with you.
First of all, I would like to know how this therapy started to be used.

Ramon: The first therapies against cancer were used in Egypt 4000 years ago. They infected tumours by piercing the tumours they saw in the body with a kind of spike to provoke suppuration and thus infection. This is the first existing treatment for cancer. They used to infect with bacteria, but when viruses were discovered at the beginning of the last century, they began to use viruses as well. For example, the rabies virus was the first vaccination campaign and it was also used to spite tumours. Then, all the discovered viruses were also used in tumours, including animal's viruses. However, this treatment was not controlled and often led to the death of the patient.

Adenovirus was discovered in 1935 and in 1956 was used to inject tumours. Then pharmacy, chemistry and chemotherapy era became, and this type of treatment was no longer used. They started to see that chemotherapy was having positive results with tumours that didn't have cure, especially leukaemia.

Mireia: When did you and your group investigation start studying this therapy?

Ramon: We started in 1993 with gene therapy using adenovirus because we wanted to re-establish the expression of oncogenes. For example, the KRAS mutation, KRAS is a gene that transmits a proliferation signal, and in many tumours it's mutated so that there's permanent expression. So, it is a protein that normally has a regulated function, but with this mutation it gives a proliferation signal. We used gene therapy to inhibit the expression of this oncogene. Then suppressor genes were discovered, such as p53. This is involved in tumour formation because when DNA has mutations, some functions are destroyed. So, if there's this destruction in genes that stop cell proliferation, there's a greater chance of uncontrolled proliferation. We tried to transfer these tumour suppressors with an adenoviral vector, so viruses were first used as vectors to do gene therapy against cancer. But the problem with this gene transfer was that it only got to where you injected the needle with the vector, so the cells that were far away from the injection didn't get the effect. So, the tumour transduction was incomplete. So, we concluded that vectors should replicate, and we returned to the idea of using intact viruses for their replication capacity. So, we changed our thinking and in 1994 we started to do virotherapy with adenovirus. We started to design viruses with specific anti-cancer properties, but we didn't use intact

adenovirus as we had done before, but we started to make mutations in the virus to increase selectivity.

Mireia: So, intact viruses are no longer used?

Ramon: Yes, they are also used, but when you start to learn the biology of that virus and cancer, you try to do genetic modifications to increase efficacy, selectivity, potency and security.

Some viruses are difficult to modify, so intact viruses are used. For example, reovirus, is used as intact virus because is what we call a segmented virus, because its genome is separated in small pieces of RNA. Other examples are Newcastle or Coxsackie virus.

So, there are 2 main groups of viruses, DNA and RNA. RNA viruses are more difficult to being genetically modify. In addition, the increase of selectivity is also more difficult, because RNA doesn't reach the nucleolus. In this part, there are mutations, cell cycle, the expression of different proteins… so more mechanisms can be used to create selectivity. DNA cells can also be driven by promoters, but RNA can't. However, RNA is faster.

Mireia: Which is the mechanism of action of oncolytic viruses?

Ramon: The mechanism of action is always direct lysis and then the induction of the immune response. This second idea is delicate because it's inevitable, as the virus stimulates the immune system, especially in people who has been in exposed to that virus previously.
The positive part is that if the virus is recognised by the immune system, this can change the escape phase and induce again the immune system against the tumour that was previously inhibited by the tumour.

Virus features must be considering because each virus has different mechanisms of entrance, selectivity mechanism, different interactions…

Mireia: Viruses cause infections, so, there is any chance of getting an infection using this therapy?

Ramon: Yes, but this is taking into account because the viruses used for this therapy are selected due to their small virulence. In addition, genetic modifications to reduce this risk can also be done, such as removing virulence genes.

Mireia: Which are the most used viruses?

Ramon: There is a preference for DNA viruses, and the most used are adenovirus (DNA), herpes virus (DNA) and vaccinia (RNA). When using vaccinia, attenuated strains are chosen.
One advantage of adenovirus is that it has a single early gene (E1), whereas herpesvirus and vaccinia have many early genes, making them more difficult to genetically modify.

Mireia: Are there any oncolytic viruses approved?

Ramon: Yes, there are 3, Oncorine, Imlygic and Delytact.

Mireia: Which is the difference between RNA and DNA in their mechanism of action?

