Abstract.

Cancer has always been an enormous threat to human health and survival. Surgery, radiotherapy, and chemotherapy could improve the survival of cancer patients, but most patients with advanced cancer usually have a poor survival or could not afford the high cost of chemotherapy.

The emergence of oncolytic viruses provided a new strategy for us to alleviate or even cure malignant tumors. An oncolytic virus can be described as a genetically engineered or naturally existing virus that can selectively replicate in cancer cells and then kill them without damaging the healthy cells, and therefore act as an in-situ cancer vaccine by releasing tumor-specific antigens. Recent evidence suggests several possible applications of OVs against cancer, especially in combination with immune checkpoint inhibitors, (ICI).

We will first overview cancer treatment from a historical perspective, followed by a brief history of oncolytic virotherapy, We will also describe the molecular mechanisms of oncolytic virotherapy and OV-induced immune responses, provide a brief summary of some of the viruses currently in clinical updates on this rapidly evolving field, and discuss a combinational strategy that is able to overcome the limitations of OV-based monotherapy.

1. Cancer.

Cancer has always been an enormous threat to human health and survival. Malignant tumors have become one of the leading causes of death all over the world. it killed over 8 million people worldwide in 2013 and have moved from the third leading cause of death in 1990 to the second leading cause behind cardiovascular diseases in 2013 [1][2].

Cancer is a genetic disease and it represents a range of manifestations. The principles of tumorigenesis are however similar across different tumors and relatively well characterized. In brief, frequent mutations occur during cell divisions or due to exogenous factors such as radiation or other carcinogens. Most of these mutations are corrected by specialized intracellular proteins. If such mechanisms are unsuccessful, mutated cells are generally cleared by apoptosis [3].

The vast majority of mutations do not help the cell to gain cancerous properties (passenger mutations). In contrast, driver mutations provide exclusive abilities to tumor cells, such as cell death resistance or metastatic capacity, for example, Most of these mutated cells are, however, recognized by our immune system and destroyed before clinical detection. Accumulating evidence supports the notion that a dysfunctional immune system is intimately associated with tumor development, progression, and recurrence. Also known as immunosuppression, this phenomenon is actively propagated by cancer cells either directly or through the tumor microenvironment [3][1][2].

This understanding has galvanized the interest in the development of immunotherapies, which aims at modifying and activating immune cells to attack cancer cells. The approach is rational as our immune system has been trained to detect, destroy, and memorize non-self-patterns. By definition, all cancer cells have multiple mutations causing non-self-structures that can potentially be detected by our immune system [3][4].

2. Tumour microenvironment and immune evasion.

Cells of the TME consist of a heterogeneous population of neoplastic cells together with a number of different non-transformed cells including mesenchymal cells, for example, cancer stem cells (CSCs), mesenchymal stem cells (MSCs), endothelial cells (ECs), fibroblasts and myofibroblasts,

hematopoietic cells, for example, innate and adaptive immune cells such as macrophages, T cells, natural killer (NK) cells, B cells, neutrophils, DCs, and mast cells (MCs) and myeloid-derived suppressor cells (MDSCs) [5] [6].

In addition to cells, the TME consists of secreted factors such as cytokines, and extracellular vesicles and proteins of the extracellular matrix (ECM).

Cancer cells, as well as non-transformed cells, for example, cancer-associated fibroblasts (CAFs), adipocytes, T regulatory cells (Tregs), MDSCs and tumour-associated macrophages (TAMs) support immune evasion and tumour growth by producing and releasing cytokines such as interleukin-10 (IL-10), chemokines such as chemokine C-X-C motif ligand 12 (CXCL12), growth factors such as transforming growth factor beta (TGF-b), matrix remodelling factors such as collagen, fibronectin and fibrin and other soluble factors such as adenosine into the TME [5] [7].

The immunosuppressive environment is established via multiple mechanisms: TGF-b and IL-10 mediate an anti-inflammatory response by dampening the activity of tumour suppressor cells such as cytotoxic T cells (CTLs) and NK cells and enhancing the activity of tumour promoting cells such as Tregs and tumour-associated neutrophils (TANs) [7].

In addition, cancer cells have acquired the ability to activate different immunosuppressive immune checkpoint pathways such as CTLA-4/CD80/86 and PD-1/ PD-L1 signalling pathways that, in normal cells, are associated with immune homeostasis and prevent an over-activated immune response leading to autoimmune reactions [6].

Despite the hostile and highly immunosuppressive environment of the TME, some tumour suppressor cells may still be activated to combat the growing lesion. Indeed, it has been shown in a variety of cancers that the number of infiltrating lymphocytes positively correlates to patient survival [5].

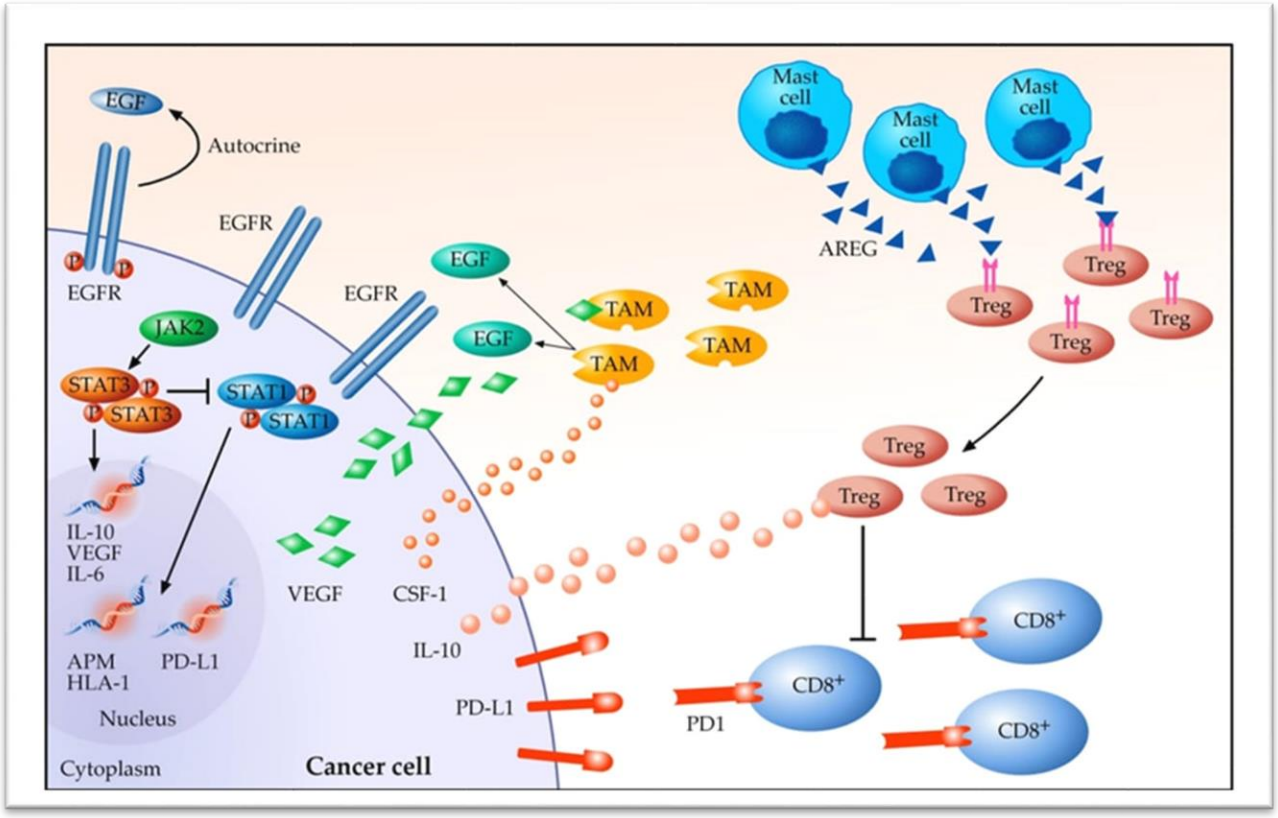

Figure 1. Tumour Microenvironment in EGFR mutated NSCLC.

3. Cancer treatment.

3.1. A historical perspective:

Seishu Hanaoka performed the first successful surgical partial mastectomy under general anesthesia for a patient with breast cancer in 1804. To date, surgery remains one of the principle mainstay ways to treat localized cancer; complete tumor removal and potential cures are possible if the cancer is detected early and has not metastasized [7][8].

Radiation therapy began to emerge as a new modality for cancer treatment with the discovery of X-ray by Wilhelm Roentgen in 1895, and the discovery of radioactive radium and polonium by Marie and Pierre Curie in 1898. In 1903, S.W. Goldberg and Efim London successfully used radium to achieve complete responses (CR) in two patients with basal cell carcinoma of the skin [9][8].

Since then, surgery and radiation therapy dominated the field of cancer treatment until the late 1940s where anti-metabolites (methotrexate) and alkylating agents (nitrogen mustard) were used as chemotherapy agents for cancer. By the 1950s, in spite of the powerful impact of combination chemotherapy in leukemia and lymphoma, physicians realized the limitations of chemotherapy to achieve the same success rates of complete remission of many advanced solid tumors [9].

An earnest effort began thereafter with the research and development of preclinical tumor models to study the basic biology of carcinogenesis, develop novel drugs and drug combinations, and the use of adjuvant chemotherapy after surgery in 1970s to improve overall survival. In the following years, cancer treatment became more targeted, focusing on specific pathways, such as antiangiogenesis, signaling pathways, or specific mutations [3][8].

Table 1: A timeline including some key steps in development of cancer treatments [3]**.**

Other cancer treatments*		Cancer Immunotherapy
Surgery	2600 BCE	Use of poultice (pharaoh Imhotep's physicians)
Surgery under ether anesthesia	1840s CE	Purposeful infection of tumors
Radiotherapy	1890s	Coley's toxins (deactivated bacteria) were injected to tumor
Hormonal therapy (estrogen, castration), chemotherapy (nitrogen mustard, antifolates)	1900–1940s	Case reports of tumor regression after natural viral infections
Linear accelerator for radiotherapy, combination chemotherapy	1950s–1970s	Hundreds of case series treating cancer with multiple viruses (e.g., varicella, measles, vaccinia, West Nile, adenovirus, mumps) BCG adopted in bladder cancer
Stereotactic radiotherapy, antiestrogens	1980s	Adoptive T cell transfer, cytokine therapies (e.g., IFN-alpha and IL-2)
Mini-invasive surgery, monoclonal antibodies (rituximab, trastuzumab)	1990s	HD-IL-2 approved by the FDA
Antiangiogenic therapies (bevacizumab), kinase inhibitors (imatinib)	2000s	First oncolytic adenovirus (H101) approved in China
Small molecular inhibitors of various proteins	2010–	Cellular immunotherapy (sipuleucel-T, TCR, CART), six different checkpoint inhibitors, oncolytic virus (T-vec)

*Many treatments in this column have also immunological properties (e.g., rituximab, trastuzumab, chemotherapy, and radiation therapy)

Cancer treatment between the present & what science aspires to achieve in the future.

3.2. Current challenges and future prospective in cancer treatment.

Although, There are already many treatments including surgical treatment, radiotherapy, chemotherapy, and the latest immunotherapy that can prolong the survival period of tumor patients, but most patients with advanced cancer usually have a poor survival or could not afford the high cost of chemotherapy [10][1].

Also, they have some limitations. Surgical treatment is mainly used for early stage cancer patients, while severe side effects make radiotherapy and chemotherapy hard for patients to tolerate. Besides, traditional immunotherapy still has many defects; for example, the objective effectiveness of patients receiving immunotherapy is only 10 to 30%, So, improving the efficiency of immunotherapy is urgently needed [3] [1].

Comprehensively, existing cancer treatment strategies are imperfect, and new treatment methods need to be proposed that should have accurate tumor targeting, powerful tumor-killing properties, and low toxic side effects [1].

3.3. Cancer immuno-therapy.

Cancer immunotherapy aims to increase the amount and function of tumour-infiltrating immune cells such as dendritic cells (DCs) and tumour-infiltrating lymphocytes (TILs) in order to elicit therapeutic efficacy. This may be achieved via multiple different strategies. For example, DC vaccinations that aim to increase tumour antigen presentation, TIL and chimeric antigen receptor (CAR) T cell therapies that aim to increase cancer killing T cells, and immune checkpoint inhibitor (ICI) therapies that aim to enhance endogenous anti-tumour immune responses [5] [3].

In particular, ICIs such as antibodies targeted against programmed cell death 1 (PD-1) (developed by Merck), or cytotoxic T-lymphocyteassociated antigen 4 (CTLA-4),(developed by Bristol Myers Squibb [BMS]) in 2011, have drastically changed the treatment paradigm for many cancers.

However, objective responses to ICI therapies have predominantly been seen in patients with prior anti-tumour immune response (10–30% of patients are responding to ICIs) [10] [5].

In addition to the clinical arrival of immune checkpoint inhibitors in recent years, other players in the field of immuno-oncology are also being explored, including adoptive cell therapeutic approaches, new approaches for cytokine therapies, and more recently, inhibition of indoleamine-2,3-dioxygenase (IDO), an inhibitor of tumour immune resistance [10]. and Finally, OV therapies which have been shown to modulate the tumour microenvironment (TME) towards a less immunosuppressive phenotype and to enhance anti-tumour immune responses. Combining ICI therapies with OVs may help patients overcome resistance to ICI therapies. OVs are currently in clinical evaluation in combination with multiple cancer immunotherapeutic platforms [8].

It is essential to note that presently therapeutic benefits are restricted to a limited fraction of patients treated with immunotherapy. In particular, solid cancers generally contain a suppressive tumor microenvironment that inhibits T cell activity and supports tumor progression. In addition, new immunotherapy treatments have led to the occurrence of new immunological adverse events, including cytokine storm and autoimmune events. Considering these challenges, further alterations to these therapeutic strategies are needed. In addition to new immunological treatment strategies, we also need better understanding of individual immune environments to provide maximal patient benefit [8].

4. The basic biology supporting oncolytic viruses as cancer therapeutic agents:

Of the nearly 1 million vertebrate viruses, approximately 320,000 are thought to infect mammalian cells. Viruses have several shared properties; these include a genetic element composed of single or double-stranded DNA or RNA and the ability to infect host cells and replicate under permissive conditions [11] [7].

