Gastroenterology and Hepatology From Bed to Bench.
2025 **RIGLD**, Research Institute for Gastroenterology and Liver Diseases

REVIEW ARTICLE

p-ISSN: 2008-2258 e-ISSN: 2008-4234

Oncolytic viral therapy as a novel potential solution for treatment of pancreatic cancer

Piruz Shadbash[1,2], Seyed Masoud Hosseini[2], Sahel Abyar[1,2], Shahrzad Shoraka[2], Amir Ghaemi[3], Nosratollah Naderi[4], Seyed Reza Mohebbi[4]

[1]Basic and Molecular Epidemiology of Gastrointestinal Disorders Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2]Department of Microbiology and Microbial Biotechnology, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, Tehran, Iran
[3]Department of Virology, Pasteur Institute of Iran, Tehran, Iran
[4]Gastroenterology and Liver Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran

ABSTRACT

Pancreatic cancer (PC) remains one of the most formidable malignancies, with survival rates showing minimal improvement over the years despite progress in chemotherapy, targeted treatments, and radiation therapy. The development of targeted agents and chemotherapy for cancer treatment has only moderately influenced clinical results and has not significantly altered 5-year survival rates. However, with the rapid discovery of the genetic and molecular functions underlying PC, new opportunities for targeted therapies are emerging. One promising approach is oncolytic viral therapy, which has shown potential as a targeted agent for the treatment of pancreatic cancer. Based on the available evidence, oncolytic viral therapy appears to be a viable treatment option for pancreatic cancer. In the present narrative review, we explore oncolytic viruses in detail, and their potential applications in cancer therapy as a future alternative treatment are investigated.

Keywords: Pancreatic cancer, Oncolytic viral treatment, Therapeutic Targets, Chemoresistance, Immunotherapy.

(Please cite as: **Shadbash P, Hosseini SM, Abyar S, Shoraka S, Ghaemi A, Naderi N, Mohebbi SR. Oncolytic viral therapy as a novel potential solution for treatment of pancreatic cancer: Oncolytic viruses and pancreatic cancer. Gastroenterol Hepatol Bed Bench 2025;18(1):53-69. https://doi.org/10.22037/ghfbb.v18i1.3066).**

Introduction

The pancreas is identified as a gland and a part of the digestive system with two different functions. It plays an important role in the endocrine system by secreting hormones such as insulin, glucagon, and somatostatin, and its function in the exocrine section is known to be producing digestive enzymes, including amylase and lipase, and alkaline fluid. The pancreas exocrine section includes two types of cells identified as acinar and ductal cells.

Received: 14 August 2024 *Accepted*: 28 October 2024
Reprint or Correspondence: **Seyed Reza Mohebbi,** *Gastroenterology and Liver Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*
E-mail: sr.mohebbi@sbmu.ac.ir
ORCID ID: 0000-0002-7020-7889

Acinar cells are recognized to be involved in storing and releasing both active enzymes (e.g., amylase, lipase) and inactive enzymes, known as zymogens, such as trypsinogen (1).

After 40 years of cancer research, pancreatic adenocarcinoma remains a highly fatal illness that is particularly difficult to diagnose and treat. The five-year survival rate shows only a slight improvement, and almost all patients relapse and succumb to the illness (2). It is the fourth leading cause of death from cancer in the United States, with approximately 66,440 new cases diagnosed in 2024 and 51,750 related deaths (3, 4). Currently, combinational treatments such as chemoradiotherapy (CRT) or stereotactic body radiation therapy (SBRT) are used for locally advanced pancreatic cancer (LAPC), although there is no clear consensus on which approach is superior. CRT is often recommended

Copyright © 2025, Gastroenterology and Hepatology From Bed to Bench (GHFBB). This is an open-access article, distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (http://creativecommons.org/licenses/by-nc/4.0/) which permits others to copy and redistribute the material just in noncommercial usages, provided the original work is properly cited.

if the patient demonstrates stability after six months of chemotherapy, with Fluoropyrimidines or Gemcitabine commonly used in conjunction with radiotherapy. However, due to pancreatic cancer's radioresistant nature, only 40%-60% of patients receiving CRT achieve one-year progression control, and over 30%-50% of LAPC patients advance to metastatic stages within three months of diagnosis despite treatment (5). Fortunately, the underlying genetic and molecular mechanisms of pancreatic cancer are being rapidly discovered, providing new targeted therapy opportunities. Oncolytic viral therapy is one of the most promising targeted agents for the treatment of pancreatic cancer which is considered as a part of immunotherapies. In oncolytic virus therapy some specific viruses including adenovirus, reovirus, poxvirus, etc. are genetically altered to become attached to only cancer cells and invade them as their host cells to replicate and make the cancer cells visible. Hence, the immune system is once again able to detect those faulty cells and adopt immune responses including cell death to eliminate the tumor cells. In this review, we generally aim to discuss the pancreatic cancer mechanism, risk factors involved in its progression, some of the current applicable treatments (including surgery, chemotherapy, radiotherapy, and immunotherapy), and the future promising therapeutic targets.

Biology of pancreatic cancer and the factors involved in its progression

Risk Factors involved in the progression of pancreatic cancer are classified into two groups modified (intestinal microflora, smoking, alcohol, chronic pancreatitis (CP), obesity, dietary factors, infection) and non-modified factors (age, sex, area, blood group, family history and genetic susceptibility, diabetes) and they have an association in the PC development in their specific way. More details on each of the risk factors are mentioned in Table 1. CP progresses through three stages: 1) Pre-Pancreatitis Phase: This initial stage is marked by risk factors like

Table 1. Important pancreatic cancer risk factors and their association with the cancer development

Classification	Risk Factor	Association	References
Non-modified Factors	Age	• PC primarily affects older adults, with rare cases under 30 • Around 90% of cases occur in individuals over 55, especially between ages 70-80	(9)
	Sex	• PC incidence is lower in women than in men globally, more pronounced in developed countries • Higher steroid levels in women might provide a protective effect	(10, 11)
	Genetic Factors	• Family history significantly increases PC risk • Over 80% of cases arise from sporadic mutations, with familial risk increasing with the number of first-degree relatives	(12, 13)
Modified Factors	Diabetes	• Type 1 diabetes, particularly with a disease duration over 10 years, raises PC risk by 5-10 times • A diabetes duration exceeding 20 years further increases the risk	(14, 15)
	Human Microbiota	• Microbiota, including bacteria, viruses, and fungi, play roles in PC occurrence and prognosis • Certain microbes (e.g., *Helicobacter pylori*, Hepatitis B virus (HBV), Hepatitis C virus (HCV)) and microbial metabolites (e.g., secondary bile acids) influence cancer cell growth and promote cell abnormalities	(13, 16, 17)
	Smoking	• Smoking is the most significant modifiable risk factor for PC • Smokers have a 74% increased risk, with nicotine-derived carcinogens enhancing stem cell characteristics in pancreatic cells	(18)
	Alcohol	• Heavy drinking (more than 30 g per day) raises PC risk • Low-to-moderate drinking has no clear link to PC, but high alcohol intake shows a dose-response relationship with increased risk	(19)
	Chronic Pancreatitis	• CP is a progressive inflammatory condition in the pancreas linked to an independent increase in PC risk • It causes pancreatic fibrosis, cell mutations, and oncogene activation	(20)
	Obesity	• Fat provides cancer cells with necessary lipids, and the KRAS mutation in obese individuals promotes PC formation • Interaction with environmental, metabolic, and nutrient stressors exacerbates the risk	(21, 22)

alcohol consumption, smoking, and genetic mutations. 2) Acute Pancreatitis (AP) Phase: This stage involves the release of inflammatory cytokines. Severe inflammation can activate macrophage-dependent pancreatic stellate cells, leading to fibrosis, especially with ongoing pro-inflammatory stimuli (1). 3) Progression to CP: In this final stage, immune-modulating factors drive the chronic inflammation seen in CP. Interactions between a weakened immune response to low-grade inflammation and environmental factors (e.g., alcohol, smoking) increase the likelihood of recurrent AP, eventually progressing to CP. CP is considered a major risk factor for pancreatic cancer. The chronic inflammation associated with CP encourages the development of precancerous lesions, such as pancreatic intraepithelial neoplasia (PanINs), intraductal papillary mucinous neoplasms (IPMNs), and mucinous cystic neoplasms (MCNs). These lesions can evolve into pancreatic ductal adenocarcinoma (PDAC) through various molecular changes (1, 6).