Ramon: RNA viruses and DNA that are sensitive to interferon are generally eliminated by the interferon pathway. The interferons act in the membrane, activating the JAK-STAT transduction signalling pathway and then activating the synthesis of proteins such as PKR. This protein recognises double- stranded RNA and phosphorylates an elongation factor, which stops protein synthesis and makes the cell refractory.
Viruses replicate in tumour cells because this type of cell is prepared not to stop the protein synthesis.

So those sensitive-interferon viruses, are naturally oncolytic virus for their selectivity for tumour cells.

Some tumour cells have mechanisms to block interferon, so you can also modify these mechanisms to make them sensitive to interferon.

However, for DNA viruses, as they reach the nucleolus, the investigators try to modify mechanisms of the nucleolus.

With adenoviruses, for example, we are trying to take advantage of their mechanism in the nucleolus. Adenovirus enters through the membrane, injects DNA, expresses early genes, which express early proteins, E1. E1 protein binds to Rb, which breaks the bond between Rb and E2F, E2F binds to E2 (promoter) and E2 genes, which make replicases, and DNA is replicated. The late promotor then activates late RNA, and these forms capsids. The new genome is packaged and released.

All tumours have E2F because it is essential for the cell cycle, so by making modifications, we can repress the promoter and the virus will not replicate because E2 will not be expressed.
In tumour cells, Rb is phosphorylated because the cycle is activated, so Rb-E2F is not together.
So, in a normal cell, the virus replication is inhibited because it cannot separate Rb-E2F and in tumour cells, the virus will replicate because E2F is separated from Rb.

Mireia: And what about the virus biodistribution?

Ramon: The virus is big, and when is administrated in bloodstream, the virus doesn't come out of the blood, it only comes out when there are fenestrations, and that happens in the spleen, in the liver and in the tumour.
In the spleen there is no problem because this virus passes through the attachment between the receptor (coxsackie adenovirus receptor) and the fibber. This receptor is typically in an epithelial cell but is not found in blood cells or in lymphocytes so it will not enter in these cells.
In the case of the liver, there is a problem because the virus can attack the hepatocytes. However, modifying Rb, we can avoid this problem.

One problem is the macrophages (Kupfer cells) because they eliminate the virus. Nowadays, there is no a solution for this problem. So little doses arrive to the tumour. What we are investigating is using mesenchymal cells to transport the virus to the tumour through tropism, avoiding the elimination.

We are also trying modifications in the capsid to have better infection of the tumours. An example is using a union domain of albumin in the exon of the virus to give ABD. So, the virus is recovered by albumin avoiding the blockage of antibodies.

Mireia: Which routes of administration are used?

Ramon: The most commonly used are intratumoral and intravenous. Intravenous is the preferred for metastasis, especially micrometastases because they are not seen.

Mireia: This therapy is proposed as monotherapy or in combination with other drugs?

Ramon: For now, they would be combined because cancer is very complex. Ideally, they should be used as monotherapy.

Mireia: Are there any contraindications for any population group?

Ramon: In principle, no, each case has to be assessed on its own merits and the selection criteria have to be evaluated.

Mireia: Which are the limitations of this therapy?

Ramon: the principal ones are biodistribution and the immunoedition.

Mireia: What is the dosage regimen that you are doing?

Ramon: Now we are doing one, the idea in phase II is to do more, separated in 3-4 months to wait for the levels of antibodies to regulate as they are increasing a lot. In the case of incorporating albumin, it could be done every few months, as it would protect against antibodies.

Mireia: Economically, what impact would it have?

Ramon: It is expensive because the viruses have to be produced with GMP, there are few companies that produce them, they have to be purified a lot... but if it works well, in the future, bigger companies will do mass production and the price will go down.

Mireia: Personally, do you think that this therapy can become a first-line treatment?

Ramon: It's difficult because the virus is bigger than an antibody, the immune system is complex... but yes, we work for it, to give a few months of survival to the patients who suffer from it.

ANNEX II

Table 4.A. The 18 new clinical trials that are overgoing this 2023 (from 01/01/2023 to 24/05/2023) (76).