Many human viruses are being evaluated for their abilities to selectively infect, replicate in and kill cancer cells and therefore be used as therapeutic oncolytic viruses (OVs) for the treatment of various human malignancies. Upon infection of a cell, viruses possess specific abilities to interact with cellular proteins to avoid early host cell death and immune system recognition in order to promote their replication and release progeny virus, and eventually killing the host cell [12][13].

Cell death can be classified according to morphologic and structural changes occurring in dying cells and viruses typically activates one or more cell death pathways during infection, replication or cell lysis. Some forms of programmed cell death lead to silent and organized uptake of dead cells by phagocytic cells and they are considered as intrinsically tolerogenic. Other forms of cell death can induce an immune response through activation of dendritic cells (DCs) and adaptive immune cells and are termed “immunogenic cell death” (ICD) [12] [7].

Inducers of ICD are characterized by their ability to stimulate the release of damage-associated molecular patterns (DAMPs) from dying host cells, such as extracellular ATP (“find-me” signal), cell surface exposure of Calreticulin (CRT) (“eat-me” signal to antigen-presenting cells), and release of high mobility group box 1 protein (HMGB1) (activation signal for immune cells). Collectively they serve as strong immune stimulants and ICD is regarded as a keystone of anti-tumor immunity [12] [13].

5. Relation between Virus and Cancer.

Most people think of viruses as pathogenic microorganisms that infect cells, overtake their DNA, RNA and protein synthetic machinery to replicate and then lyse their host cell to spread their progeny, thereby propagating the infection throughout a tissue.Viral infection results in cytopathic effects, such as induction of cell death and/or dysfunction. In the 1990s, researchers began reinvestigating an old hypothesis: could viruses be used to kill tumour cells?

Although the cytotoxic effects of viruses are usually viewed in terms of pathogenicity, it is possible to harness this activity for therapeutic purposes. Viral genomes are highly versatile, and can be modified to direct their cytotoxicity towards cancer cells. These viruses are known as oncolytic viruses [14].

Several oncolytic viruses (OVs) that selectively infect or replicate in cancer cells, but spare normal cells have been identified. Some of these are naturally attenuated viral strains (such as some strains of reovirus or vesiculostomatitis virus) that more effectively infect or replicate in cancer cells. Others are genetically modified (such as herpes simplex virus type 1 or adenovirus) to mediate oncolytic effects [14] [15].

Reports of viruses having therapeutic benefits in cancer started appearing early last century with multiple reports of leukemia patients becoming disease-free after viral infections. Typically, the reported patients were young and the remissions were short-lived lasting for 1 or 2 months. These observations did not go unnoticed by the medical community, who subsequently begun utilizing viruses for the treatment of cancer [3] [15].

Especially during the 1950s and 1960s, multiple wild type viruses (e.g., hepatitis, Epstein-Barr, West Nile, Uganda, dengue, yellow fewer) were used to treat different cancers in hundreds of case series. Results were variable and occasionally poorly documented. However, during this time, it was becoming clear that most wild type viruses lacked efficacy or safety. [3]

6. Oncolytic virotherapy:

Oncolytic virus therapy is perhaps the next major breakthrough in cancer treatment following the success in immunotherapy using immune checkpoint inhibitors [12] [16].

An oncolytic virus is defined as a genetically engineered or naturally occurring virus that can selectively replicate in and kill cancer cells without harming the normal tissues. In contrast to gene therapy where a virus is used as a mere carrier for transgene delivery, oncolytic virus therapy uses the virus itself as an active drug reagent [3] [16].

OVs are able to exploit cancer-specific changes in cellular signaling to specifically target cancers and their microenvironment. The direct cytolytic effect of OVs on cancer cells is known to release antigens, which can begin a cascade of events that results in the induction of anti-cancer adaptive immunity. This response is now regarded as the most critical mechanism of OV action and harnessing it can lead to the elimination of distant micrometastases as well as provide long-term anti-cancer immune surveillance [5] [17] [10].

Some viruses such as myxoma virus or reovirus have inherent selectivity to tumor cells, while being nonpathogenic in healthy human cells. On the other hand, other OVs, including adenovirus, herpes simplex virus type-1 (HSV-1), and vesicular stomatitis virus (VSV), have been genetically engineered to function as vectors to boost anti-tumor immune responses. The anti-tumor efficacies

of these OVs have been evaluated in many preclinical and clinical studies as monotherapy and combination therapy [15] [16][12].

6.1. Does any virus can be oncolytic?

There are more than 3,000 species of viruses but not all are suitable as oncolytic agents. The typical features of these OVs must include being non-pathogenic, having intrinsic cancer selective killing activity, and the capacity of being transformed to express tumor-killing factors through genetic engineering methods[8].

Tumor selectivity could be at the level of receptor-mediated cell entry, intracellular antiviral responses and/or restriction factors that determine how susceptible the infected cell is to support viral gene expression and replication [8].

6.2. Historical snap shots of oncolytic virus.

The concept of a clinically advantageous link between viruses and cancer treatment emerged in the early twentieth century (Fig.2) [10] due to observations surrounding individual case reports of cancer regressions following natural virus infection. At that time, viral biology was poorly understood and the ability to pursue laboratory-based studies on such agents was limited [10][16].

The history of treating cancer with microbes dates back to 1890; a surgeon named William B. Coley in the Memorial Hospital in New York was the first to observe the regression of tumors in several patients infected with the pathogen. Moreover, he called the pathogen antitumor agents [1][15].

In 1935, Clostridium histolyticum was used by Connell to treat advanced cancers, and tumor regression was observed not long after that. Later, in the 1950–1970s, live viruses were deliberately injected into cancer patients and showed positive activity, such as Egypt 101 West Nile virus (4/34 transient regressions), adenovirus lysates (26/40 showing localized tumor necrosis), and Urabe strain mumps virus (37/90 complete remission or partial responses) [8].

The results of those trials were interesting; however, many at the time dismissed the clinical utility of this novel class of anti-cancer agent due to the inherent safety risks and demonstrated lack of

successful human translation [10] [18]. These viruses were not deemed useful as therapeutics reagents and, some side effects were emerging in these early researches by using natural viruses, because in those days, there was no known method to control the virulence and yet retain viral replication in cancer cells[16]. And, the viruses were not engineered for tumor selectivity, especially in immunosuppressed patients with leukemia or lymphoma (five of eight patients had severe encephalitis after being treated with West Nile virus) [1][18].

The next several decades of research into basic virology and cancer-targeted viruses yielded many fundamental insights; however, major clinical steps were delayed until the 1990s. An important step in the clinical advancement of OVs was the Chinese approval of the genetically modified adenovirus H101 in 2005, which was followed by a second generation of OVs that have now begun to mature in the clinic [10] [16].

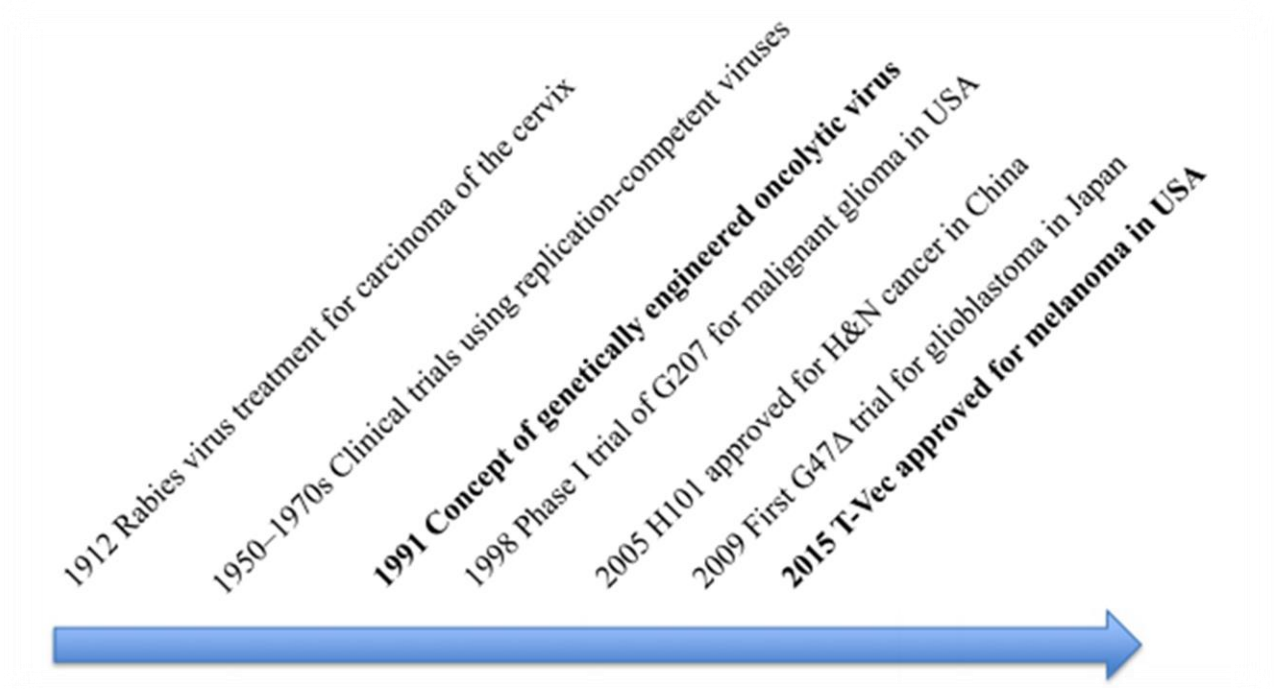

Figure (2): milstones of oncolytic viruses therapy development[16]**.**

The clinical evolution of oncolytic viruses.

7. Aspects of OVs clinical development.

More recently, clinical trials have provided support for improved therapeutic responses when OVs are given in combination with immune checkpoint blockade.

Despite the clinical trial results supporting the potential therapeutic benefit of OVs, there are many aspects of OV clinical development, including the viral species, genetic modifications, transgene expression, route and schedule of administration, type and stage of patients with cancer, optimal combination agents and predictive biomarkers of response that remain to be elucidated [17][19].

Preclinical studies have supported a large number of both DNA and RNA viruses as potential candidates for OV drug development. Indeed there is no standard method for OV selection with some viruses exhibiting natural tropism and predilection for preferential replication in tumors cells and others demonstrating improved replication in tumor cells following genetic modification [17] [20].

Since some viral genes are considered nonessential, in some viruses genetic deletions can help attenuate pathogenicity of viral infection and may promote tumor cell replication. In addition, larger viruses are able to express eukaryotic genes and, especially when non-essential viral genes have been deleted, OVs can be engineered to deliver additional gene expression to help promote anticancer activity [17] [20].

There has been considerable preclinical studies supporting expression of a variety of genes that help promote cytotoxic killing of tumor cells, induction of immune responses, inhibition of tumor neoangiogenesis, enhancing radiosensitization and other strategies[17] [20].

Other considerations in OV development includes selection of how to deliver the virus to the patient with cancer and, while initial studies used direct intratumoral (IT) injections, this may be logistically challenging for visceral and central nervous system (CNS) tumors [17].

strategies have included intravenous administration which is logistically simple and allows targeting of multiple metastatic lesions but may be complicated by rapid dilution in the circulation,

neutralization by antiviral antibodies and other serum proteins, and ultimately limited biodistribution to tumors. Other factors, such as which combination agents and how to sequence them with OVs, how to best select appropriate patients and lesions for OV therapy, and the need for alternative endpoint assessment criteria for IT therapy are highly controversial and require further clinical study [17][19].

8. OVs anti-tumor activity.

Generalized Overview of Mechanisms of Action of Oncolytic Viruses.

Although incompletely understood OVs molecular and cellular mechanisms of action. oncolytic viruses are thought to mediate antitumour activity through two distinct mechanisms of action:

1) selective replication within neoplastic cells, resulting in a direct lytic effect on tumour cells.

2) And, induction of systemic antitumour immunity [11] [13].

The relative contribution of these mechanisms may differ depending on : the nature and type of cancer cell, the characteristics of the viral vector, and the inter-action between the virus, tumour microenvironment and host immune system [11] [13][15].