Pancreatic cancer treatment is challenging due to various genetic changes, even with targeted therapies. Research indicates that pancreatic tumors have a high concentration of cancer stem cells (CSCs), which play a role in metastasis and recurrence. This abundance of CSCs makes the cancer resistant to chemotherapy, often leading to relapse. Additionally, the epithelial-to-mesenchymal transition (EMT) contributes to pancreatic cancer's aggressiveness. During EMT, epithelial cells transform into mesenchymal cells, which are more resistant to cell death and have increased migration and invasion capabilities (7, 8).

Oncogenic transformation in the pancreas is now considered to be a multi-step process involving the accumulation of inherited and acquired mutations of specific cancer-related genes in precancerous lesions. Three types of precursor lesions of pancreatic ductal adenocarcinoma (PDAC) are described as pancreatic intraepithelial neoplasia, intraductal papillary neoplasia, and mucinous cystic neoplasms. All follow a stepwise progression from preneoplastic stages to invasive cancer, characterized by an increased degree

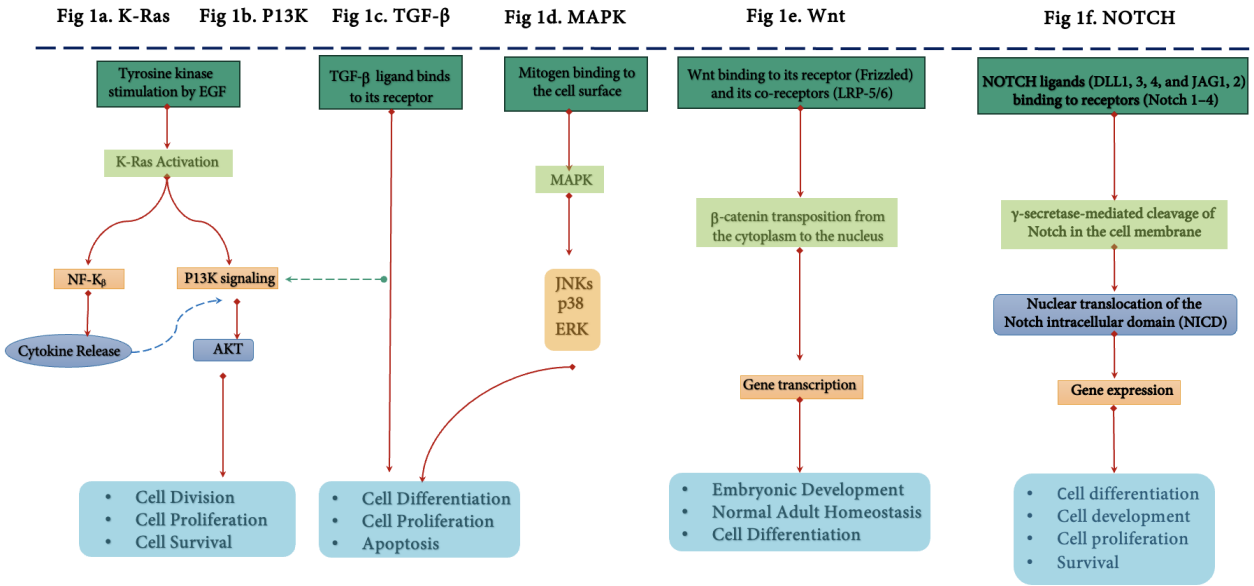

Figure 1. Signaling pathways controlling normal cell function. Fig 1a) K-Ras: KRAS, a key gene in the RAS family, drives cell growth through the MAPK and PI3K/Akt pathways. Mutations, present in over 80% of pancreatic cancers, lead to chemotherapy resistance and tumor progression, but effective inhibitors remain challenging to develop. Fig 1b) PI3K: The PI3K pathway, activated by KRAS mutations or growth factors, supports cancer cell survival and is linked to chemo- and radiotherapy resistance in PDAC. Fig 1c) TGF-β: TGF-β acts as a tumor suppressor early but promotes metastasis in advanced stages via EMT. Its upregulation in pancreatic cancer correlates with poor prognosis, though clinical targeting has shown limited success. Fig 1d) MAPK: The MAPK pathway, disrupted in pancreatic cancer through KRAS mutations, drives disease progression. Targeting its downstream effects shows therapeutic promise. Fig 1e) Wnt: The Wnt/B-catenin pathway promotes EMT, tumor growth, and apoptosis resistance in pancreatic cancer, maintaining cancer stem cells and supporting the tumor microenvironment (37, 38). Fig 1f) Notch: Notch signaling, critical for cell proliferation and EMT, drives pancreatic cancer progression and chemoresistance, making it a potential therapeutic target.

of morphological and cytological atypia (23, 24). Most commonly, it begins with an activating mutation in the KRAS gene, followed by a somatic mutation in one or more of the tumor suppressor genes TP53, p16/CDKN2A, and SMAD4 (25). About 10% of cases may be partially associated with one of several germline mutations, including BRCA2, STK11/LKB1, or p16/CDKN2A (26). This model of PDAC stepwise development has been supported in animal models by transgenic mice with point mutations in the Kirsten rat sarcoma virus (KRAS) oncogene. It was found that these mice developed preneoplastic lesions of the pancreas similar to those found in humans (27). Progression to metastatic carcinoma requires both KRAS mutations and loss of tumor suppressor genes (27-29). Researchers have identified a total of 12 core signaling pathways that are commonly dysregulated in PDAC. Of these, KRAS, TP53, p16/CDKN2A, and SMAD4 are found in two-thirds of the tumors examined (30). The dense extracellular matrix of pancreatic cancer distorts normal tissue structures and causes abnormal configurations of blood vessels and lymphatics, leading to tumor hypoxia, which is one of the reasons why many systemically delivered therapies, such as systemic chemotherapy and viral therapy, do not work well in pancreatic cancer (31). Two other factors involved in PC progression are identified to be signaling pathways and miRNAs. These signaling pathways include K-Ras, phosphatidylinositol 3 kinase (P13K), mitogen-activated protein kinase (MAPK), transforming growth factor beta (TGF-β), canonical Wnt pathway (Wnt/B-catenin), and NOTCH signaling, which can be targeted in the therapeutic strategies including (discussed in Figure 1). In addition, micro ribonucleic acids (miRNAs) such as miR-301, miR-296, miR-21, miR-200 family, miR-125a-3p, etc. are associated with the development of PC. More details on miRNAs association are provided in Table 2.