	Title	Status	Study Results	Conditions	Interventions	Locations
1	A Clinical Study on Oncolytic Virus Injection (R130) for the Treatment of Relapsed/Refractory Cervical and Endometrial Cancer	Recruiting	No Results Available	•Cervical Cancer •Endometrial Cancer •Advanced Cancer	•Drug: Recombinant oncolytic herpes simplex virus type # (R130)	•Shanghai Tenth People's Hospital, Shanghai, Shanghai, China
2	A Study of CodaLytic, an Intratumoral Oncolytic Virus, in Patients With Breast Cancer	Not yet recruiting	No Results Available	•Breast Neoplasms •Neoplasm Metastasis	•Biological: CodaLytic	•Dana Farber Cancer Institute, Boston, Massachusetts, United States •Gabrail Cancer Center, Canton, Ohio, United States
3	Study to Assess the Safety and Preliminary Efficacy of STI-1386 Oncolytic Virus in Relapsed or Refractory Solid Tumors	Not yet recruiting	No Results Available	•Cancer •Cancer of Pancreas •Sarcoma •Hepatic Metastasis •Solid Tumor	•Drug: STI-1386	
4	A Clinical Study on Oncolytic Virus Injection (R130 OV) for the Treatment of Relapsed/Refractory Head and Neck Cancer	Recruiting	No Results Available	•Head and Neck Cancer •Esophageal Cancer •Otorhinolaryngologic Neoplasms •Ear Cancer •Nose Cancer •Laryngeal Cancer •Pharyngeal Cancer	•Drug: Recombinant oncolytic herpes simplex virus type 1 (R130)	•Eye & ENT Hospital of Fudan University, Shanghai, China
5	A Clinical Study on Oncolytic Virus Injection (R130) for the Treatment of Relapsed/Refractory Bone and Soft Tissue Tumors	Recruiting	No Results Available	•Osteosarcoma •Sarcoma •Sarcoma,Soft Tissue •Bone Tumor	•Drug: Recombinant oncolytic herpes simplex virus type # (R130)	•Xi'an Honghui Hospital, Xi'an, Shaanxi, China
6	A Clinical Study on Oncolytic Virus Injection (R130) for the Treatment of Advanced Solid Tumors	Recruiting	No Results Available	•Sarcoma •Carcinoma •Breast Cancer •Pancreatic Cancer •Colorectal Cancer •Gastric Cancer •Liver Cancer •Lung Cancer •Gynecologic Cancer	•Drug: Recombinant oncolytic herpes simplex virus type 1 (R130)	•Xuzhou Second People's Hospital, Shanghai, Jiangsu, China
7	OH2 Oncolytic Viral Therapy Via Transcatheter Intraarterial Infusion in Patients With Advanced Liver Cancer	Recruiting	No Results Available	•Advanced Liver Cancer	•Biological: OH2 injection	•Tongji Hospital, Tongji Medical College of Huazhong University of Science and Technology, Wuhan, Hubei, China
8	First-line Maintenance of OH2 injection for Advanced Colorectal Cancer	Not yet recruiting	No Results Available	•Advanced Colorectal Carcinoma	•Biological: OH2 •Drug: Capecitabine •Drug: Bevacizumab	•National Cancer Center/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, Beijing, China
9	Safety and Efficacy of KM1 in Subjects With Recurrent or Refractory Ovarian Cancer	Not yet recruiting	No Results Available	•Ovarian Cancer	•Biological: KM1 •Drug: Chemotherapy	•Tongji Hospital of Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, China
10	Oncolytic Adenovirus TILT-123 and Avelumab for Treatment of Solid Tumors Refractory to or Progressing After Anti-PD(L)1	Recruiting	No Results Available	•Melanoma •Head and Neck Squamous Cell Carcinoma	•Biological: TILT-123 •Drug: Avelumab	•Docrates Cancer Center, Helsinki, Finland