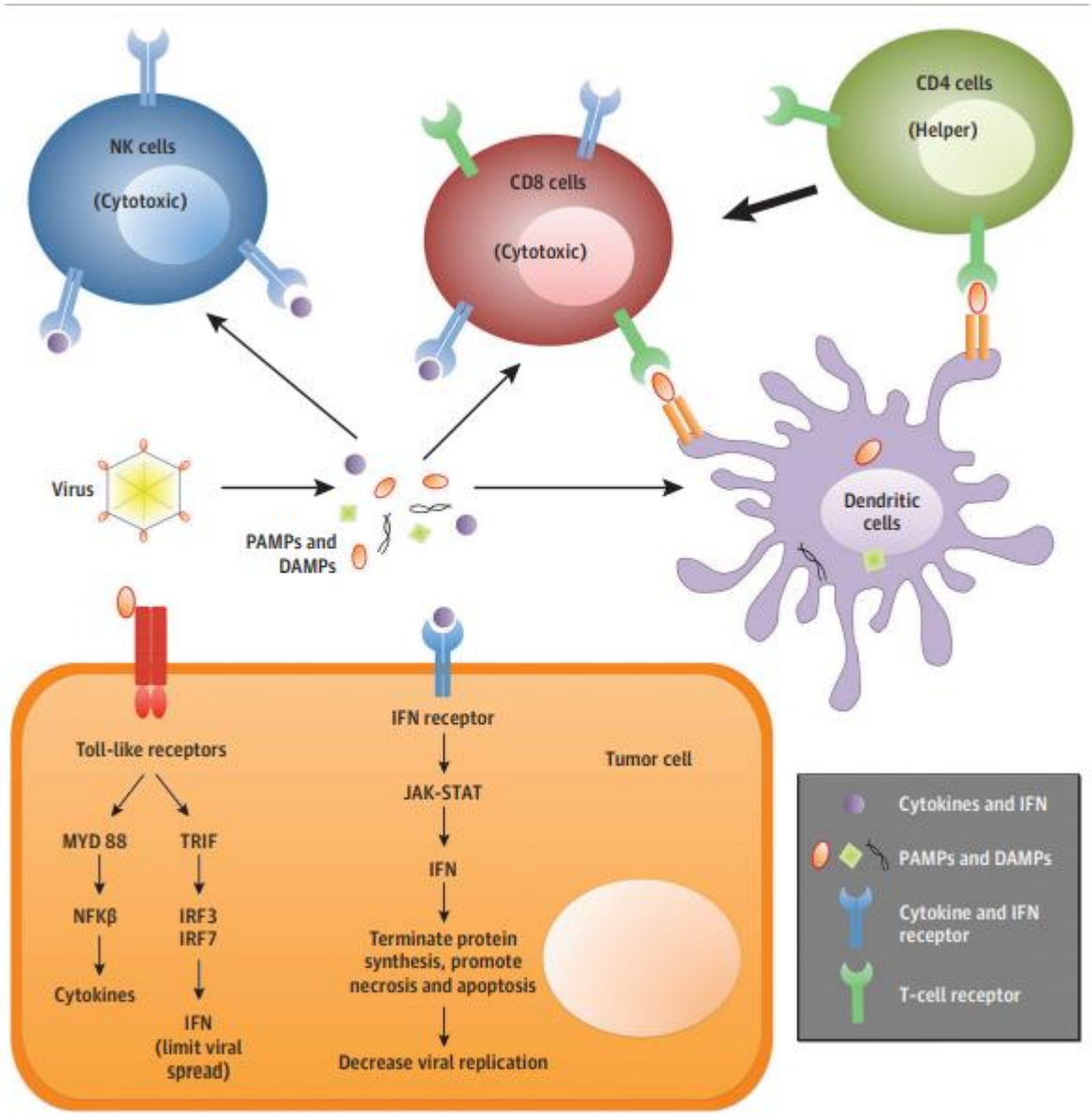

Figure (3). Generalized Overview of Mechanisms of Action of Oncolytic Viruses.

It is thought that most oncolytic viruses function through a combination of tumor cell lysis and stimulation of innate and adaptive immunity through presentation of viral and tumor antigens. DAMPs indicates (damage-associated molecular patterns); IFN, interferon; JAK-STAT, Janus kinase–signal transducer and activator of transcription; MYD 88, myeloid differentiation primary response gene 88; NFKβ, nuclear factor κB; NK, natural killer; PAMPs, pathogen-associated

molecular patterns; RF, Interferon regulatory factor; and TRIF, TIR (toll/II-1 receptor)–domain-containing adapter-inducing interferon-β [13].

It is now recognized, that most viruses can replicate to a much greater extent in cancer cells than in normal cells. because protection mechanisms against viral infection (e.g. interferon-beta signal pathway) are impaired in the majority of cancer cells [16].

Therefore, getting a virus to replicate in cancer cells is not a problem, What is difficult is making a virus not replicate in normal cells at all, while retaining its replication capability in cancer cells. Attempts to achieve cancer cell-specific replication have been undertaken either by selecting a virus that is non-virulent in humans or by engineering the virus genome [16].

8.1. Anti-Cancer mechanism of Oncolytic Virus.

As promising cancer gene therapy agents, OVs have the unique ability to selectively replicate in cancer cells and cause the inflammation and even death of cancer cells, further leading to host immune responses because of cancer-associated antigen exposure. As is shown in Figure 4, [1].

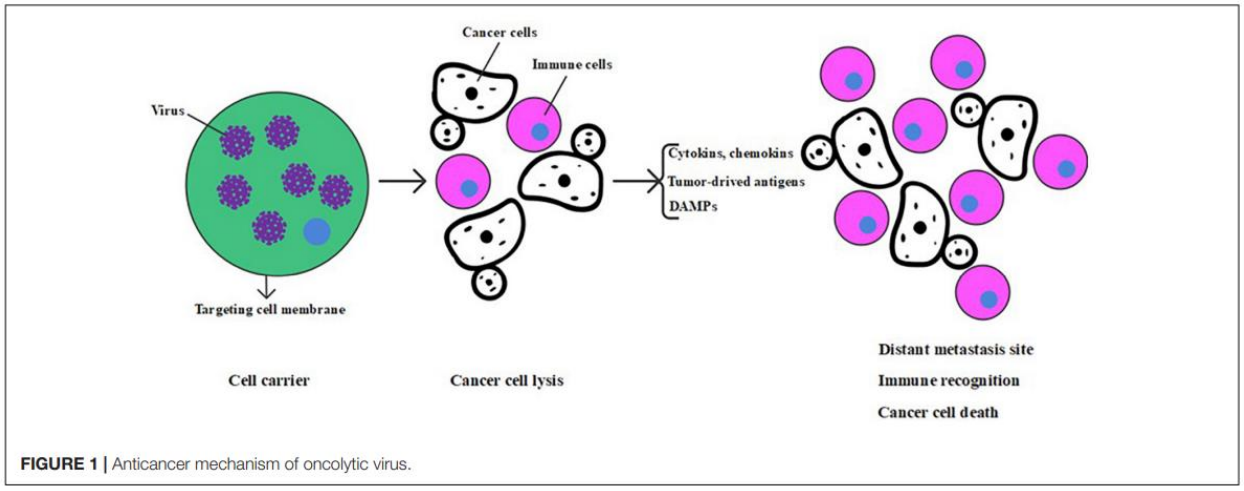

Figure (4): anti-cancer mechanism of ocolytic virus [1].

The anticancer mechanism of the OV includes direct oncolysis or cytotoxicity toward the cancer cells or indirect induction of bystander effects (including the destruction of tumor blood vessels) and immunotherapeutic toward tumors.

After infection, the viruses can hijack the tumor cell's protein factory and prevent tumor cells from producing enough protein to meet growth needs, thus destroying the normal physiological process of tumor cells. Besides, tumor cells can also be killed through the induction of immune response [15][11] [1].

Infected tumor cells can produce cytokines or chemokines, release tumor-derived antigens after apoptosis, and then attract a collection of immune cells including cytotoxic T lymphocytes, natural killer cells, dendritic cells, and phagocytic cells, which induce a tumor-specific immune response and potentially resulting in the elimination of uninfected cancer cells.

Eventually, it is worth noting that the immune response associates with an "immune-associated" bystander effect, in which local release of cytokines may cause the immune responses of nearby tumor cells, even without direct antigen expression. Except for the ones above, OVs can also destroy tumor blood vessels, reducing or even disrupting tumor blood supply, leading to tumor hypoxia and lack of nutrients. (As shown in figure:5)

The necrosis induced by OVs can also cause the release of damage-associated molecular patterns (DAMPs), which stimulate dendritic cells and acquired immune responses [3] [13][15].

Different viruses can also manipulate distinct aberrant signalling factors within tumour cells to block apoptosis, which allows more time for the virus to complete its life cycle. Following viral replication, most oncolytic viruses induce cell death, which can directly eliminate viable tumour cells but also sets the stage for initiating systemic immune responses. Induction of host immune responses can be greatly aided by both the type of cell death and the release of danger signals from virus-infected cells. For example, necrosis or pyroptosis are more immunogenic forms of cell death than apoptosis [11].

Besides tumor cells and immune cells, other cellular components within the TME also respond to OV therapy. Many OVs can infect and destroy tumor endothelial cells, thus showing direct vascular disruption. This anti-angiogenic effect was selective for tumor endothelial cells but not for endothelial cells of normal tissues, indicating the targeted destruction of pathologic tumor vasculatures [15].

Arulanandam et al. suggested a potential mechanism for this viral tropism of tumor blood vessels. In their study, activated vascular endothelial growth factor receptor 2 (VEGFR2) signaling within tumor endothelial cells upregulated the transcriptional repressor, positive regulatory domain I–binding factor 1 (PRD-BF1), which suppresses genes involved in type I interferon-mediated anti-viral activity, thereby making tumor vessels sensitive to OV infection.

Cancer-associated fibroblasts (CAFs) also respond to OV therapy. Although normal fibroblasts are refractory to OV infection, CAFs have increased sensitivity to OV therapy. Tumor cell-derived transforming growth factor beta (TGF-β)-reprogrammed CAFs suppress their innate anti-viral and type I interferon signaling, thereby rendering CAFs sensitive to OV infection. In turn, CAFs dampen the anti-viral response within tumor cells by secreting high levels of fibroblast growth factor 2 (FGF2). Therefore, cellular crosstalk between CAFs and tumor cells promotes OV growth and killing in both cell types [15].

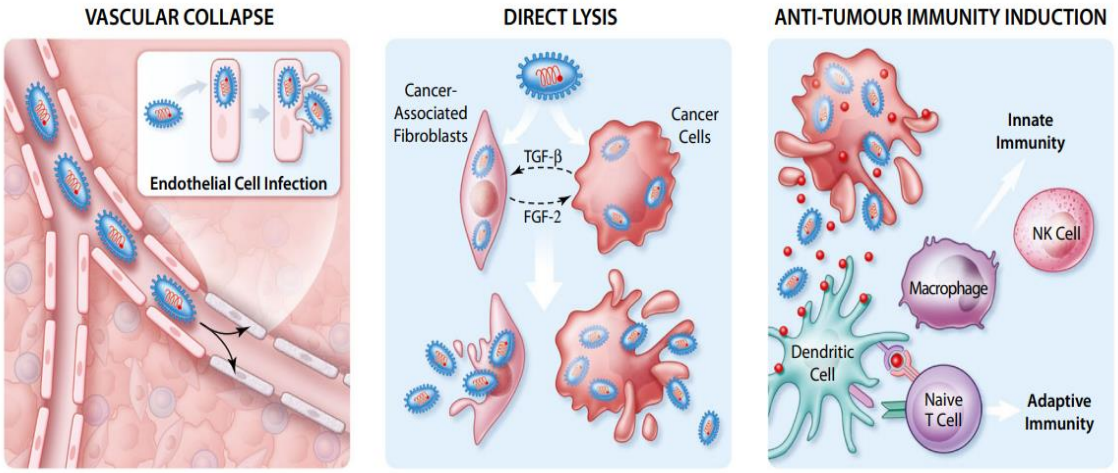

Fig:(5) Multi-faceted cancer attack of OVs.

Tumour-associated active endothelial cells are vulnerable to OV infection through a VEGF-mediated mechanism, which leads to vascular collapse and tumour starvation. The traditional mechanism of action of direct cancer cell oncolysis can now also be applied to cancer-associated fibroblasts (CAFs) as we have come to understand their vulnerability to OV attack. Finally, released tumour-specific antigens can be ingested by various antigen presenting cells, which can lead to the initiation of both innate and adaptive anti-tumour immunity [10].

However, the diminished IFN response, a fundamental biological property of the malignant phenotype, also leaves the cancer cell vulnerable to OV infection. These multiple mechanisms of action are summarized in Fig. 5 and take advantage of a number of changes in the tumour microenvironment to facilitate vascular collapse, cause the direct cellular lysis of both cancer cells (oncolysis) and cancer-associated fibroblasts (CAFs), and perhaps most importantly, to initiate or augment existing anti-tumour immunity [10].

8.2. Difference between Cold and hot tumors in their response to OVs.

Tumors can be divided into immunologically “cold” tumors and immunologically “hot” tumors according to the level of tumor antigen, CD8+ T cells, and immune-suppressive cells or cytokines [1][15].

The non-inflamed cold tumors are described as “immune deserts” because they are poorly immunogenic and have very few anti-tumor immune effector cells within the TME. Therefore, there have been tremendous efforts to develop a novel immunotherapeutic agent that can not only enhance tumor immunogenicity but also augment immune cell trafficking into the TME to covert non-inflamed cold tumors to inflamed hot tumors that can respond favorably to ICI therapy [15].

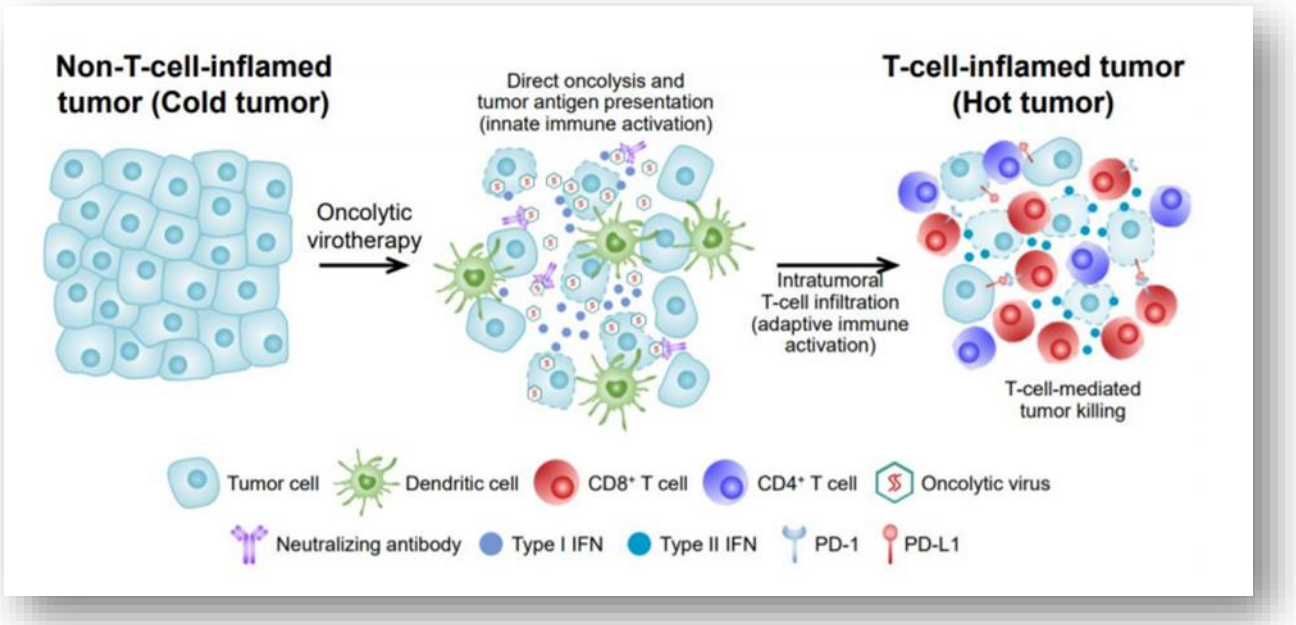

Figure (6): mechanisms of oncolytic virus (OV) anti-tumor effects [15]**.**

The tumor microenvironment of advanced cancers is "cold" due to the lack of immunological activity. Oncoytic virus therapy restores the immunological activity of immune tumor infiltrates [3].