K-Ras

The KRAS gene, part of the RAS family, encodes the K-Ras protein, a GTPase involved in signal transduction that regulates cell division. Upon activation by epidermal growth factor (EGF) binding to the EGF receptor (EGFR), K-Ras transmits signals through the MAPK and PI3K/Akt pathways, promoting cell proliferation and transformation. Mutations in KRAS, observed in over 80% of pancreatic cancers and more than 90% of pancreatic ductal adenocarcinoma (PDAC) cases, are associated with chemotherapy resistance and tumor growth. Despite being a critical therapeutic target, effective inhibitors for KRAS remain elusive (32, 33).

PI3K

Mutated KRAS activates the PI3K signaling pathway, which is pivotal in regulating oncogenic processes in PDAC. PI3K can also be activated independently by growth factors and cytokines via receptor tyrosine kinases (RTKs). This pathway supports cancer cell survival, metabolism, and migration. In PDAC, PI3K activity is linked to resistance against chemo- and radiotherapy (34).

TGF-β

Transforming growth factor-beta (TGF-β) plays dual roles in cancer. Early in tumor development, it suppresses tumors by halting the cell cycle and promoting apoptosis. However, at advanced stages, TGF-β facilitates metastasis, particularly through epithelial-to-mesenchymal transition (EMT). In pancreatic cancer, elevated TGF-β expression correlates with advanced stages, liver metastases, and poorer prognosis. While preclinical trials targeting

Table 2. MiRNAs involved in PC development

miRNA	miRNA Interference	Reference
miR-301 overexpression	Regulates EMT leading to gemcitabine resistance by suppressing the expression of E-cadherin	(41)
miR-296 overexpression	Downregulates BOK gene 1 and facilitates epithelial-to-mesenchymal transition (EMT), cancer invasion, and drug resistance	(42)
miR-21 overexpression	Enhances NOTCH signaling leading to promotion of EMT signaling and PC progression	(43)
miR-125a-3p overexpression	Decrease the chemosensitivity by targeting EMT related genes	(44)
miR-200b & miR-509-5b downregulation	Increase chemosensitivity	(41, 42)
miR-148a under expression	Reduce pro-apoptotic function	(45)

TGF-β have shown promise, clinical outcomes remain inconsistent (35).

MAPK

The MAPK pathway, essential for cell proliferation, differentiation, and apoptosis, is frequently dysregulated in cancer. It includes kinases like ERK, JNK, and p38. In pancreatic cancer, KRAS and BRAF mutations, along with reduced DUSP6 expression, hyperactivate MAPK, driving disease progression. Targeting MAPK-regulated genes holds therapeutic potential for pancreatic cancer (13, 36).

Wnt

The Wnt/B-catenin signaling pathway, originally linked to embryonic development and adult tissue maintenance, regulates processes like EMT in pancreatic cancer, promoting carcinogenesis, angiogenesis, and apoptosis resistance. This pathway supports the survival of cancer stem cells (CSCs), contributing to apoptosis resistance and maintaining the tumor microenvironment (37, 38).

Notch

The Notch signaling pathway, conserved across species, regulates cell proliferation, differentiation, and survival. In pancreatic cancer, Notch enhances EMT, increasing tumor invasiveness and chemoresistance. It plays a key role in the progression of pancreatic intraepithelial neoplasia (PanIN) to invasive cancer. Notch is often overexpressed in pancreatic cancer and holds promise as a biomarker and therapeutic target (39, 40).

Current PC treatments and future perspectives

Many strategies are being studied that target the stromal matrix to increase the effectiveness of systemic therapies. Inhibition of hedgehog signaling involved in desmoplasia has been shown to increase gemcitabine penetration into pancreatic xenografts. The use of hyaluronidase for enzymatic remodeling of the stromal matrix has shown increased delivery of systemic therapies to tumor tissue. The initial preclinical success of these therapies suggests that the dense extracellular matrix in pancreatic cancer is partly responsible for the innate chemoresistance of pancreatic tumors, by inducing barriers that protect tumor cells from systemic circulating therapeutic compounds (46-49). It is becoming increasingly clear that pancreatic stellate cells (PSCs) are an important component of the tumor microenvironment. These stromal cells have been shown to increase both the proliferation and migration of pancreatic cancer cells by releasing growth factors and cytokines (50). This has been confirmed in “in vivo” studies in which simultaneous injection of PaSCs with tumor cells in orthotopic models of PDAC results in increased tumor size and increased metastasis (51). PaSCs are also detected in metastatic foci in the liver of nude mice, showing that PaSCs may co-migrate with cancer cells to create a favorable microenvironment for distant tumors (51). Recent studies have also shown that PaSCs in vitro increase the stem cell phenotype of pancreatic cancer cells, offering a therapeutic target that may reduce the metastatic potential of primary PDAC (52). Some studies show that more than 50% of tumor mass is made up of inflammatory cells, and many studies indicate that long-term pancreatitis is a driving force in the development of PDAC (53, 54). The PDAC tumor microenvironment is significantly pro-tumorigenic, with the majority of cells consisting of immunosuppressive cells such as regulatory T cells and myeloid-derived suppressor cells (53). For successful immunotherapy, cytotoxic T cells must have a high affinity for cancer cell antigens without causing autoimmunity. Cytotoxic T cells constitute a significant minority of tumor-infiltrating immune cells, which significantly limits immunogenicity in response to oncogenic virus infections. This suggests that enhancing the virus with systemic or local cytokines to boost the immune response could be beneficial. Another important factor in the development of chemoresistance and the propensity to metastasis in pancreatic cancer is that the cancer can undergo an epithelial-to-mesenchymal transition (EMT) (55, 56). EMT is characterized by reduced expression of E-cadherin and increased expression of mesenchymal markers such as vimentin, N-cadherin, and zinc finger transcription factors (Snail, Slug, Zeb1, and Twist) (57, 58). Gene expression profiling has shown that EMT cells are important contributors to chemoresistance in PDAC (56, 59). Cells with mutated E-cadherin or expressing mesenchymal markers displayed significantly reduced growth inhibition with the epidermal growth factor receptor (EGFR) inhibitor erlotinib compared with cells with the epithelial phenotype (60). The microenvironment of pancreatic cancer makes it difficult for systemic therapies to reach cancer cells. Significant

therapeutic effects can be achieved using strategies that deplete desmoplastic stroma and activate the immune system to target tumor cells and target cells that enhance the metastatic potential of PDACs. For patients with metastatic PDAC, FOLFIRINOX—a chemotherapy regimen consisting of folinic acid, 5-fluorouracil, irinotecan, and oxaliplatin—has shown the most efficacy, extending life expectancy to approximately 11.1 monthsMost patients undergoing surgery have only a slight improvement in survival, and virtually all patients have a recurrence of the tumor (61-63). Because of the high recurrence rate, adjuvant therapy after surgery is recommended, although the long-term effects of adjuvant radiation therapy are inconclusive (64-66). The high rate of systemic recurrence following surgery and radiation therapy has led to the idea that metastases may be present early in pancreatic tumor genesis (67). Gemcitabine and erlotinib are the only two agents authorized for use in advanced disease; nevertheless, both have only modest benefits. Gemcitabine was shown to prolong patient survival by 5.6 months compared with 4.4 months with 5-fluorouracil (68, 69). Adding erlotinib to gemcitabine resulted in a minimal increase in median survival from 5.9 to 6.2 months (70). A summary of the previously developed treatments and their weaknesses are provided in Table 3.