	Title	Status	Study Results	Conditions	Interventions	Locations
11	A Study of ONCOS-102 in Combination With Other Novel Immune-therapies in Advanced Treatment-resistant Melanoma Patients	Not yet recruiting	No Results Available	•Melanoma	•Biological: ONCOS-102 •Biological: Balstilimab	
12	RP2/RP3 in Combination With Atezolizumab and Bevacizumab for the Treatment of Patients With CRC	Not yet recruiting	No Results Available	•Refractory Metastatic Colorectal Cancer •pMMR •MSS	•Biological: RP2 •Biological: RP3 •Biological: atezolizumab •Biological: bevacizumab	•Mayo Clinic Phoenix AZ, Phoenix, Arizona, United States •USC Norris Comprehensive Cancer Center, Los Angeles, California, United States •Mayo Clinic Jacksonville FL, Jacksonville, Florida, United States •Moffitt Cancer Center, Tampa, Florida, United States •Mayo Clinic Rochester MN, Rochester, Minnesota, United States •University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, United States •University of Cincinnati Medical Center, Cincinnati, Ohio, United States •University of Pennsylvania, Abramson Cancer Center, Philadelphia, Pennsylvania, United States •MD Anderson Cancer Center, Houston, Texas, United States •University of Washington Seattle Cancer Care Alliance, Seattle, Washington, United States •and 12 more
13	Study of Nab-Paclitaxel and Gemcitabine and Plus/Minus VCN-01 in Patients With Metastatic Pancreatic Cancer	Recruiting	No Results Available	•Pancreatic Adenocarcinoma •Metastatic	•Drug: Nab-paclitaxel •Drug: Gemcitabine •Genetic: VCN-01	•University of California - Davis Cancer Center, Sacramento, California, United States •University of Lousville - Brown Cancer Center, Louisville, Kentucky, United States •Weill Cornell Medical Center, New York, New York, United States •Hospital Duran i Reynals (ICO), Hospitalet de Llobregat, Barcelona, Spain •Hospital Universitario Marqués de Valdecilla, Santander, Cantabria, Spain •Hospital Universitari Vall d'Hebron, Barcelona, Spain •Hospital Gregorio Marañon, Madrid, Spain •Hospital Universitario Ramon y Cajal, Madrid, Spain •Hospital Universitario 12 de Octubre, Madrid, Spain •Hospital Universitario Virgen de la Victoria, Málaga, Spain •Hospital General Univesitario de Valencia, Valencia, Spain •Hospital Miguel Servet, Zaragoza, Spain
14	Oncolytic Virus Ad-TD-nsIL12 for Primary Pediatric Diffuse Intrinsic Pontine Glioma	Recruiting	No Results Available	•Oncolytic Virus •Diffuse Intrinsic Pontine Glioma •Adverse Drug Event	•Biological: Ad-TD-nsIL12	•Sanbo Brain Hospital, Capital Medical University, Beijing, Beijing, China
15	Oncolytic Virus Ad-TD-nsIL12 for Progressive Pediatric Diffuse Intrinsic Pontine Glioma	Recruiting	No Results Available	•Oncolytic Virus •Diffuse Intrinsic Pontine Glioma •Adverse Drug Event	•Biological: Ad-TD-nsIL12	•Sanbo Brain Hospital, Capital Medical University, Beijing, Beijing, China

	Title	Status	Study Results	Conditions	Interventions	Locations
16	HSV G207 With a Single Radiation Dose in Children With Recurrent High-Grade Glioma	Not yet recruiting	No Results Available	•Neoplasms •High Grade Glioma •Glioblastoma Multiforme •Malignant Glioma of Brain •Anaplastic Astrocytoma of Brain •High-grade Glioma •Anaplastic Glioma •Giant Cell Glioblastoma	•Drug: Biological G207	•Children's of Alabama, Birmingham, Alabama, United States
17	RP3 in Combination With 1L or 2L Therapy in Patients With Locally Advanced Unresectable or Metastatic HCC	Not yet recruiting	No Results Available	•Locally Advanced Hepatocellular Carcinoma •Recurrent Hepatocellular Carcinoma •Metastatic Hepatocellular Carcinoma	•Biological: RP3 •Biological: atezolizumab •Biological: bevacizumab	•University of Maryland Medical Center, Baltimore, Maryland, United States •Roswell Park Comprehensive Cancer Center, Buffalo, New York, United States •University of Cincinnati, Cincinnati, Ohio, United States •University of Pennsylvania Abramson Cancer Center, Philadelphia, Pennsylvania, United States •Université Claude Bernard Lyon 1, Centre Hospitalier Lyon Sud, Lyon, France •CHU Bordeaux, Pessac, France •Centre Eugène MARQUIS, Rennes, France •Uniklinikum Heidelberg National Center for Tumors Heidelberg (NCT), Heidelberg, Germany •University Hospital Leipzig Clinic and Polyclinic for Oncology, Gastroenterology, Hepatology, Pneumatology, Infectiology, Leipzig, Germany •Leeds Teaching Hospital NHS Trust / St James's University Hospital, Leeds, United Kingdom •Clatterbridge Cancer Center NHS Foundation Trust, Liverpool, United Kingdom •Oxford University Hospitals NHS Foundation Trust, Oxford, United Kingdom