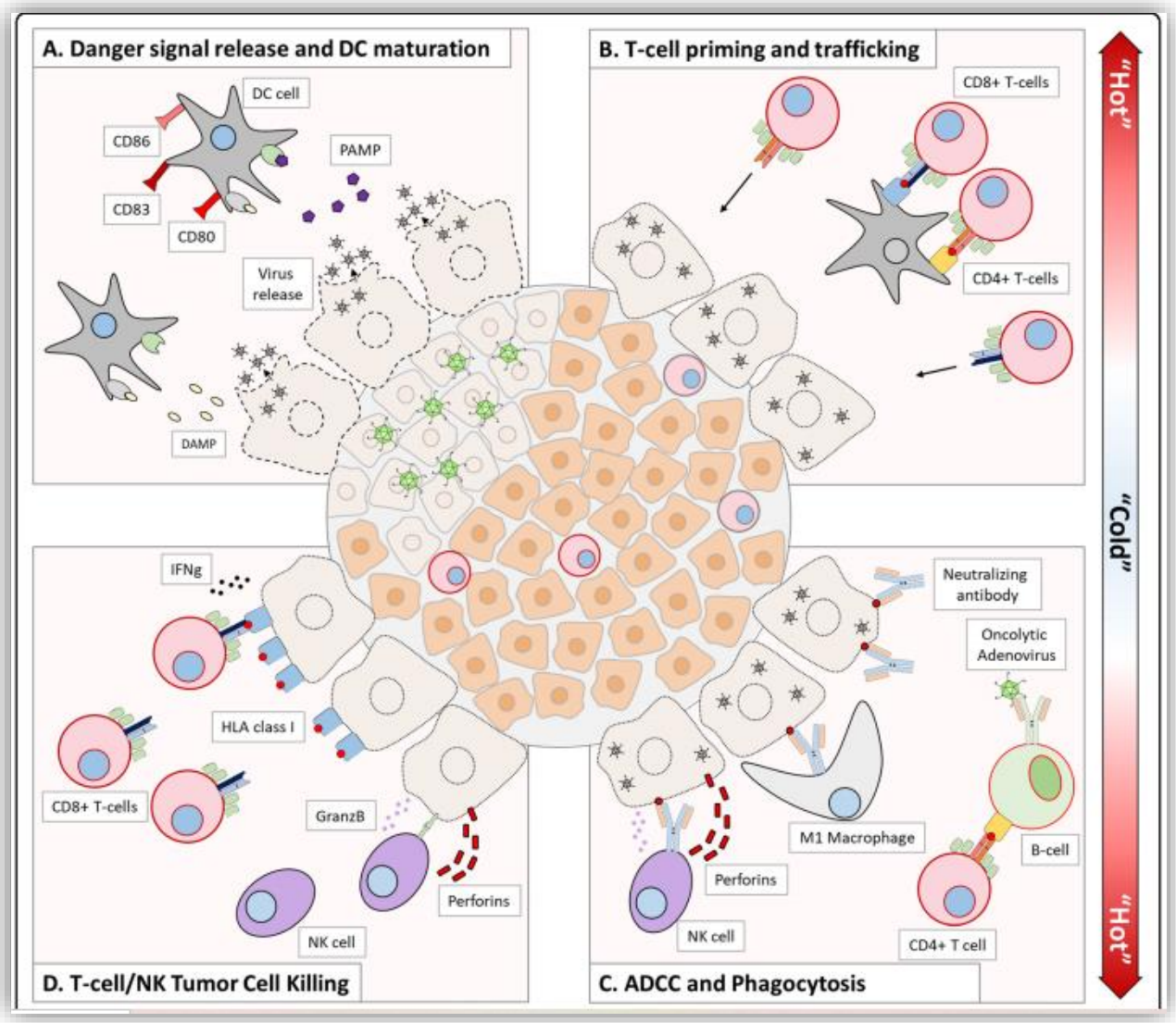

Fig. (7): Activating the immune system for cancer rejection with oncolytic virus therapy.

a) Danger signal release and DC maturation. Oncolytic adenoviruses infect tumor cells and cause oncolysis, releasing new virus progeny but also DAMPS and PAMPS, which will activate nearby dendritic cells and foster their maturation by upregulating co-stimulatory markers, such as CD80, CD83, and CD86.

b) Mature dendritic cells will process tumor debris and present tumor-associated and virus antigens to local and distant T cells. Concurrently, the ongoing virus infection attracts T cells to the tumor site.

c) The activation of B cells by CD4+ T cells or BCR-virus interaction causes the release of neutralizing antibodies, which mark infected tumor cells for ADCC by NK cells, or phagocytosis by M1 macrophages.

d) CD8+ T cells and NK cells destroy infected and non-infected tumor cells through INFg/GranzB and GranzB/Perforins, respectively. The oncolytic adenovirus infection also upregulates class I HLA in tumor cells, allowing for increased exposure to CD8+ T cells. Overall, the components of this modulation allow the tumor microenvironment to become "hot" with increased immunological activity. DAMP danger-associated molecular patterns, PAMP pathogen-associated molecular patterns, HLA human leukocyte antigen, BCR B cell receptor [3].

9. Tumor selectivity of OVs.

Tumor selectivity is an essential prerequisite of OVs to guarantee maximal oncolysis while minimizing off-target effects on normal tissue. Since the 1990s, with the development of molecular virology, the genomes of wild-type viruses have been engineered to enhance their tumor selectivity [21].

There are several ways to enhance the tumor selectivity of OVs. Because tumor cells activate various oncogenic signaling pathways during carcinogenesis, engineering viruses that depend on these oncogenic pathways can remarkably increase their tumor selectivity without affecting normal tissues.

For example, the oncolytic vaccinia virus, pexastimogene devacirepvec (Pexa-Vec), was engineered to inactivate its own thymidine kinase (TK) gene for tumor selectivity. Because TK is essential for nucleic acid metabolism, Pexa-Vec can preferentially replicate within TK-overexpressing cancer cells, while not being able to do it within normal healthy cells where TK activity is absent or minimal, thus showing tumor selectivity [15] [21].

9.1. The Difference between normal and cancer cells in their interaction with OVs.

Certain viruses have the ability to enter cancer cells and selectively replicate within such cells. Although oncolytic viruses can enter both normal and cancer cells, the inherent abnormalities in the cancer cell response to stress, cell signalling and homeostasis provide a selective advantage for viral replication [11].

The normal host cell anti-viral machinery, which is responsible for the detection and clearance of viruses, may also be abnormal in cancer cells. For example, the protein kinase R (PKR) is a critical factor that helps in clearing intracellular viral infections. PKR may be absent in some cancer cells, allowing increased viral replication, whereas it may be active in other cancer cells, such as low-grade tumours, and these differences can influence the therapeutic activity of an oncolytic virus [11].

(PKR: is an intracellular protein kinase that recognizes double-stranded RNA and other viral elements) [11].

One of the well-characterized changes that occurs in the process of malignant transformation in many cancers is the accumulation of mutations affecting IFN signaling [10].

Recently, an interesting mechanistic discovery has linked commonly-observed mutations in the tumour-suppressor gene encoding PTEN with defects in the anti-viral IFN response. As summarized in Fig. 8, the results of these changes are directly implicated in oncogenesis by preventing the cell from initiating apoptosis, growth arrest, or immune stimulation [10].

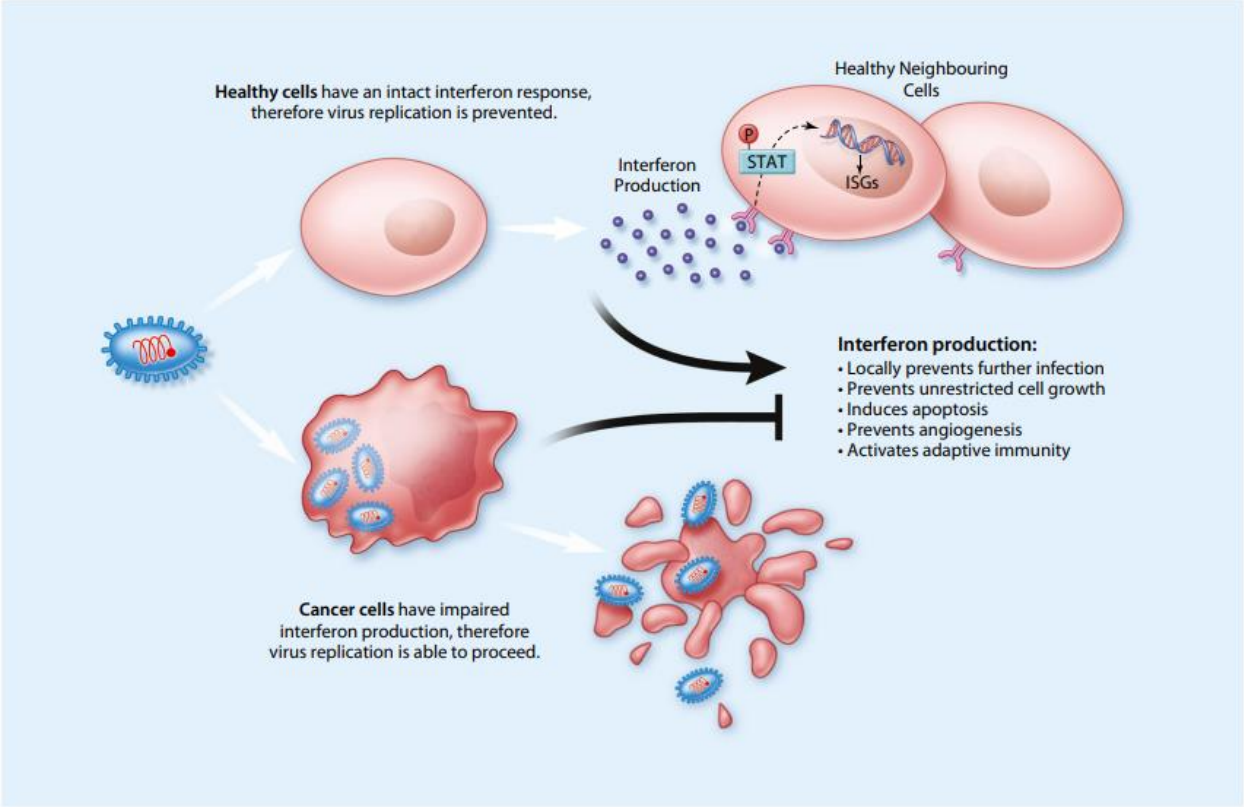

Fig. (8): difference between normal and cancer cell in their response for OVs.

Upon viral infection, the intact interferon response in healthy cells is initiated. This leads to the induction of an anti-viral state in nearby healthy cells, primarily through the JAK/STAT pathway, amongst others. This prevents the oncolytic virus infection from spreading in healthy cells, leaving normal tissues intact. In addition, an intact interferon response prevents uncensored cell growth, induces apoptosis, prevents angiogenesis, and activates an adaptive immune response—all properties that the efficient cancer cell seeks to evade. Conversely, many malignantly transformed cells have developed impairments in their cell signaling pathways necessary to initiate an interferon response, which has left them vulnerable to oncolytic virus attack. (ISG: interferon stimulated genes) [10].

10. Challenges and achievements of OVs for cancer treatment.

Although oncolytic virotherapy could kill cancer cells through direct oncolysis and activation of the immune response, the tumor can hinder antitumor immune response by interfering almost every step of immune activation and acquiring an immune-suppressive tumor microenvironmen [1][5][21].

The OV can destroy the immune-suppressive environment through arming with immune-modulating genes including genes encoding inhibitors of immune checkpoints, tumor antigens, and targets for chimeric antigen receptor T cells, to further improve overall immune responses especially for immunologically “cold” tumors. However, solid tumors are complex, heterogeneous structures that hinder the oncolytic function of OVs [1].

OVs can be engineered to increase their oncolytic ability by expressing modulatory molecules that target the structure of the tumor microenvironment to destroy tumor cells and impair the support for the growth of the tumor. Besides, the combination of OVs and immunostimulatory molecules can promote the development of antitumor immune responses [1].

T-VEC, which was approved by the US FDA, recently can express granulocyte-macrophage colony-stimulating factor (GM-CSF) to treat melanoma. Treatment of advanced melanoma with T-VEC was safe and resulted in a 10.8% complete response rate, which was significantly higher than the systemic administration of GM-CSF alone. Thus, oncolytic virotherapy represents a new period of promising cancer virotherapy candidates [1].

11. Designing & engineering viruses for cancer therapy.

Viral gene and noncoding sequences can be modified in a variety of ways to add or eliminate functions and nonviral genes or noncoding regulatory elements, whether synthetic or naturally occurring, can be added into viral genomes to confer additional desirable properties [22][16].

New ways to regulate viral replication Substitution of endogenous regulatory sequences in the viral genome by promoters that are preferentially active in cancer cells is a standard method to gain tumour specificity in OVs. (as shown in table.2)

Some promoters confer specificity against a wide array of cancers, such as the telomerase reverse transcriptase (TERT), survivin promoters or those containing response elements for hypoxia inducible factors (HIF) or E2F transcription factors.

Others are more specific for particular types of cancer, such as the alpha-fetoprotein promoter for liver cancer or the carcino-embryonic promoter (CEA) for gastrointestinal cancers.

However, the ability of promoters to regulate replication will largely depend on the viral context. It has been recently described that a HIF-responsive promoter fails to control the replication of HSV-1 vectors, whereas adenoviruses can be efficiently controlled by similar sequences. Another potential problem is the relatively low potency of some natural or artificial tumour-specific promoters, which can lead to virus attenuation also in cancer cells.