Oncolytic viruses

Oncolytic viruses selectively target tumor cells through engineered mutations that prevent the virus from attaching and replicating in normal cells and by expressing foreign genes that directly or indirectly cause cell death (77). Oncolytic virus replication in tumor cells produces infectious progeny that can further spread and destroy tumor masses (78). The effectiveness of the oncogenic virus depends on the direct lysis of the tumor, the ability of the virus to spread to surrounding cancer cells, and the ability of the virus to direct an immune response against the tumor. The optimal oncolytic virus must be highly selective in the cells it infects and replicates. There are several methods to improve their selectivity: 1) by blocking viral genes necessary for replication in normal cells but not needed in cancer cells; 2) by transcriptional targeting, where viral replication is controlled by specific tissue promoters; and/or 3) transfection targeting, where the virus is specifically retargeted to tumor cells. One of the important advantages of oncogenic viruses over other cancer treatments is the great potential for genetic

Table 3. An overview of the current treatments applied as a cure for PC.

Treatments	Information	References
Surgery	• Surgical resection is the only curative approach for PC, significantly extending survival • PC is categorized into resectable, borderline, unresectable (local progression), or with distant metastasis	(13, 71)
Chemotherapy	• Chemotherapy, particularly post-surgical adjuvant chemotherapy, enhances disease-free and overall survival • Regimens include MFOLFIRINOX (leucovorin, 5-FU, irinotecan, oxaliplatin) and gemcitabine plus capecitabine for resectable cases, with FOLFIRINOX and gemcitabine/NAB-paclitaxel for metastatic cases	(13, 72, 73)
Cooperation with Neoadjuvant Therapy	• Full-dose chemotherapy before surgery improves tumor control, survival, and quality of life • Preoperative chemotherapy/radiation can eliminate potential metastatic lesions and may be more effective than postoperative treatment	(13, 72)
Radiotherapy	• Radiation therapy uses X-rays to destroy cancer cells, primarily for locally advanced PC 1. External Beam Radiation Therapy: Uses external sources like X-rays, gamma rays, electrons, or heavy particles 2. Brachytherapy: Internal radiotherapy applied directly to or near the pancreas	(13, 74)
Targeted Therapy	• Targeted therapies like EGFR/VEGF-directed antibodies are effective in other cancers, but have had limited success in PC • The food and drug administration (FDA) recently approved pembrolizumab for PC, but other drugs like aflibercept, cetuximab, sorafenib, and axitinib have been ineffective	(13, 75)
Immunotherapy	• Immune checkpoint blockade (ICB) therapy is approved for various cancers but not yet for PC due to PC's immunosuppressive tumor microenvironment	(13, 76)

manipulation of candidate viruses to induce greater potency against a specific cancer.

1. Adenovirus

One of the most commonly used viral vectors to treat cancer is adenovirus. Adenovirus is a double-stranded DNA virus surrounded by an icosahedral protein capsid. Upon contact with a cell, the virus attaches to the cell's receptor with high affinity (79). Once inside the cell, the virion migrates to the nuclear pore complex, where its genome is released into the nucleoplasm, and gene expression and genome replication happens. In the final step, progeny viruses are released due to membrane disintegration and infection of neighboring tumor cells happens. To obtain cancer cell selectivity in adenovirus, two main strategies have been used. The first involves the deletion of essential viral genes required for replication in normal cells. These deletion mutations take advantage of the dysregulation of specific cell signaling pathways in cancer cells, allowing the virus to replicate in cancer cells even after the deletion of important genes. One such virus is ONYX-015, the first replication-competent oncolytic virus employed in clinical trials for pancreatic cancer (80). It is engineered to lack the E1B gene, which encodes a 55-kDa protein that binds to the tumor suppressor p53 in normal cells and causes cell cycle progression and viral replication (81). It is known that 50 to 75% of pancreatic tumors lack p53, thereby allowing replication of E1B-deleted viruses. ONXY15 has been seen to be effective in a mouse model of human xenografts with anti-tumor efficacy and increased survival (82). In phase I/II clinical trials, ONYX15, when combined with gemcitabine, was found to be a viable and well-tolerated treatment option for patients with pancreatic cancer (80). Oncorine is another clinically used virus that has the ability to delete the E1B gene and partially delete the E3 gene. Another deletion mutation in pancreatic cancer adenovirus is the E1A gene. The E1A protein binds to the retinoblastoma protein (pRb), a regulatory protein in normal cells that promotes cell cycle progression from the G1 to the S phase. This interaction allows E2F1, a critical transcription factor in viral replication, to function effectively. Many commonly mutated pancreatic cancer genes, such as CDKN2A, are involved in the regulation of the G1-S cell cycle. These mutations result in dysregulation of the cell cycle, rendering E1A binding to pRb unnecessary for E2F1 to support viral replication in pancreatic cancer. Therefore, cancer adenoviruses that use this gene deletion for tumor selection are excellent candidates for use in pancreatic cancer (83). E1B 19 kDa is another good candidate to induce tumor selectivity in adenovirus. The E1B 19 kDa gene encodes an antiapoptotic B-cell lymphoma 2 homologs, which is not required in the abnormal apoptotic environment of pancreatic cancer (30). Deletions or mutations in some genes further improve the virus's selectivity for cancer and prevent it from returning to wild type. Deletion of both the E1A and E1B19kDa genes greatly improves tumor selectivity. Importantly, viral potency was retained compared with wild-type adenovirus without gene deletion. Viral efficacy has been enhanced when combined with gemcitabine and other chemotherapeutic agents (84, 85). A second way that adenoviruses have been engineered to improve selectivity in pancreatic cancer is by placing key proteins behind tumor-specific promoters. At least five tumor-specific promoters have been used to create targeted adenoviruses for the treatment of pancreatic cancer. The promoters used were the cyclooxygenase 2 promoter, the urokinase-type plasminogen activator receptor promoter, the telomerase reverse transcriptase promoter, the hypoxic responsive promoter, the KRAS promoter, and the human carcinoembryonic antigen promoter (86-94). One of the main drawbacks of adenovirus as an oncolytic vector is its low intratumoral spread and infectivity inherent in the virus. Indeed, most oncolytic adenoviruses are constructed from wild-type adenovirus serotype 5. The adenovirus serotype 5 binds to the coxsackie adenovirus receptor, with little or no expression in pancreatic cancer, requiring high viral doses to overcome the low infectivity. Oncolytic adenoviruses with this novel binding motif have been shown to infect tumor tissue independently of coxsackievirus receptor levels. This new mutant adenovirus uses the desmoglein2 protein as a binding site and thus has increased viral infectivity in pancreatic cancer (90, 95, 96). As improved infectivity and selectivity improve, oncolytic adenoviruses are now empowered by arming them with therapeutic genes that boost the immune system against pancreatic cancer and improve oncolysis. Interleukin 24 (IL24) is

a prime example of a potential therapeutic protein that could enhance the immune response against tumor antigens and reduce potential side effects. Unfortunately, there can be serious side effects with systemic use, which limits its usefulness as a cancer treatment. An adenovirus, ZD55-IL24, has been engineered to locally produce IL24 in tumor cells, thereby avoiding systemic effects. In addition to cytokine delivery, therapeutic genes can be used to inhibit tumor differentiation, silence oncogenes, activate prodrugs, and image the existence of viruses. Using an adenovirus with an inserted thymidine kinase gene, the researchers imaged microscopic images of tumors in the pancreatic flank using an 18FFEAU radiometer (2′-[18F]-fluoro-5-ethyl-1-beta-D-arabinofuranosyluracil), show the existence of the virus (97). Quantification of tumor thymidine kinase activity correlates with virus anti-tumor effects (97).