	Title	Status	Study Results	Conditions	Interventions	Locations
18	Study of RP3 in Combination With Nivolumab and Other Therapy in Patients With Locoregionally Advanced or Recurrent SCCHN	Not yet recruiting	No Results Available	•Squamous Cell Carcinoma of Head and Neck •Locally Advanced Head and Neck Squamous Cell Carcinoma •Recurrent Head and Neck Squamous Cell Carcinoma	•Biological: RP3 •Other: CCRT(concurrent chemoradiation therapy) •Other: carboplatin and paclitaxel •Biological: nivolumab	•University of California San Diego, UCSD, La Jolla, California, United States •USC Norris Comprehensive Cancer Center, Los Angeles, California, United States •UCLA Medicine Division of Hematology-Oncology, Los Angeles, California, United States •University of Iowa Hospitals and Clinics, Iowa City, Iowa, United States •University of Cincinnati Medical Center, Cincinnati, Ohio, United States •Cleveland Clinic, Cleveland, Ohio, United States •Thomas Jefferson University City Center and Abington, Philadelphia, Pennsylvania, United States •University of Pittsburgh Medical Center, UPMC, Pittsburgh, Pennsylvania, United States •Jefferson Health Abington Asplunhd Cancer Pavillion, Willow Grove, Pennsylvania, United States •Sarah Cannon Research Institute, Nashville, Tennessee, United States •and 24 more

ANNEX III

Table 4.B. Paediatric oncolytic virus therapy clinical trials for the brain tumour treatment (75).

Table 2.

Pediatric Oncolytic Virotherapy Clinical Trials

Class	Virus (Dose)	Phase; Status	Delivery/ Location	Combinations	Age (Years)	Disease	Novel Aspects; Key findings	NCT
Herpes Simplex Virus	HSV1716 (10^5 PFU)	1; terminated	Peri- and intratumoral after tumor resection	IV dexamethasone prior to and 6 and 12 hrs post-surgery	12–21	Refractory/ recurrent HGG	Well tolerated but trial retracted after 2 pts enrolled	NCT02031965
	HSV G207 (10^7 or 10^8 PFU)	1; completed	IT infusion over 6 hrs via 3–4 catheters	5 Gy radiation to tumor within 24 hrs after virus	3–18	Progressive/ recurrent supratentorial malignant glioma	First completed trial of OV for pediatric brain tumor; 12.2 month median overall survival. 5/12 patients lived ≥18 months post treatment. 10^8 PFU safe and tolerable. Marked increase in TILs	NCT02457845
	HSV G207	1; recruiting	IT via catheter	5 Gy radiation to tumor within 24 hrs of virus	3–18	Refractory/ recurrent malignant cerebellar tumors; includes LMD	First OV delivered via catheter to the cerebellum; first trial of oHSV for infratentorial tumors	NCT03911388
	HSV G207 (10^8 PFU)	2; not yet recruiting	IT infusion over 6 hrs via ≤4 catheters	5 Gy radiation to tumor	3–21	Progressive/ recurrent malignant HGG	First phase 2 trial of OV in children	NCT04482933
Adenovirus	DNX-2401 (≤ 5×10^{10} viral particles in 1mL)	1; completed	IT infusion via catheter in cerebellar peduncle	Standard radiation and/or chemotherapy 3–4 wks after virus	1–18	Naïve DIPG	Including OV as upfront therapy; Safe and tolerable. All patients showed reduced tumor volume	NCT03178032
	AloCELYVIR (500 cells/kg)	1b/2; recruiting	IV infusion; weekly × 8	Radiotherapy for naïve DIPG	1 – 21	Naïve DIPG; Relapsed/refractory MB	Cellular therapy	NCT04758533
Poliovirus	PVSRIPO (5×10^7 $TCID_{50}$)	1b; active, not recruiting	IT infusion via intracerebral CED		12 – 21	Recurrent HGG/MB/ATRT	Disease involving cerebellum, pituitary, leptomeninges, brainstem, spinal cord, or requiring ventricular access can be included at discretion of neurosurgeon	NCT03043391
Reovirus	Pelareorep	1; active, not recruiting	IV infusion over 60 min on days 3–5 of 28-day cycle × ≤ 12	SQ GM-CSF on days 1 and 2 of 28 day cycle. Total cycles ≤ 12	10–21	Refractory/relapsed HGG/MB/ATRT/ PNET	IV virus delivery	NCT02444546
Measles Virus	MV-NIS	1; recruiting	Locally for recurrent tumors; Lumbar puncture for disseminated disease		1 – 39	Disseminated or locally recurrent MB; refractory ATRT	NIS allows noninvasive spatial and temporal virus tracking Lumbar puncture delivery	NCT02962167