A solution can be to incorporate the key responsive elements that confer tumour specificity in the endogenous viral promoter, in combination with other ways to restrain replication in normal cells, such as partial deletions in viral genes.

If the sequence used to direct viral replication is not only active in cancer cells but also in tumour-associated stromal cells (for instance, the SPARC promoter), it can facilitate the spread of the virus inside the tumours, because stromal cells will not act as a barrier for the virus.

Table(2): tumour-specific promoters [11].

Virus	Tumour-specific promoter	Viral gene regulated by tumour-specific promoter	Effect	References
Adenovirus CV706	*PSA*	*E1A*	Replication restricted to prostate tissue	142
Adenovirus CN787	Rat probasin; *PSA*	*E1A, E1B55kD*	Replication restricted to prostate tissue	143,144
Adenovirus CV980	*AFP* (expressed by hepatocellular carcinoma)	*E1A, E1B55kD*	Replication restricted to hepatic tumours	61
Adenovirus ONYX-411*	*E2F1*	*E1A, E4*	Increased dependence of virus replication on overactive E2F	50
Adenovirus 01/PEME*	*p53*	*E2F* antagonists	Expression of E2F-viral genes dependent on loss of p53 function	29
CG8840	*Uroplakin-II* (bladder specific)	*E1A, E1B55kD*	Replication restricted to bladder cancer	145
KD1-SPB	*Surfactant protein B*	*E4*	Replication improved in lung tumours	146
HSV1 Myb34.5*	*B-Myb*	*γ34.5* (ICP34.5)	Improved replication in tumours	57,58
HSV1 DF3γ34.5	*DF3*	*γ34.5* (ICP34.5)	Improved replication in MUC-positive pancreatic and breast tumour cells	147
HSV1 G92A	*Albumin*	*ICP4*	Replication restricted to hepatoma	148

2) Transcriptional control of replication is also a versatile method to target cancer-initiating cells and can be used in cases in which detailed information regarding gene expression profiles is available. Using this approach, an oncolytic HSV-1 that had replication regulated by the nestin promoter demonstrated the ability to destroy neuroblastoma-initiating cells [22][23].

An alternative strategy, based on specific viral gene deletions, led to the identification of a multi-mutated HSV-1 virus (G47δ) that was active against glioblastoma stem cells. Incorporating target sequences for endogenous micro (mi)RNAs into the 3'UTRs of essential viral genes reduces the production of the corresponding viral protein and, consequently, reduces viral replication in cells expressing the specific miRNA. (as shown in **fig.9**).

This approach has been used to inhibit the replication of wild-type Ad in healthy livers. Binding sites for the hepatocyte-specific miRNA-122 were introduced into the 3'UTR of the early viral gene E1A, resulting in a strong inhibition of viral replication in hepatocytes and a concomitant

reduction in liver damage, without decreasing the oncolytic potency of the virus in cancer cells that did not express miRNA-122.

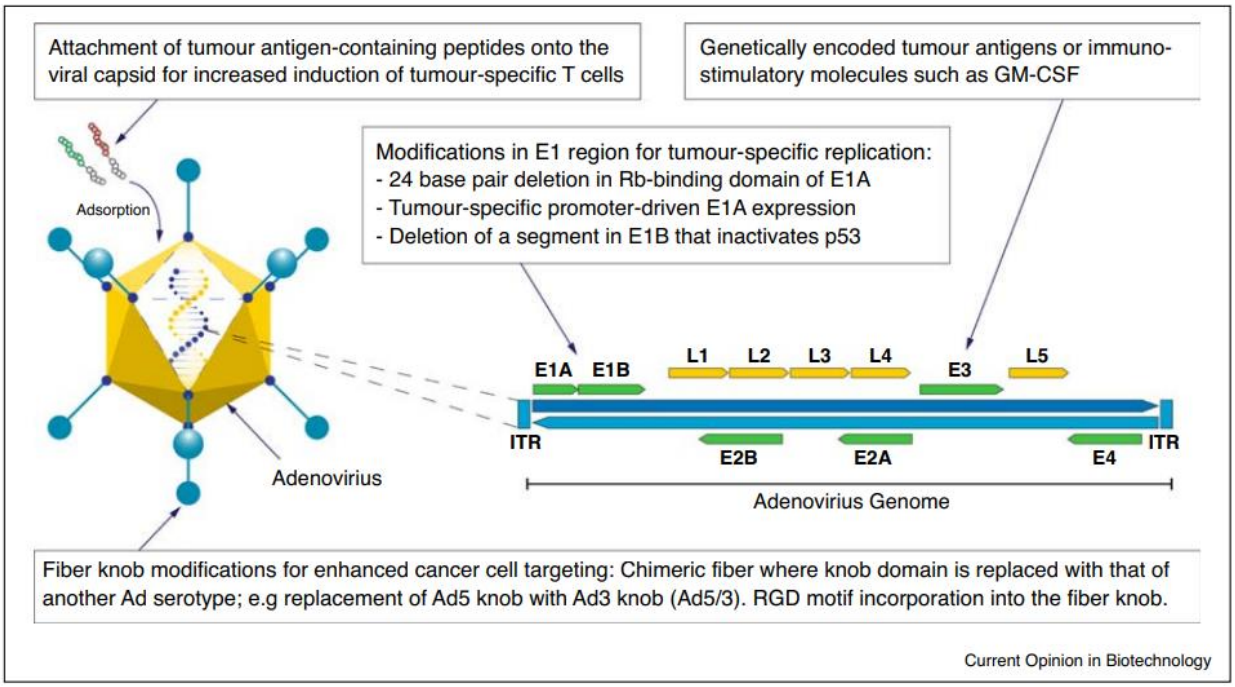

fig.(9): Schematic representation of various strategies for the design of an oncolytic adenovirus.

Modifications in the viral E1, E3 and fiber knob regions are commonly used in oncolytic adenoviruses used in clinical trials. Ad, adenovirus; Rb, retinoblastoma protein; p53, cellular tumour antigen p53; ITR, inverted terminal repeat [5].

A parallel strategy was used to avoid the replication of HSV-1 in normal cells. Multiple copies of complementary target sequences for miR-143 or miR-145 (which are expressed in normal cells, but are down-regulated in prostate cancer cells) were inserted into the 3'UTR of the ICP4 viral gene. Selective viral replication was observed in prostate cancer cells, with a >80% reduction in tumour volume in mice bearing LNCaP human prostate tumours.

Although miRNA-mediated inhibition appears to be a general method that is useful for the selective reduction of virus replication, some viruses, such as VSV, are relatively resistant to this approach. Nevertheless, following the introduction of four tandem copies of the neuronal miR-125 target sequence into the 3'UTR of the VSV polymerase (L) gene, the neurotoxicity of the virus

was reduced in mice. Interestingly, the post-transcriptional regulation of viral genes by miRNAs can be used in combination with classical promoter replacement strategies to achieve an optimal control of viral replication.

Transcriptional control of viral genes can be combined with specific deletions that impair replication in normal cells. For example, deletions in the pRB-binding CR2 domain of the Ad E1A gene eliminate the ability of E1A to interfere with the pRB pathway but do not affect its function as a pan-activator of virus transcription.

Therefore, placing this E1A mutant under the transcriptional control of optimised E2F1 or hypoxia-responsive promoters can produce highly tumour-specifi c OAVs. Deletion of the E1B 19K gene causes early apoptosis in normal cells infected with adenovirus, thus blocking viral spread. This modification has been used in conjunction with replacement of the E1A promoter by the regulatory sequence of the AFP protein plus additional HREs.

Drug-inducible replication of HSV-1 has been recently achieved by placing the essential ICP27 viral gene under the control of a tetO-bearing promoter. The ICP0 gene was substituted by the tetR repressor and the basal expression of ICP27 was further reduced by insertion of a self-cleaving ribozyme. The resulting virus KTR27 shows efficient replication in cancer cells upon tetracycline treatment. Cleavage of the envelope fusion (F) protein of MV by cellular furin is needed for activation of its fusogenic properties. Introduction of matrix metalloproteinase (MMP) substrate motifs in the F protein resulted in oncolytic MV that forms syncytia preferentially among MMP-expressing cancer cells [23].

12. Naturally occurring oncolytic viruses.

The idea of using naturally occurring viruses for the treatment of cancer was almost abandoned after vigorous attempts during the 1960s and 1970s because of the lack of means to control viral pathogenicity at the time.

However, the idea was revived along with the emerging development of genetically engineered viruses, and newly developed naturally occurring viruses are typically those that are not pathogenic in humans. Reolysin. Reoviruses are double-stranded RNA viruses that replicate preferentially in transformed cell lines but not in normal cells [16][24].

In theory, oncolytic properties of reovirus depend on activated Ras signaling. Reolysin is the T3D strain of reovirus, which has been most extensively studied among several serotypes as an anticancer agent, and is currently the only therapeutic wild-type reovirus in clinical development.

The first phase I trial involved intralesional administration of Reolysin in patients with advanced solid tumors. The most common treatment-related adverse events were nausea (79%), vomiting (58%), erythema at the injection site (42%), fevers/ chills (37%) and transient flu-like symptoms (32%).

Further phase I studies demonstrated the safety and broad anticancer activity of Reolysin in prostate cancer, malignant glioma, metastatic colorectal cancer, multiple myeloma and solid cancers.

Multiple phase II studies have investigated intralesional injection of Reolysin together with local irradiation for the treatment of refractory or metastatic solid tumors, intravenous administration of Reolysin for metastatic melanoma and intravenous administration of Reolysin in combination with chemotherapy for head and neck cancer or lung squamous cell carcinoma.

A randomized double-blinded phase III trial has been performed, comparing intravenous Reolysin in combination with paclitaxel and carboplatin versus chemotherapy alone, in patients with metastatic and/or recurrent head and neck cancer. Patients were treated with intravenous administration of tissue culture infectious dose-50 (TCID50) of Reolysin on days 1–5 with standard doses of intravenous paclitaxel and carboplatin on day 1 only every 21 days, versus standard doses of intravenous paclitaxel and carboplatin alone.

According to a report by the company developing Reolysin, of 165 patients analyzed, 118 patients had regional head and neck cancer with/without distant metastases and 47 patients had distant metastases only. In patients with regional cancer, a significant improvement in OS was observed for the Reolysin group versus the control group ($P = 0.0146$). The FDA in the USA granted Reolysin an orphan drug designation for malignant glioma, ovarian cancer and pancreatic cancer in 2015 [25] [24].

13. Genetically Engineered Oncolytic Virus.

To enhance the therapeutic effect, modifications in OVs through genetic engineering, including insertions and deletions in the genome, can deliver additional therapeutic molecules to cancer cells and effectively avoid the widespread resistance of single-target anticancer drugs [1] [24][16].

At present, there are nearly a hundred therapeutic exogenous genes in research, such as cell death-related molecules, anti-angiogenic molecules, and small RNA molecules (including miRNA, siRNA, shRNA, and lncRNA) that inhibit tumor-related genes.

It is well known that the resistance to oncolytic virotherapy of tumor is related to the over-expression of PD-L1 expression on tumor cells and immune cells. In a study by Guan Wang, an OV that expressed PD-L1 inhibitor and GM-CSF was generated by genetic engineering technology. The PD-L1 secreted by the engineered OV could block PD-L1 on tumor cells and immune cells.

The result showed that the OV could enhance the activity of cancer neoantigen-specific T cell responses and acquire more effective antitumor effects, especially for cancer patients insensitive to PD-1/PD-L1 blockade therapy. Besides, an OV armed with IL-7, IL-12, and IL-24 master pro-inflammatory cytokine interleukin or Beclin-1 was all proved to have a superior antitumor activity than the parent OV.

Suicide gene therapy is also one of the methods of tumor gene therapy, also known as viral-mediated enzyme hydrolytic drug precursor therapy (VDEPT). So-called suicide gene therapy is the introduction of a gene encoding a sensitive factor into tumor cells, so that the cells have a specific sensitivity to a nontoxic or low-toxicity drug, resulting in the death of tumor cells.

In a study by Su-Nam Jeong, researchers constructed a novel oncolytic vaccinia virus by replacing the vaccinia growth factor (VGF) and viral TK (vTk) genes with genes expressing TNFrelated apoptosis-inducing ligand (TRAIL) and angiopoietin 1 (Ang 1). This gene transition could enhance the ability of tumor-targeted apoptosis and immune response of the novel oncolytic vaccinia virus with high biosafety.

Different from other combined therapies, the OV can achieve specific local expression effects through being armed with therapeutic transgenes, which, to a certain extent, means more accurate

tumor killing. Besides, direct modification of foreign genes on reproducible OVs can obtain a long-term expression effect of related genes.

Due to the heterogeneity of tumor cells, it is unlikely to achieve a satisfying effect to treat tumors with monotherapy. Therefore, the combination of OV therapy and other therapies may be a better way to improve the efficacy and maximize the survival of patients [1].

14. Clinically approved OVs for treatment cancer.

Clinically, OVs have moved beyond a field of investigative laboratory-based research to becoming acknowledged as validated therapeutics [10] [1]. To date, three OVs have been approved globally for the treatment of advanced cancers.

1) The first in 2004 was an RNA virus derived from the native ECHO-7 strain of a picornavirus, called Rigvir, and achieved approval for melanoma treatment in Latvia [26][16].

2) Then, in November 2005, China approved a genetically modified adenovirus, (An E1B-deleted adenovirus), called Oncorine (H101, the same construct as ONYX-015) [11], for the treatment of nasopharyngeal carcinoma in combination with cytotoxic chemotherapy[16]. Which was approved for head and neck cancer and esophagus cancer. The use and clinical data of Oncorine is so far limited to China [11].

3) The other is the herpes simplex virus 1-based Talimogene Laherparepvec (T-Vec; Imlygic™, formerly OncoVEXGMCSF), which was approved for melanoma by the U.S. Food and Drug Administration (FDA) in the USA in October 2015 and was subsequently approved in Europe in January 2016 and in Australia in May 2016 [16].

The field is now moving towards discovering optimal viral backbones to limit toxicity and maximize anti-cancer potency, while clinical studies are being conducted to define the clinical contexts in which OVs will fit [10] [8][6].