2. Herpes Simplex Virus

The herpes simplex viruses (HSVs) HSV1 and HSV2 are large, enveloped, double-stranded DNA viruses. As an oncolytic virus, HSV has been genetically modified to replicate exclusively in cancer cells and has been shown to be promising as a virotherapy agent for the treatment of pancreatic cancer. The genome of HSV is large compared to other viruses, with many non-essential genes that can be mutated and replaced with therapeutic genes. Several anti-HSV drugs are available (acyclovir, ganciclovir) to treat patients who experience adverse reactions to virotherapy. HSV does not integrate into the cellular DNA, reducing the risk of inserting foreign genes into the patient's DNA. Compared to other virus families, HSV is known to infect and kill tumor cells faster. HSV has the ability to infect many different types of cancer cells, making it a possible candidate for many different forms of cancer (98). Finally, HSV exhibits strong T cell-mediated tumor reactivity and indirectly stimulate an immune response against cancer, leading to tumor regression through cytotoxic T cell and natural killer (NK) cell-mediated mechanisms. One potential drawback is that exposure to HSV is common in the general population. There is, therefore, a risk that carriers with pre-existing immunity to HSV may clear the virus before it can exert its oncolytic effect. Although, marine animal studies have not shown that this anti-HSV immunity has a significant deleterious effect on oncolysis (99, 100). The HSV oncolytic viruses use two main strategies to engineer selectivity for cancer cells: 1) deletion of viral genes required for replication (i.e., gene γ34,5); and 2) deletion of genes that regulate protein kinase response (PKR) pathways (i.e., ICP6 genes). Oncolytic viruses R3616 and HSV1716 have a deletion of the γ34.5 gene, which renders virus-infected cells unable to inhibit PKR phosphorylation. This consequence is the inactivation of eukaryotic translation initiation factor 2 alpha (eIF2α), stopping translation within normal cells. In pancreatic cancer cells, eIF2α is consistently activated because of mutations in glycogen synthase kinase-3, an alternative pathway that is independent of the PKR pathway and allows viral replication. Interestingly, R3616 replication and oncolysis in pancreatic cancer cells are because of dysregulation and activation of the phosphatidylinositide 3-kinase pathway and not to dysregulation of PKR or enhancement of Ras signaling (101). The hrR3 virus lacks the UL39 gene, which encodes for the ICP6 protein, the viral homolog of the cellular ribonucleotide reductase; in pancreatic cells, this protein is upregulated, allowing viral replication. The virus also has active HSV thymidine kinase, which allows the simultaneous treatment of cancer cells with ganciclovir. Ganciclovir disrupts cellular and viral DNA replication when activated by viral thymidine kinase and has been demonstrated to increase tumor lysis. In a xenograft model of pancreatic carcinomatosis, in which mice received hrR3 virus and ganciclovir together, there was a 30% increase in survival at 150 days compared to treatment with the virus alone (102). One study compared R3616 (γ34.5 deletion) with hrR3 (UL39 deletion) with or without gemcitabine. Treatment with R3616 was found to have better long-term survival than hrR3 with or without gemcitabine. Remarkably, when combined with gemcitabine, R3616 demonstrated improved long-term survival, while hrR3 combined with gemcitabine demonstrated decreased long-term survival. These data show that chemotherapy may affect viral replication and kill cancer cells differently depending on the viral targeting mechanism (103). Another study investigating L1BR1 (US3 locus-deficient HSV2) showed synergistic effects with the anticancer drugs fluorouracil and cisplatin (104). G207 virus is a mutated HSV1 virus with deletions at both loci γ34.5 loci and a lacZ insertion that disrupts the ICP6 gene encoding for

HSV ribonucleotide reductase. NV1020, a virus closely related to a single copy of the deleted γ34.5 loci, had a higher proliferation rate than G207 in pancreatic cancer. NV1023 and NV1066, which are derivatives of NV1020, are being actively studied for pancreatic cancer. NV1066 and hyperthermia significantly increased the death of pancreatic cancer cells as a result of enhanced replication by heat shock proteins (98). NV1020 in combination with radiation showed a synergistic effect on tumor oncolysis (105). One particularly promising HSV oncolytic is FusON-H2, another HSV-2 virus. This virus deleted the ICP10 gene encoding the serine/threonine protein kinase activity involved in the activation of the Ras/protein kinase pathway activated by a mitogen. When used to treat pancreatic cancer, intratumoral injection of FusON-H2 has been demonstrated to completely remove subcutaneous pancreatic xenografts. When the virus was administered intraperitoneally, 75% of tumors were eliminated and metastasis was prevented in the animals (106). Two oncolytic viruses HSV1, HF10, and OncoVex granulocyte-macrophage colony stimulating factor (GM-CSF) are in clinical trials for pancreatic cancer. HF10 is a naturally happening replication mutant of HSV1 that has been shown to replicate and spread efficiently in cancer cells (107). A phase I clinical trial was completed in which patients were treated with three doses of HF10 (108). No side effects were noted in the trial. Of the six treated patients, three were stable, one regressed, and two progressed. The second HSV oncolytic in the clinical trial was OncoVex GM-CSF, a γ34.5 and ICP47 deletion mutant expressing human GM-CSF. ICP47 encodes a protein, US11, that inhibits PKR activation, further enhancing transcriptional selectivity. Importantly, this virus was also engineered to express human GM-CSF. Human GM-CSF is involved in the recruitment and differentiation of activated dendritic cells in the tumor microenvironment (109, 110). In phase I/II clinical trials in solid tumors (head and neck cancers, squamous cell carcinoma, breast cancer, gastrointestinal cancers, and melanoma), OncoVex GM – CSF was well tolerated at high and repeated doses (111, 112).

3. Poxviruses

The family Poxviridae includes enveloped double-stranded DNA viruses, of which many members consist of vaccine virus, myxoma virus (MYXV), and raccoon pox. The most widely studied is the vaccinia virus, which played a key role in one of medicine's greatest achievements: the eradication of smallpox. The ability of poxviruses to express foreign antigens and stimulate an immune response against the tumor is one of the reasons it is currently being studied as an oncolytic virus for cancer treatment (113). In pancreatic cancer, vaccinia has been genetically modified to increase the cytotoxicity of the tumor against pancreatic cancer. One such modified vaccinia expresses the endostatin-angiostatin fusion protein, which has been reported in pancreatic cancer to inhibit angiogenesis. Current studies with this endostatin-angiostatin fusion protein expressing vaccinia virus have demonstrated remarkable antitumor efficacy in vivo against pancreatic cancer (114). GLV1h68 is a replication virus with attenuation mutations in F14.5L, J2R (codes for thymidine kinase), and A56R (codes for hemagglutinin). It has been reported to infect, replicate, and suppress pancreatic cancer cells both in vivo and in vitro. Importantly, when combined with gemcitabine or cisplatin, tumors demonstrated an enhanced and accelerated therapeutic effect. The protein survivin is related to chemotherapy resistance in pancreatic cancer and has been shown to be overexpressed in 70-80% of tumors. A modified vaccinia Ankara virus expressing murine survivin induces significant tumor regression and increased survival in mice when combined with gemcitabine (115). One of the factors of pancreatic cancer resistance to systemic therapies is the hypoxic nature of the tumor. The Lister strain of vaccinia, when allowed to infect pancreatic cells under hypoxic conditions, was found to show no signs of impaired viral replication, showing that vaccination may be possible. Particularly suitable as a viral vector to the hypoxic conditions of pancreatic cancer (116).