ATRT, atypical teratoid/rhabdoid tumor; CED, convection enhanced delivery; DIPG, diffuse intrinsic pontine glioma; GM-CSF, granulocyte-macrophage colony-stimulating factor; HGG, high-grade glioma; hrs, hours; IT, intratumoral; IV, intravenous; LMD, leptomeningeal disease MB, medulloblastoma; oHSV, oncolytic herpes simplex virus therapy; OV, oncolytic virotherapy; PNET, primitive neuroectodermal tumor; SQ, subcutaneous; wks, weeks

ANNEX IV.

Oncolytic viruses are being studied for the treatment of haematological neoplastic pathologies such as multiple myeloma, acute and chronic myeloid leukaemia, and lymphoproliferative diseases. Table 1C shows the main in vitro and in vivo studies in Hematologic Malignancies.

Table 4.C. Main in vitro and in vivo studies in Hematologic Malignancies (77).

Study	Disease	Virus	Possible Disadvantages	Ref.
In vitro	Multiple Myeloma cell line	Measles virus		[78]
	Multiple myeloma and breast cancer cells	Adenovirus		[81,82,83]
	Multiple myeloma cell lines	Reovirus		[85]
In vivo	Multiple Myeloma	Adenovirus	Induction of proinflammatory cytokines. Neutralization by serum factors Sequestration in liver and spleen	[83,182]
	Multiple myeloma	Reovirus	Unknown	[85]
	Multiple myeloma	Measles virus	Increased unwanted pathology	[80,182]
In vitro	Acute Myeloid Leukemia	Measles virus		[93]
	Acute myeloid leukemia	Reovirus		[95]
	Acute myeloid leukemia, FLT3 mutant acute myeloid leukemia cells	Myxoma virus		[98,99,100]
	Kasumi-1 (AML), SD-1 (BCR-ABL-positive ALL)	Cytomegalovirus		[101]
	Acute myeloid leukemia cells	Coxsackievirus		[105]
	Kasumi-1, KG-1, HL-60, U937 AML cell lines	Adenovirus		[106,107]
	High-burden multidrug-resistant AML cells A549, HEPG2, Huh-7 cell lines	Non-replicating rhabdovirus-derived particles, Vesicular Stomatitis Virus		[109,111]
	Wild-type leukemia cells, Multiple myeloma cell lines	Vaccinia virus		[115]
	Baby hamster kidney-21 cells	Herpes Simplex Virus-1		[118]
In vivo	Acute Myeloid Leukemia	Adenovirus	Sequestration in liver and spleen	[107]
	Acute myeloid leukemia	Vesicular Stomatitis Virus	Neurotoxicity	[111,182]
	Acute myeloid leukemia cells	Measles virus	Increased unwanted pathology	[116]
In vitro	Chronic Myeloid Leukemia cells	Adenovirus		[119]
	Human and canine lymphomas	Newcastle disease virus		[126]
	Burkitt lymphoma cells, Cutaneous T-cell lymphoma	Measles virus		[128,142]
	Burkitt's tumor cells, Chronic lymphocytic leukemia	Reovirus type 3		[130,134]
	Chronic lymphocytic leukemia	Adenovirus		[137]
In vivo	Mantle cell lymphoma, Cutaneous T-cell lymphoma	Measles virus	Increased unwanted pathology	[140,142,182]
	Non Hodgkin lymphoma- A20 lymphoma	Sindbis virus	Unknown	[144]