Below, we will describe the Engineering/ development of (T-Vec) Oncolytic virus, *(the most successful OV to date):*

14.1. Imlygic™ and beyond—biological strategies for engineering superior oncolytic viruses.

Amgen's Imlygic™ recently gained FDA and European approval for the treatment of advanced melanoma, with clinical trials underway to expand both the clinical indications and the strategic approach for its use.

Imlygic™ (formerly OncoVEX) is built on the JS1 strain of HSV-1, which was isolated from the infected cold sore of an otherwise healthy volunteer. The ICP-34.5 gene was deleted to increase both malignant cell specificity and potency, co-incident with an ICP-47 gene deletion to increase antigen presentation [26].

The human cytokine granulocyte-macrophage colony-stimulating factor (GM-CSF) was added to the HSV-1 vector in an effort to enhance anti-tumour adaptive immunity and provide long-lasting anticancer immune surveillance [12] [1][8].

This approach has proven clinically effective, which justifies its continued investigation (Table 1). Despite the clinical activity of Imlygic™, many believe this iteration of oncolytic HSV-1 poorly capitalizes on the therapeutic potential of the OV platform and, thus, laboratory research designed to improve OV therapeutics continues[16] [10].

Many clinical trials using T-Vec are currently performed worldwide by the pharmaceutical company in order to expand its application and also to expand countries for marketing[16] [26].

14.2. Other oncolytic viruses that are closing in on drug approval.

In North America and Europe include vaccinia virus JX-594 (pexastimogene devacirepvec) for hepatocellular carcinoma, GM-CSFexpressing adenovirus CG0070 for bladder cancer, and Reolysin (pelareorep), a wild-type variant of reovirus, for head and neck cancer [8].

In Japan, a phase II clinical trial of G47Δ, a third-generation oncolytic HSV-1, is ongoing in glioblastoma patients. G47Δ was recently designated as a "Sakigake" breakthrough therapy drug in Japan. This new system by the Japanese government should provide G47Δ with priority reviews and a fast-track drug approval by the regulatory authorities [16] [11].

15. Strategies to maximize anti-tumour immunity.

A recent focus of the OV field has been to optimize virus design or combination therapeutic strategies to improve the ability of existing platforms to induce anti-tumour immunity.

One effective approach has been the inclusion of immunostimulatory transgenes and/or cytokines into candidate OVs. For instance, Imlygic™ unquestionably the most successful OV to date encodes and expresses human GM-CSF cDNA.

The addition of GM-CSF promotes monocyte-to-dendritic cell differentiation, thereby facilitating antigen presentation on the surface of dendritic cells following viral-induced oncolysis.

Another widely utilized strategy in HSV-1 is the addition of various interleukins to activate adaptive immunity through T cell activation (IL2) or by doubly activating both T cells and NK cells (IL12, IL15, IL18). The ability to direct the co-stimulation of T cells has also been exploited in the development of HSV-1-based OVs.

Specifically, B7- 1 (CD80) has been cloned into the HSV backbone to help activate antigen presentation, a strategy that has also been utilized with the ligand of CD40, likely working through a PI3K-dependent mechanism.

Similarly, approaches have been taken to create more immunostimulatory oAd and VacV OV candidates.

As with Imlygic™, human GM-CSF has been encoded into both oAd and VacV candidates. Specifically in the oncolytic VacV Pexa-Vec, ongoing phase III clinical efforts are being made following several promising phase I and II studies.

Although there is a clear trend in the OV field of encoding human GM-CSF cDNA and/or that of other cytokines in clinical candidate strains, it is important to understand the fundamental immunology behind this strategy.

The mechanisms are incompletely understood, but some evidence suggests that we can trigger the activation of immune inhibitory cells such as myeloid derived precursor cells upon cytokine-encoding viral infection.

More fundamental discovery must occur to understand the control of these approaches, but we believe it is important for the field to consider potential unwanted outcomes caused by stimulating undesirable immune targets. Since the generation of oAds began prior to VacV OV development, many more strategies have been attempted in this context, especially as they relate to immune cell recruitment and activation.

From the CC chemokine sub-family, two proteins have been encoded into oAds—CCL3 and RANTES (CCL5), which work to attract PMN leukocytes and T cells, respectively—positively affecting OV efficacy while recruiting immune cells to the tumour microenvironment.

Additionally, IL4 and IL12 have been used to elicit anti-tumour immunity; however, the toxicity risk of the latter was recently called into question in the context of oAd in a hamster pancreatic cancer model, so questions remain. Similar to the approach in HSV-1 of using B7-1 to increase T cell co-stimulation, this approach has been adapted for both VacV and oAd with the tumour necrosis factor receptor family member CD137 (4-1BB).

Interestingly, this approach has also been adapted as a cotherapeutic approach in which the TK/VGF double-deleted strain of VacV is co-administered with a CD137 agonist, which resulted in a decreased tumour burden and increased intratumoural immune infiltrate [10].

The trend in the OV field of combining strategies to make more immunogenic clinical candidate viruses extends beyond those discussed in this review. However, this tendency reveals a fundamental truth that researchers in the OV field are accepting—current OVs are most likely to be clinically successful when they have been optimized for their ability to induce anti-tumour immunity [10] [27].

16. Drugs being used in combination with OVs.

Overall, of the 97 clinical trials reviewed, 61 (62.9%) clinical trials were conducted with OV monotherapy while 36 (37.1%) reported OV was given in combination with at least one other treatment or anticancer drug. Of the combinations **(see table.3),** the most common other drugs were cytotoxic chemotherapy agents (n=36; 37.1%) and chemotherapy prodrugs (n=7; 7.2%) [25].

Table (3): Combination agents used with oncolytic viruses (OVs) in clinical trials.

Treatment	N	N	Agents
Oncolytic Virus alone	61		
Oncolytic virus and other therapy	36		
	Chemotherapy	36	
	Radiation therapy	6	
	Immunotherapy	5	Interferon-alpha; IL-2; Ipilimumab (n=2), Pembrolizumab
	Pro-drugs	7	5-FU (n=3); Ganciclovir (n=2); Valganciclovir (n=2)
	Targeted therapy	4	Bevacizumab; Bortezomib; Erlotinib; Rituximab

(A) The number of clinical studies using monotherapy OVs (n=61) or combination trials (n=36) with the breakdown by types of other drugs or regimens combined with OVs. The specific agents are listed for immunotherapy, prodrugs and targeted therapy [10].

Other modalities used in OV combination studies included radiation therapy (n=6; 6.2%), immunotherapy (n=5; 5.2%) and targeted therapy (n=4; 4.1%). The types of chemotherapy agents used are shown in (figure.10) with the most common not reported and largely from studies that allowed investigator choice or standard chemotherapy to be given with OV treatment and the type of chemotherapy was not explicitly reported.

Where specific agents were prespecified within the clinical protocol, the most common agents used were paclitaxel (n=5) and carboplatin (n=4), often used together. In addition, four studies used cyclophosphamide, which was given as preconditioning chemotherapy to help promote antitumor immune responses.

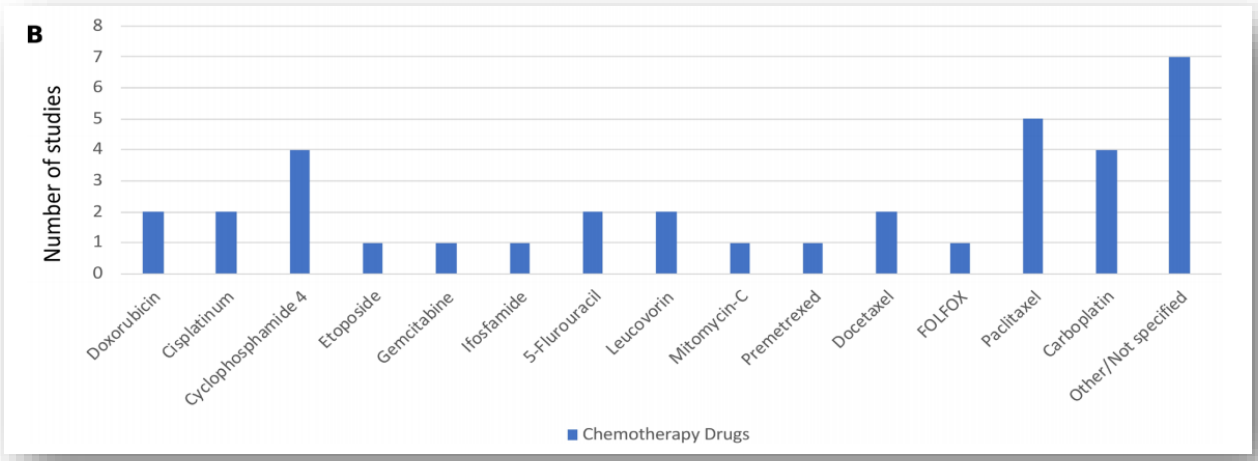

Fig.(10): chemotherapy agents used in combination OV clinical trials. IL-2, interleukin 2; 5-FU, 5-fluorouracil [17].

There were seven studies that combined OV treatment with a prodrug, including three studies with 5-fluorocytosine, a precursor to 5-fluorouracil, two studies with ganciclovir and two studies with valganciclovir. There were few clinical studies reporting on the combination of OV and immunotherapy, but all studies used immune checkpoint blockade or cytokines.

Two trials used ipilimumab and one study used pembrolizumab. There was one study each using interferon-alpha and IL-2. There were four studies that reported on OV and targeted therapy with one each evaluating combinations with erlotinib, rituximab, bortezomib and bevacizumab [13] [17] [23].

16.1. Synergy between oncolytic viruses and immune checkpoint blockade.

As has been clinically well-documented, current standards of immune checkpoint inhibition (ICI) therapy, while impressive as monotherapies, are effective in only a minority of cancer patients.

This is likely because of the mechanism of action of ICIs, which by design Bremove the brakes from existing anti-tumour immune responses. Likely in many patients, pre-existing bona fide anti-tumour immunity is not present, and thus, there is little for an ICI therapeutic approach to augment.

An objective appraisal of the OV literature will also demonstrate their limitations as monotherapies in many patients; however, it is important to understand that we are in the midst of improving early-generation products, and that by their mechanistic nature, OVs are optimally positioned to work in synergy with existing ICIs.

As discussed, numerous pre-clinical and clinical studies are beginning to demonstrate this. One such example occurred in a mouse tumour model with intratumoural NDV infection in combination with systemic anti-CTLA-4 administration, leading to a systemic antitumour effect.

Another example of OV synergy with ICI therapy was observed in difficult-to-treat mouse cancer models using a Semliki Forest virus platform encoding IL12 in concert with anti-mouse-PD-1. These and other data have led to clinical studies designed to test OVs in combination with ICIs.

Ongoing studies include the combination of Imlygic™ with the anti-CTLA-4 product Yervoy® , and in another study with the anti-PD1 therapy Keytruda®.

An exciting recent development in the OV field was the publication of results from the first phase Ib trial of combination Imlygic™ and Yervoy® in advanced melanoma, which demonstrated significant improvements over either monotherapy. In addition, the chimeric oAd Enadenotucirev and the naturally oncolytic Reolysin® virus are being tested in combination with Keytruda®.

While the clinical benefits of ICIs began to emerge, it became evident that systemic administration of these agents carried the potential to cause immune-related adverse events in some patients that required acute immunosuppressive therapies to manage.

Given that OVs can often harbor and express therapeutic payloads, it was hypothesized that the intratumoural OV-induced production of an antibody against CTLA-4 could reduce toxicity while improving anti-tumour immunity. Thus, a transcriptionally targeted oAd expressing anti-CTLA-4 was generated, which demonstrated preferential tumoural replication, effective anti-CTLA-4 delivery to the tumour, and mechanistically linked T cell activation. This approach of intratumoural viral-induced anti-CTLA-4 production was replicated in an oAd, which demonstrated efficacy in an immunocompetent B16 melanoma model.

More recently, antibodies against CTLA-4 and PD-L1 were cloned into the measles virus platform, which improved therapeutic outcomes in terms of both tumour size and overall survival. This effect

was observed to be maintained when systemic administration of immune checkpoint blockade was alternatively utilized, strongly supporting the clinical rationale for co-treatment with OVs and immune checkpoint blockade, (fig:11) [10] [23] [7].

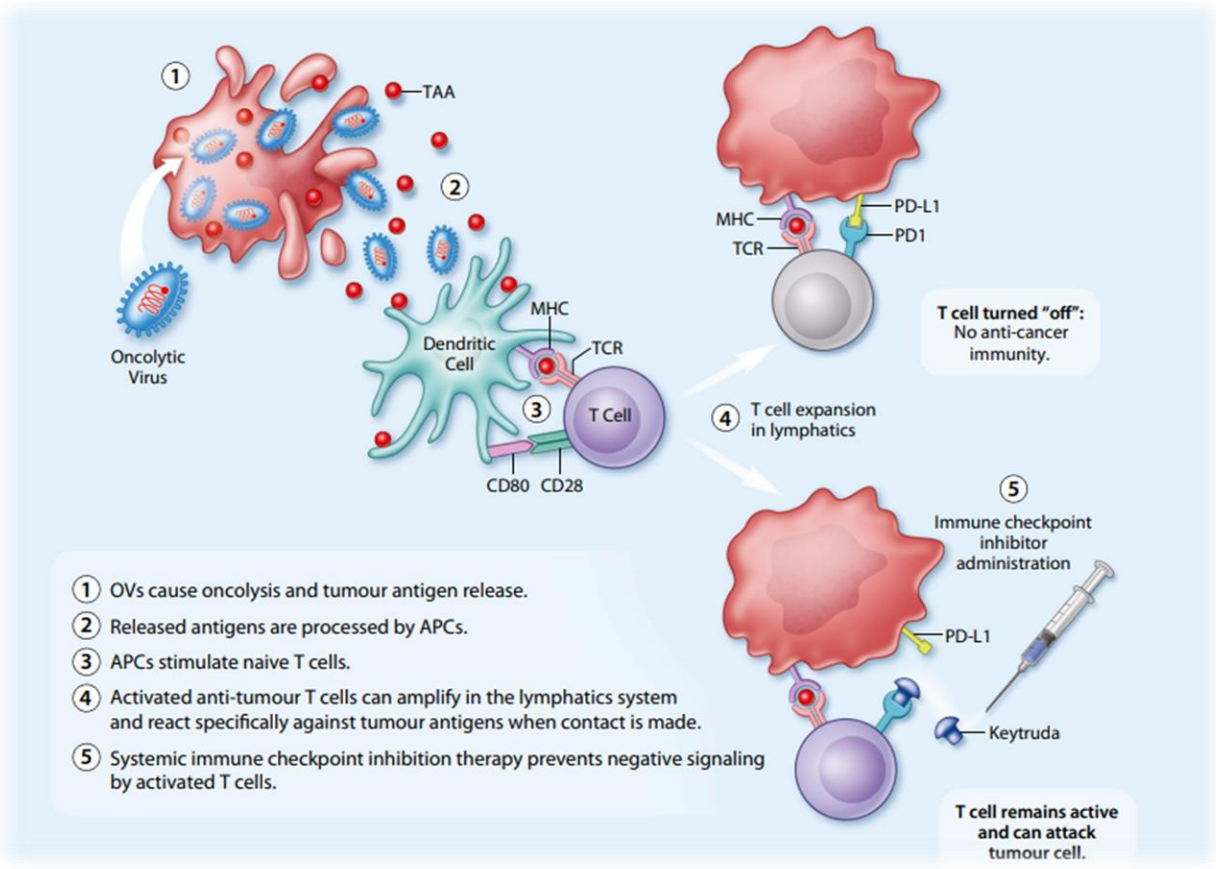

Fig. (11): Synergy between oncolytic viruses with existing immunotherapies.