MYXV is a rabbit-specific poxvirus that has been demonstrated to be able to replicate in human cancer cells. In pancreatic cancer, when combined with gemcitabine, MYXV can demonstrate excellent replication and significant oncolysis. In an immunocompromised mouse model of diffuse pancreatic cancer, MYXV in combination with gemcitabine resulted in 100% long-term survival. These data show that MYXV is an effective oncolytic virus for pancreatic cancer and can be combined with gemcitabine to improve survival, especially in the

presence of an intact host immune system (117). The newly engineered, replicative MYXV, vMyxgfp, contains a green fluorescent protein construct. The researchers were able to demonstrate the replication and oncolysis of chemotherapy-resistant cell lines, making it a promising new virus for the treatment of pancreatic cancer (118). There is a poxvirus currently in clinical trials as a vaccine treatment. It is the vaccinia virus that expresses carcinoembryonic tumor antigen and mucin2, two molecules known to be expressed by pancreatic cancer cells. It is packaged with three co-stimulating molecules, B7.1 (differentiation group 80),

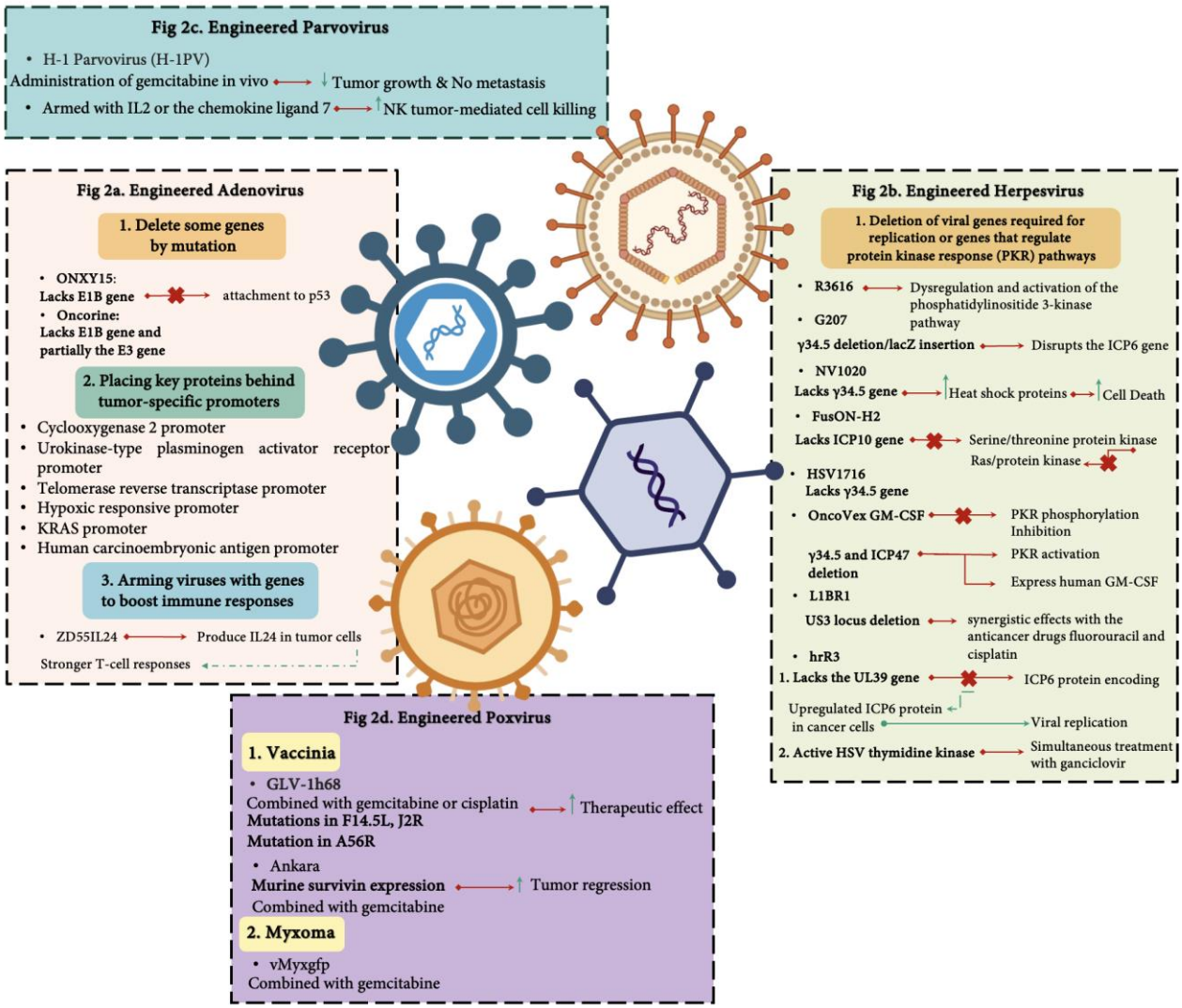

Figure 2. Oncolytic DNA Viruses Involved in the Treatment of Pancreatic Cancer. Fig.2a) Adenovirus: A double-stranded DNA virus used in cancer treatment. Modified versions like ONYX-015 (E1B-deleted) and Oncorine selectively target cancer cells with p53 mutations. Engineered adenoviruses with tumor-specific promoters and enhanced infectivity (e.g., desmoglein2 targeting) show improved efficacy. Therapeutic genes like IL24 are being incorporated for immune modulation. Fig.2b) Oncolytic Herpes Simplex Virus (HSV): HSV-1 and HSV-2 are modified to replicate in cancer cells while sparing normal ones. Deletions in genes such as γ34.5 (e.g., R3616) and ICP6 (e.g., hrR3) enhance selectivity and efficacy. Some HSV strains (e.g., OncoVex GM-CSF) express immune-stimulating genes like GM-CSF. Studies indicate synergistic effects with chemotherapy and radiation. Fig.2c) Poxviruses: Enveloped double-stranded DNA viruses like vaccinia and myxoma virus (MYXV) show promise. Vaccinia expressing anti-angiogenic proteins or survivin inhibitors improves outcomes in combination with chemotherapy. MYXV enhances survival, especially with gemcitabine. Engineered poxviruses (e.g., PANVAC-V) also induce strong immune responses. Fig.2d) Parvovirus: Small, non-enveloped DNA viruses (e.g., H-1PV) are inherently oncolytic, replicating in dividing cancer cells. In pancreatic cancer, they exhibit direct oncolysis, immune activation, and synergy with gemcitabine, enhancing natural killer (NK) cell activity.

ICAM1 (intracellular adhesion molecule1), and LFA3 (leukocyte functional binding antigen) (TRICOM) (PANVACV), to help increase the immune response to the vaccine. To further improve vaccine treatment, interferon alpha is also used. Results are promising with a significant increase in patient survival with mild injection site reactions being the most common side effect. This shows that in pancreatic cancer, the vaccinia virus is able to induce antigen-specific immune responses (119, 120).

4. Parvovirus

Parvoviruses, such as H-1 (H-1PV), are small, non-enveloped, icosahedral particles with a single-stranded DNA genome that require actively dividing cells for replication, making them innately oncolytic. Although they are not pathogenic to animals or humans, animal models have demonstrated that they possess a tumor-suppressing effect (122). Parvoviruses cause both direct oncolysis and immunomodulatory effects and have been shown to support the immune system of animals against infected tumors (123). When given after the administration of gemcitabine, H-1PV in vivo demonstrated decreased tumor growth, prolonged animal survival, and no metastasis on computed tomography scans. This shows that parvovirus could be a useful adjuvant in the multimodality treatment of pancreatic cancer (48). Studies with parvovirus show that infection can increase NK tumor-mediated cell killing in pancreatic ductal carcinoma (124). When the virus is armed with IL2 or the chemokine ligand 7, the anti-tumor response of NK cells and monocytes to pancreatic ductal carcinoma is enhanced (125).

5. Measles Virus

As an oncolytic virus, measles virus has demonstrated promising results in a variety of tumor models. They depend on the overexpression of CD46, a viral entry receptor present in many cancer cells (126). An engineered measles virus expressing the sodium iodine symporter gene (MV-NIS) has demonstrated oncolytic activity in pancreatic tumor xenografts in mice with tumor regression and increased survival. Unfortunately, when this was combined with radiotherapy, no synergy was observed (127). In several studies, the authors were able to use iodinated contrast and micro-single-photon emission computed tomography to accurately image initial viral distribution in a solid tumor (128, 129). In addition, another modified measles virus was developed to target prostate stem cell antigen (PSCA), which is a protein expressed in pancreatic cancer. To improve the effectiveness of the virus, it is armed with a suicide gene, purine nucleoside phosphorylase (PNP) convertase, which activates the prodrug fludarabine. The authors succeeded in showing that MVPNPantiPSCA has beneficial therapeutic effects in pancreatic cancer. Importantly, they were able to demonstrate that gemcitabine-resistant pancreatic carcinoma was sensitive to both the virus and the activated prodrug (130).

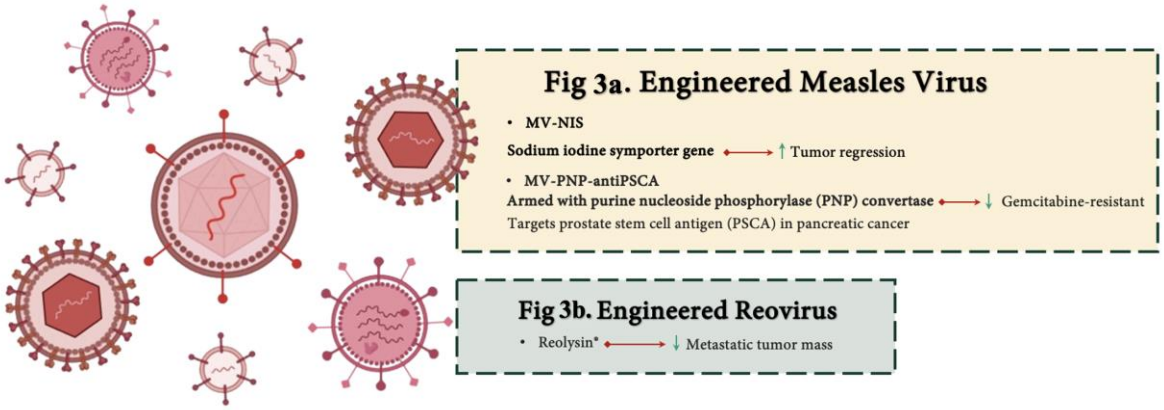

Figure 3. Oncolytic RNA Viruses Involved in the Treatment of Pancreatic Cancer. Fig.3a) Measles Virus: The measles virus targets cancer cells through overexpressed CD46. Modified strains, like MV-NIS, showed tumor regression in pancreatic cancer models but lacked synergy with radiotherapy. Another variant, MVPNPantiPSCA, targets PSCA in pancreatic cancer and uses a suicide gene to activate fludarabine, proving effective even against gemcitabine-resistant tumors. Fig.3b) Reovirus: Reovirus exploits KRAS mutations (common in pancreatic cancer) to replicate in cancer cells. In animal models, it reduced tumor size and ascites. Reolysin®, a reovirus-based therapy, is undergoing phase II trials for pancreatic cancer.

6. Reovirus

Reovirus is a double-stranded RNA virus. The replication of this virus depends on the cellular activity of Ras. KRAS mutations in pancreatic cancer are prevalent (85% to 90%), making reovirus a promising candidate as an oncolytic virus in pancreatic carcinoma (87). In immunocompetent animal models, in vivo, administration of reovirus targets cells with Ras mutations and reduces the mass of metastatic tumors in the liver (131, 132). It also resulted in reduced tumor mass and decreased ascites when administered intraperitoneally (131). Reolysin® (Oncolytics Biotech Inc., Calgary, AB, Canada) is the name of the reovirus that is currently undergoing phase II clinical trials for pancreatic cancer (133-135).

Conclusion

Although the current methods of surgery treatment, chemotherapy, and radiotherapy, the prognosis of pancreatic cancer remains poor. The microenvironment of pancreatic cancer presents many unique challenges that will require to be overcome if we are going to successfully treat pancreatic cancer. Randomly, several distinctive oncolytic viruses have appeared to be valuable in treating pancreatic cancer in preclinical animal models. Clinical trials of oncolytic infections have shown that oncolytic virotherapy is both a safe and feasible method for pancreatic cancer therapy in humans. However, there is still much opportunity to optimize oncolytic viruses in the treatment of pancreatic cancer. Future research should focus on several key strategies to enhance outcomes. First, improving virus selectivity and oncolytic potency by targeting pathways such as epithelial-to-mesenchymal transition (EMT) could help reduce the population of cancer stem cells involved in therapy resistance and metastasis. Second, the combination of oncolytic viruses with systemic treatments designed to eliminate the desmoplastic matrix could improve viral penetration into tumors, enabling more effective treatment. Additionally, engineering viruses to locally deliver immune-stimulating cytokines could overcome systemic immune suppression while minimizing side effects. Finally, expanding clinical trials to validate the efficacy of oncolytic virotherapy across diverse patient populations and in combination with standard-of-care treatments will be essential.

Conflict of interests

There is no conflict of interest for authors of this article.

References

1. Kolodecik T, Shugrue C, Ashat M, Thrower EC. Risk factors for pancreatic cancer: underlying mechanisms and potential targets. Front Physiol 2014;4:415.

2. Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. CA: CA Cancer J Clin. 2014;64:9-29.

3. Stathis A, Moore MJ. Advanced pancreatic carcinoma: current treatment and future challenges. Nat Rev Clin Oncol 2010;7:163-72.

4. Gopal S, Sharpless NE. Cancer as a global health priority. JAMA 2021;326:809-10.

5. Nisar M, Paracha RZ, Adil S, Qureshi SN, Janjua HA. An extensive review on preclinical and clinical trials of oncolytic viruses therapy for pancreatic cancer. Front Oncol 2022;12:875188.

6. Whitcomb DC. Genetics of alcoholic and nonalcoholic pancreatitis. Curr opin Gastroenterol. 2012;28:501-6.

7. Luu T. Epithelial-mesenchymal transition and its regulation mechanisms in pancreatic cancer. Front Oncol 2021;11:646399.

8. Walcher L, Kistenmacher A-K, Suo H, Kitte R, Dluczek S, Strauß A, et al. Cancer stem cells—origins and biomarkers: perspectives for targeted personalized therapies. Front Immunol 2020;11:1280.

9. McMenamin ÚC, McCain S, Kunzmann AT. Do smoking and alcohol behaviours influence GI cancer survival? Best Pract Res Clin Gastroenterol 2017;31:569-77.