Cancer cell infection and direct oncolysis leads to the release of tumour-specific antigens, which can be processed by dendritic cells for antigen presentation to naïve T cells. Now activated against the tumour antigen, these T cells can aggressively replicate within lymphoid organs before being circulated to intact tumour cells. The concomitant systemic administration of checkpoint inhibitors (ex. Keytruda®; antiPD-1) will block the negative checkpoints on the surface of cytotoxic T cells, providing a competitive advantage for the positive regulators of T cell function to interact with the tumour cell, leading to their specific destruction. TAA tumour-associated antigen, TCR T cell receptor, APC antigen presenting cell [10].

17. Routes of OVs administration.

The delivery of OVs has been a controversial area and so we sought to determine which routes of administration were used in the reported OV clinical trials (figure. 12).

The most common route was **IT delivery** used in 48 of the clinical trials (49.5%) followed by **intravenous delivery** used in 34 of the clinical trials (35%). Other routes of delivery included **hepatic artery infusion** in six studies (6.2%), **intraperitoneal delivery** in five studies (5.1%) and additional delivery modalities included **intravesical delivery** (n=2), **direct injection** of a resected tumor bed (n=1), **convection-enhanced delivery** to brain tumor bed (n=1), **intradermal injection** (n=1), ex vivo infection of tumor cells (n=1), and two studies did not report how the OV was given.

There were no reported clinical trials using stem cell or nanovesicle delivery, although these have been described in preclinical studies. When delivery was evaluated by numbers of patients **(fig.12),** the most common approaches were again IT (n=1482; 45.8%) and intravenous (n=1147; 35.5%). Fifty-four patients received OVs by multiple routes in the same study, most commonly a combination of intravenous and IT. Another 550 (17%) of patients received OVs through other routes,as described above [17].

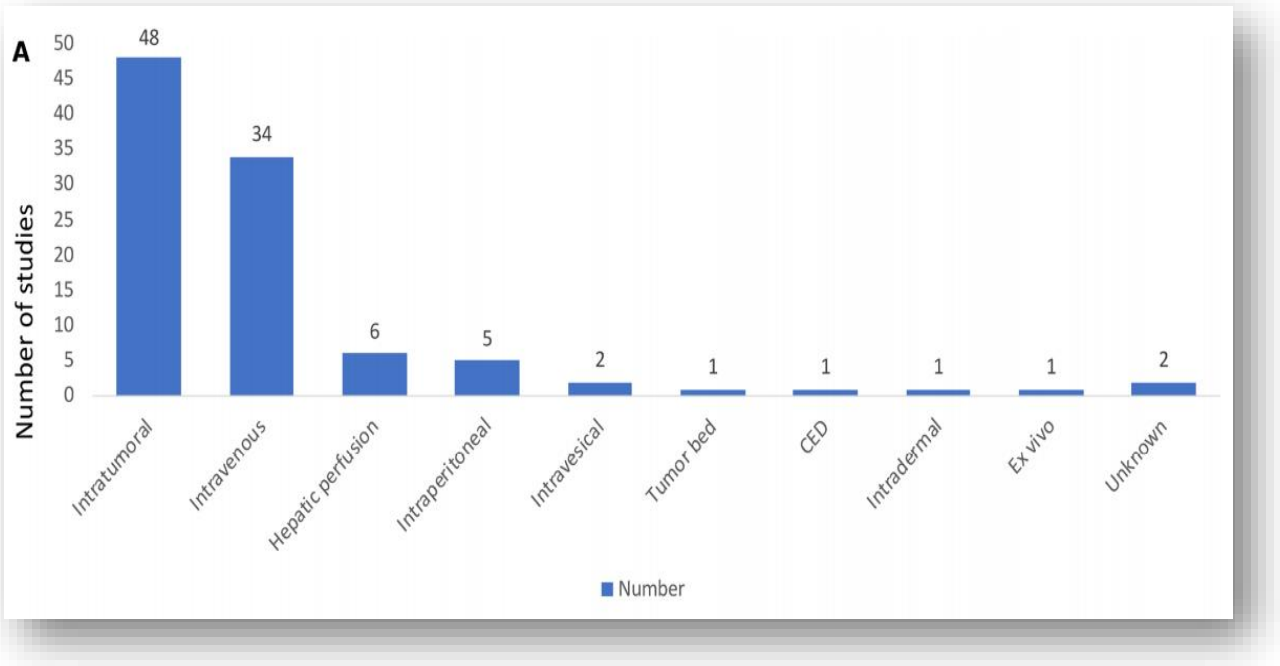

Figure (12): Routes of adminstration for oncolytic viruses in clinical trials.

Method of oncolytic virus delivery in clinical trials; most were by intratumoral (n=48) or intravenous (n=34) routes of administration with 18% using alternative delivery routes. CED, convection-enhanced delivery [15].

18. Current limitation of Ovs.

Currently, the two most challenging problems of oncolytic virotherapy are as follows:

(i) to ensure that the virus can maximize the ability of invasion and replication in tumor cells without infecting healthy tissues and cells to minimize the damage to the body.

(ii) to prevent the virus from being eliminated by the body's strong immune system, which leads to a significant reduction in the efficacy.

For these two problems, on the one hand, the specificity of the OV can be enhanced by further modification of the genome; on the other hand, an attempt can be made to construct appropriate cell vectors for the OV. Healthy cells of the body can be selected to help the OV achieve immune evasion [3] [15] [5] [21].

On this basis, targeted drug therapy can combine with oncolytic virotherapy to enable OV-carrying targeted drugs in a certain way, thus enhancing the anticancer effect. It is believed that the future development direction of oncolytic virotherapy will be an organic combination of gene modifications, construction of virus carriers, and targeted drug therapy [1].

19. Advantages and Disadvantages of OVs.

A wide variety of oncolytic viruses are currently under clinical development worldwide, and, as described in this review, each oncolytic virus carries the characteristics of the parental wildtype virus, not only the advantages but also the disadvantages [14] [16] [15].

Compared with other tumor immunotherapies, OVs have many advantages, such as: high killing efficiency, precise targeting, fewer side effects or drug resistance, and low cost. All of these make oncolytic virotherapy a promising therapy to fight cancer compared with surgical therapy, chemoradiotherapy, and targeted therapy [1].

For example, in regards to oncolytic HSV-1, such as T-Vec and G47Δ, because HSV-1 spreads from cell to cell and does not naturally cause viremia, oncolytic HSV-1 is best administered intralesionally and may not be well suited for intravenous delivery.

However, as proven by the phase III study of T-Vec in melanoma patients at advanced stages, local intralesional injections with oncolytic HSV-1 can act on remote lesions via induction of systemic antitumor immunity and prolong survival. It has been shown that expression of GM-CSF does not augment the efficacy of oncolytic HSV-1, while IL-12 expression does, in immunocompetent mouse tumor models.

Therefore, it is likely that the systemic effect via antitumor immunity was due to the characteristics of HSV-1 itself rather than the effect by GM-CSF. One major concern of oncolytic virus therapy has been that the efficacy may be diminished by the presence of circulating antibodies.

Viruses that naturally cause viremia are likely vulnerable to neutralizing antibodies; therefore, for such viruses, the antitumor effect of intravenous administration may be limited in patients who have had previous treatment or vaccination.

An unfavorable effect of circulating antibodies was well documented in a clinical trial using oncolytic measles virus (MVNIS) in patients with multiple myeloma. In this dose escalation study, it was only after the dosing level reached a very high dose of 1011 TCID50 that intravenous infusion with MV-NIS showed efficacy.

In a preclinical study using tumor-bearing immunocompetent mice, intravenous treatment with reovirus resulted in regrowth of tumors 3 weeks after initial tumor growth inhibition, which coincided with the rise in serum antireovirus antibody titers.

Phase I data showed that the maximum neutralizing anti-reovirus antibody titers were reached by day 7 in 12 (36%) of 33 patients and at day 14 in 20 patients (61%).

It was, therefore, recommended that, for systemic treatment, reovirus should be administered in rapid, repeated, high doses within the first week of treatment before the rise of serum neutralizing antibodies, and that it should be used in combination with other anticancer therapies [16].

Below, we will in brief mention the advantage and disadvantages of different oncolytic viruses: (shortened in table.4.)

Table.(4): advantages and disadvantages of different oncolytic viruses [14]**.**

Virus	Oncolytic strain occurrence	Advantages	Disadvantages
HSV1	Laboratory engineered	Can be easily manipulated genetically; clinical trial experience; drugs exist to shut-off unwanted viral replication	Side effects include serious or potentially fatal disease; unknown action of many HSV1 genes
Adenovirus	Laboratory engineered	Can be manipulated genetically; clinical trial experience; good knowledge of viral protein function; associated with relatively mild diseases	Replication cannot be easily shut-off
Reovirus	Naturally occurring	Associated with relatively mild diseases; good knowledge of viral gene function	Cannot be easily manipulated genetically; no clinical trial experience; undesirable viral replication cannot be easily shut-off
Vaccinia virus	Laboratory engineered	Can be easily manipulated genetically; clinical trial experience	Undesired viral replication cannot be easily shut-off; unknown action of many genes; side effects might include potentially fatal or seriously morbid disease
Vesiculostomatitis virus	Naturally occurring	Associated with relatively mild disease; good knowledge of viral gene functions	Cannot be easily manipulated genetically; no clinical trial experience; undesirable viral replication cannot be easily shut-off
Poliovirus	Laboratory engineered	Good knowledge of viral gene functions	Cannot be easily manipulated genetically; no clinical trial experience; undesirable viral replication cannot be easily shut-off; associated with fatality or serious disease

HSV1, herpes simplex virus type 1.

20. Considerations for OVs Clinical Trials.

With the emergence of increasing numbers of OVs and combinatorial studies in the clinical trial arena, it is worth considering issues involved in clinical trial design and execution. Areas evolving as the field develops are delivery, viral pharmacokinetics and pharmacodynamics, and biomarkers.

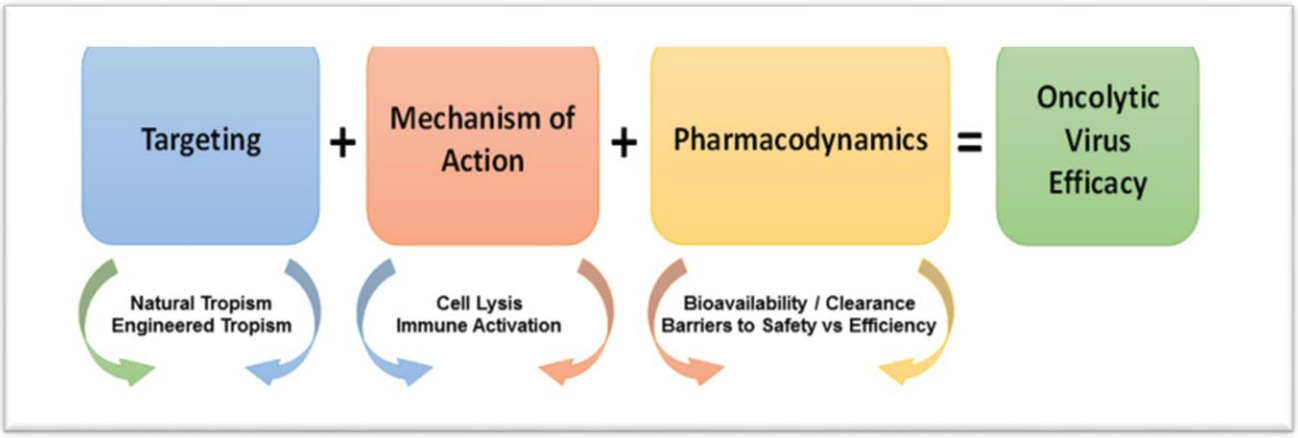

FIGURE (13) : Considerations in the development of oncolytic viruses (OVs).

Considerations in the development of efficacious OV immunotherapy include targeting, mechanism of action, and pharmacodynamics. Targeting (blue box) is dependent on the natural and engineered tropism of viruses for tumor vs. normal cells. The mechanism of action (red box) of OVs is dependent on the immune mechanisms and the non-immune mechanisms of OVs, which are further enhanced by the combination of OVs with traditional and emerging antitumor therapeutics. OVs share pharmacodynamic considerations (orange box) with other small molecule drugs as well as raise new fundamental issues in terms of bioavailability vs. clearance and barriers to safety vs. efficiency. Overlapping arrow colors signify the existent overlap between the listed considerations [24].

Safety:

Although mortality has been reported occasionally, published trial data have not shown significant general safety issues. However, as OVs with greater potency are developed and used in novel combinations, safety remains a concern.

Despite engineering for tumor cell specificity, there is the possibility of off-target effects, and genetic manipulation may result in unexpected toxic effects. Other concerns include virus mutation, evolution, and recombination; cytotoxic gene products; and viral transmissibility.

Oncolytic HSVs have retained their native thymidine kinase gene, which facilitates virus replication and is also the target of the antiviral drug ganciclovir. The retention of thymidine kinase allows the possibility of controlling infection and is seen as an important advantage in terms of safety. Potential safety concerns are reflected in clinical trial criteria, which do not allow inclusion of immunocompromised patients or those with active viral infections.

Toxic and Adverse Effects

Local delivery of OVs is generally well tolerated. The most common adverse effects reported are mild flulike symptoms, which may be more severe after systemic administration, and local reaction at the injection site. These reactions can be reduced by acetaminophen administration before treatment.

Dose

In contrast to results in conventional drug clinical trials, many OVs do not reach an Maximum tolerated dose (MTD) owing to the concentration of virus stock that is possible to achieve or very high tolerance for the virus. Maximum tolerated dose may need to be re-established for trials using novel therapeutic combinations.

Viral Pharmacokinetics, Pharmacodynamics, and Biomarkers

Effectiveness of OV therapy is monitored by standard approaches, including imaging and tumor-specific biomarkers. In addition, viral pharmacokinetics and pharmacodynamics (shedding, viremia, replication, genomes, and viral load) are frequently included in OV trials. These approaches allow inpatient tracking of viral fate in patients.

Additional viral pharmacokinetic and pharmacodynamic variables include analysis of intratumoral viral replication and immune infiltrates by immunohistochemistry and circulating immune cell status. In multi-institutional trials, it may be optimal to use centralized testing to ensure reproducibility.

Resistance Mechanisms

One of the most fascinating features of OV therapy is the battle between the virus and the host, which is vital in determining the therapeutic outcome. The major resistance mechanisms in OV therapy result from the ability of the host to rapidly shut down viral replication.

Host antiviral mechanisms include the presence of neutralizing antibodies and the rapidmobilization of innate immune cells in response to OVs. Cells recruited after OV treatment include neutrophils, natural killer cells, macrophages, and microglia in the brain.

Innate immunity is a major resistance mechanism, which can restrict the ability of the virus to replicate and spread within tumors. The existence of these innate immune resistance mechanisms has led to the idea that inhibition of immune responses early in treatment may be beneficial, and immuno-suppressants such as cyclophosphamide promote viral replication.

However, this approach should be treated with caution for safety reasons and also to ensure that the ultimate anti-tumor response is not blocked. Studies have shown that vascular endothelial growth factor also plays an important, although virus dependent, role in OV action.

In the case of oHSV, vascular endothelial growth factor can limit efficacy, potentially because of recruitment of myeloid cells into the tumor microenvironment. Conversely, vascular endothelial growth factor sensitizes tumor vasculature to vaccinia and VSV via a novel mechanism.

Delivery

Therapeutic delivery of OVs is dependent on virus and tumor type. Most often, OVs are injected directly into the tumor site.

For example, tumors of the brain are treated using local delivery by multiple injections at a single time point (during surgery). Other, more accessible tumors can be treated with multiple doses and multiple injection sites over time. The experience with talimogene laherparepvec,(T-vec) provides an indication that OV administration at a single tumor site can lead to regression of distant tumors, implying that the induction of local antitumor immunity can have systemic effects.

Intravenous injection is also commonly used and allows systemic administration to multiple tumor sites. Cellular carriers may also be used, which may protect the virus from recognition by the host immune system before reaching the tumor.

Ongoing OV Trials

A search of clinicaltrials, performed April 1, 2016, listed approximately 40 clinical trials currently recruiting patients for treatment with OVs. There is representation from multiple countries across 4 continents, with most trials being conducted in the United States. These trials have been almost exclusively performed in adults, with studies in young adults and pediatric patients just beginning.

Most trials are early-phase, dose-finding, and exploratory studies, although increasing numbers of late-phase trials are anticipated. Trials increasingly incorporate viral pharmacokinetics and pharmacodynamics, and a consistent feature is monitoring of the immune response to virus and tumor.

Few viruses in the present trials express human transgenes. Based on encouraging preclinical data, numerous combination studies are under way using small molecules and chemotherapy [13] [22] [24].

21. Executive summary.

Viruses as anticancer drugs

● Viruses naturally possess many properties that favor infection of cancer cells. Enhancing these natural properties and adding new properties through directed evolution and genetic engineering are used to create oncolytic viruses (OVs), which are emerging as a new anticancer drug class.

● OVs target tumor tissues, kill tumor cells directly, amplify antitumor immunity and must be safe for the patient and healthcare workers.

● The diversity of virus families and engineering techniques allows for the creation of OVs with a wide range of properties that can be tailored for each type of cancer.

Delivery of OVs

● OVs do not obey conventional pharmacological principles due to their ability to be biologically amplified after administration.

● Intravenous delivery allows a virus to reach distant sites of metastasis via the circulation, but extravasation into the tumor parenchyma is inefficient.

● Intratumoral injection can concentrate virus at a site of tumor growth, but regression of distant tumors requires that the virus spread systemically or induce a systemic antitumor immune response.

● Neutralizing antibodies, hepatosplenic sequestration of the virus by macrophages and dilution of the virus in blood or tissue may limit the effectiveness of treatment. **Viral spread**

● Targeting viral spread to tumor cells can be accomplished by transductional targeting (modifying receptor tropism), transcriptional targeting (controlling virus gene expression with tumor-specific promoters), physiologic targeting (disrupting viral immune combat proteins), apoptosis targeting (disrupting viral antiapoptotic proteins) or miRNA targeting.

● Viral replication in the tumor and subsequent spread from infected to uninfected cells is critical for tumor eradication.

Arming viruses with transgenes

● The addition of transgenes allows tumor cells that escape viral infection to be killed by bystander effects or be better targeted by the immune system.

● Secreted toxins, prodrug convertases and immunostimulatory proteins have been incorporated into OVs to increase treatment efficacy.

Safety

● Careful steps must be taken to avoid the creation of OVs that might evolve to become serious pathogens.

● Contingency plans to terminate the spread and/or transmission of an infection can increase clinical confidence in viral therapy. ● Ideally, OVs should be nontransmissible [22].

Conclusion.

It would not be too early to say that oncolytic virus therapy is now established as an approach to treat cancer. Because an induction of specific anti-tumor immunity in the course of oncolytic activities is the common feature that plays an important role in presenting antitumor effects, the efficacy of oncolytic virus therapy is expected to improve further when combined with immunotherapy.

By arming oncolytic viruses with functional transgenes, a whole panel of oncolytic viruses with a variety of antitumor functions would be available in the future, from which a combination of appropriate viruses can be chosen according to the type and stage of cancer. A new era of cancer treatment seems at dawn, where cancer patients can freely choose oncolytic virus therapy as a treatment option [15].

In the future, researchers will develope new combination therapies with other agents, generate newly genetically engineered OVs, and produce new delivery systems.

Future perspectives:

The past decade in the OV field has been one of great progress as OVs have demonstrated themselves to be excellent potential partners to boost existing and emerging immunotherapies, and as discussed herein are poised to enhance the growing anti-cancer armament available to today's oncologist.

However, there are still many unanswered questions in the OV field and there is a need to continue funding fundamental research into virus-host interactions. For instance, how can we best design vectors that overcome natural adaptive and innate anti-viral responses facilitating successful systemic delivery and tumour lysis without compromising the excellent safety record OVs have enjoyed to date? These approaches have to be balanced with the need to also stimulate the immune systems of individuals with cancer to generate effective and long lasting anti-tumour immunity. Whatever the answers to these questions, it is clear the OV field has entered 'primetime', and we now seek to deliver the best possible next-generation therapeutics for our patients [10]

Referances:

[1] G. D. Cao *et al.*, "The Oncolytic Virus in Cancer Diagnosis and Treatment," *Front. Oncol.*, vol. 10, no. September, pp. 1–12, 2020, doi: 10.3389/fonc.2020.01786.

[2] A. M. L. S. M. Christopher, "ONCOLYTIC VIROTHERAPY," *Physiol. Behav.*, vol. 176, no. 1, pp. 100–106, 2016, doi: 10.1038/nbt.2287.ONCOLYTIC.

[3] O. Hemminki, J. M. Dos Santos, and A. Hemminki, "Oncolytic viruses for cancer immunotherapy," *J. Hematol. Oncol.*, vol. 13, no. 1, pp. 1–15, 2020, doi: 10.1186/s13045-020-00922-1.

[4] W. Q. Chen, R. S. Zheng, S. W. Zhang, H. M. Zeng, and X. N. Zou, "The incidences and mortalities of major cancers in China, 2010," *Chin. J. Cancer*, vol. 33, no. 8, pp. 402–405, 2014, doi: 10.5732/cjc.014.10084.

[5] E. Ylösmäki and V. Cerullo, "Design and application of oncolytic viruses for cancer immunotherapy," *Curr. Opin. Biotechnol.*, vol. 65, no. Ici, pp. 25–36, 2020, doi: 10.1016/j.copbio.2019.11.016.

[6] A. Santaniello *et al.*, "Tumour Microenvironment and Immune Evasion in EGFR Addicted NSCLC : Hurdles and Possibilities," pp. 1–15.

[7] P. K. Bommareddy, M. Shettigar, and H. L. Kaufman, "Integrating oncolytic viruses in combination cancer immunotherapy," *Nat. Rev. Immunol.*, vol. 18, no. August, pp. 1–16, 2018, doi: 10.1038/s41577-018-0014-6.

[8] L. Russell and K. Peng, "The emerging role of oncolytic virus therapy against cancer.," vol. 7, no. 2, 2019, doi: 10.21037/cco.2018.04.04.The.

[9] W. K. Decker *et al.*, "Cancer immunotherapy: Historical perspective of a clinical revolution and emerging preclinical animal models," *Front. Immunol.*, vol. 8, no. AUG, 2017, doi: 10.3389/fimmu.2017.00829.

[10] B. A. Keller and J. C. Bell, "Oncolytic viruses—immunotherapeutics on the rise," *J. Mol. Med.*, vol. 94, no. 9, pp. 979–991, 2016, doi: 10.1007/s00109-016-1453-9.

[11] H. L. Kaufman, F. J. Kohlhapp, and A. Zloza, "Oncolytic viruses: A new class of immunotherapy drugs," *Nat. Rev. Drug Discov.*, vol. 14, no. 9, pp. 642–662, 2015, doi: 10.1038/nrd4663.

[12] J. Ma *et al.*, "Characterization of virus-mediated immunogenic cancer cell death and the consequences for oncolytic virus-based immunotherapy of cancer," *Cell Death Dis.*, vol. 11, no. 1, 2020, doi: 10.1038/s41419-020-2236-3.

[13] S. E. Lawler, M. C. Speranza, C. F. Cho, and E. A. Chiocca, "Oncolytic viruses in cancer treatment.," *JAMA Oncol.*, vol. 3, no. 6, pp. 841–849, 2017, doi: 10.1001/jamaoncol.2016.2064.

[14] E. A. Chiocca, "Oncolytic viruses," *Nat. Rev. Cancer*, vol. 2, no. 12, pp. 938–950, 2002, doi: 10.1038/nrc948.

[15] C. M. Oh, H. J. Chon, and C. Kim, "Combination immunotherapy using oncolytic virus for the treatment of advanced solid tumors," *Int. J. Mol. Sci.*, vol. 21, no. 20, pp. 1–16, 2020, doi: 10.3390/ijms21207743.

[16] H. Fukuhara, Y. Ino, and T. Todo, "Oncolytic virus therapy : A new era of cancer treatment at dawn," vol. 107, no. 10, 2016, doi: 10.1111/cas.13027.

[17] N. Macedo, D. M. Miller, R. Haq, and H. L. Kaufman, "Clinical landscape of oncolytic virus research in 2020," *J. Immunother. Cancer*, vol. 8, no. 2, 2020, doi: 10.1136/jitc-2020-001486.

[18] H. Kasuya, S. Takeda, S. Nomoto, and A. Nakao, "The potential of oncolytic virus therapy for pancreatic cancer," *Cancer Gene Ther.*, vol. 12, no. 9, pp. 725–736, 2005, doi: 10.1038/sj.cgt.7700830.

[19] R. M. Eager and J. Nemunaitis, "Clinical development directions in oncolytic viral therapy," *Cancer Gene Ther.*, vol. 18, no. 5, pp. 305–317, 2011, doi: 10.1038/cgt.2011.7.

[20] J. Burke, J. Nieva, M. J. Borad, and C. J. Breitbach, "Oncolytic viruses: perspectives on clinical development," *Curr. Opin. Virol.*, vol. 13, pp. 55–60, 2015, doi: 10.1016/j.coviro.2015.03.020.

[21] E. Vacchelli *et al.*, "Trial watch: Oncolytic viruses for cancer therapy," *Oncoimmunology*, vol. 2, no. 6, 2013, doi: 10.4161/onci.24612.

[22] J. Maroun, M. Muñoz-alía, A. Ammayappan, A. Schulze, K. Peng, and S. Russell, "Designing and building oncolytic viruses," vol. 12, pp. 193–213, 2017.

[23] R. Hernández-alcoceba, "Recent advances in oncolytic virus design," pp. 229–239, 2011, doi: 10.1007/s12094-011-0647-4.

[24] S. R. Jhawar *et al.*, "Oncolytic viruses-natural and genetically engineered cancer immunotherapies," *Front. Oncol.*, vol. 7, no. SEP, pp. 1–11, 2017, doi: 10.3389/fonc.2017.00202.

[25] S. Chaurasiya, N. G. Chen, and Y. Fong, "Oncolytic viruses and immunity," *Curr. Opin. Immunol.*, vol. 51, pp. 83–90, 2018, doi: 10.1016/j.coi.2018.03.008.

[26] S. Harcharik *et al.*, "Oncolytic virus therapy for cancer," pp. 31–46, 2013.

[27] L. Aurelian, "Oncolytic virotherapy : the questions and the promise," pp. 19–29, 2013.