10. McGuigan A, Kelly P, Turkington RC, Jones C, Coleman HG, McCain RS. Pancreatic cancer: A review of clinical diagnosis, epidemiology, treatment and outcomes. World J Gastroenterol 2018;24:4846.

11. Masoudi S, Sanat ZM, Saleh AM, Nozari N, Ghamarzad N, Pourshams A. Menstrual and reproductive factors and risk of pancreatic cancer in women. Middle East J Dig Dis 2017;9:146.

12. Yeo TP, editor Demographics, epidemiology, and inheritance of pancreatic ductal adenocarcinoma. Semin Oncol; 2015: Elsevier.

13. Zhao Z, Liu W. Pancreatic cancer: a review of risk factors, diagnosis, and treatment. Technol Cancer Res Treat 2020;19:1533033820962117.

14. Bosetti C, Rosato V, Li D, Silverman D, Petersen G, Bracci P, et al. Diabetes, antidiabetic medications, and pancreatic cancer risk: an analysis from the International Pancreatic Cancer Case-Control Consortium. Ann Oncol 2014;25:2065-72.

15. Gardner TB, Hessami N, Smith KD, Ripple GH, Barth RJ, Klibansky DA, et al. The effect of neoadjuvant chemoradiation on pancreatic cancer-associated diabetes mellitus. Pancreas 2014;43:1018-21.

16. Raza MH, Gul K, Arshad A, Riaz N, Waheed U, Rauf A, et al. Microbiota in cancer development and treatment. J Cancer Res Clin Oncol 2019;145:49-63.

17. Staley C, Weingarden AR, Khoruts A, Sadowsky MJ. Interaction of gut microbiota with bile acid metabolism and its influence on disease states. Appl Microbiol Biotechnol 2017;101:47-64.

18. Molina-Montes E, Van Hoogstraten L, Gomez-Rubio P, Löhr M, Sharp L, Molero X, et al. Pancreatic cancer risk in relation to lifetime smoking patterns, tobacco type, and dose–response relationships. Cancer Epidemol Biomarkers Prev 2020;29:1009-18.

19. Wang Y-T, Gou Y-W, Jin W-W, Xiao M, Fang H-Y. Association between alcohol intake and the risk of pancreatic cancer: a dose–response meta-analysis of cohort studies. BMC cancer 2016;16:1-11.

20. Samokhvalov AV, Rehm J, Roerecke M. Alcohol consumption as a risk factor for acute and chronic pancreatitis: a systematic review and a series of meta-analyses. EBioMedicine 2015;2:1996-2002.

21. Downes DP, Daurio NA, McLaren DG, Carrington P, Previs SF, Williams KB. Impact of extracellular fatty acids and oxygen tension on lipid synthesis and assembly in pancreatic cancer cells. ACS Chem Biol 2020;15:1892-900.

22. Eibl G, Rozengurt E, editors. KRAS, YAP, and obesity in pancreatic cancer: A signaling network with multiple loops. Semin Cancer Biol; 2019: Elsevier.

23. Koorstra J-BM, Hustinx SR, Offerhaus GJA, Maitra A. Pancreatic carcinogenesis. Pancreatology 2008;8:110-25.

24. Gnoni A, Licchetta A, Scarpa A, Azzariti A, Brunetti AE, Simone G, et al. Carcinogenesis of pancreatic adenocarcinoma: precursor lesions. Int J Mol Sci 2013;14:19731-62.

25. Iacobuzio-Donahue CA, Velculescu VE, Wolfgang CL, Hruban RH. Genetic basis of pancreas cancer development and progression: insights from whole-exome and whole-genome sequencing. Clin Cancer Res 2012;18:4257-65.

26. Lowenfels AB, Maisonneuve P. Epidemiology and risk factors for pancreatic cancer. Best Prac Res Clin Gastroenterol 2006;20:197-209.

27. Grippo PJ, Nowlin PS, Demeure MJ, Longnecker DS, Sandgren EP. Preinvasive pancreatic neoplasia of ductal phenotype induced by acinar cell targeting of mutant Kras in transgenic mice. Cancer Res 2003;63:2016-9.

28. Aguirre AJ, Bardeesy N, Sinha M, Lopez L, Tuveson DA, Horner J, et al. Activated Kras and Ink4a/Arf deficiency cooperate to produce metastatic pancreatic ductal adenocarcinoma. Genes Deve 2003;17:3112-26.

29. Bardeesy N, Aguirre AJ, Chu GC, Cheng K-h, Lopez LV, Hezel AF, et al. Both p16Ink4a and the p19Arf-p53 pathway constrain progression of pancreatic adenocarcinoma in the mouse. Proc Natl Acad Sci U S A 2006;103:5947-52.

30. Jones S, Zhang X, Parsons DW, Lin JC-H, Leary RJ, Angenendt P, et al. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. Science 2008;321:1801-6.

31. Feig C, Gopinathan A, Neesse A, Chan DS, Cook N, Tuveson DA. The pancreas cancer microenvironment. Clin Cancer Res 2012;18:4266-76.

32. Kulkarni AM, Kumar V, Parate S, Lee G, Yoon S, Lee KW. Identification of new KRAS G12D inhibitors through computer-aided drug discovery methods. Int J Mol Sci 2022;23:1309.

33. Bannoura SF, Uddin MH, Nagasaka M, Fazili F, Al-Hallak MN, Philip PA, et al. Targeting KRAS in pancreatic cancer: new drugs on the horizon. Cancer Metastasis Rev 2021:1-17.

34. Mehra S, Deshpande N, Nagathihalli N. Targeting PI3K pathway in pancreatic ductal adenocarcinoma: rationale and progress. Cancers 2021;13:4434.

35. Teixeira AF, Ten Dijke P, Zhu H-J. On-target anti-TGF-β therapies are not succeeding in clinical cancer treatments: what are remaining challenges? Front Cell Dev Biol 2020;8:605.

36. Furukawa T. Impacts of activation of the mitogen-activated protein kinase pathway in pancreatic cancer. Front Oncol 2015;5:23.

37. Neiheisel A, Kaur M, Ma N, Havard P, Shenoy AK. Wnt pathway modulators in cancer therapeutics: An update on completed and ongoing clinical trials. Intl J Cancer 2022;150:727-40.

38. Ram Makena M, Gatla H, Verlekar D, Sukhavasi S, K. Pandey M, C. Pramanik K. Wnt/β-catenin signaling: the culprit in pancreatic carcinogenesis and therapeutic resistance. Intl J Mol Sci 2019;20:4242.

39. Gao J, Long B, Wang Z. Role of Notch signaling pathway in pancreatic cancer. Am J Cancer Res. 2017;7:173.

40. Disis ML, editor Mechanism of action of immunotherapy. Semin Oncol; 2014: Elsevier.

41. Funamizu N, Lacy CR, Kamada M, Yanaga K, Manome Y. MicroRNA-200b and-301 are associated with gemcitabine response as biomarkers in pancreatic carcinoma cells. Intl J Oncol 2019;54:991-1000.

42. Pan G, Liu Y, Shang L, Zhou F, Yang S. EMT-associated microRNAs and their roles in cancer stemness and drug resistance. Cancer Commun 2021;41:199-217.

43. Bao B, Wang Z, Ali S, Kong D, Li Y, Ahmad A, et al. RETRACTED: Notch-1 induces epithelial–mesenchymal transition consistent with cancer stem cell phenotype in pancreatic cancer cells. Cancer Lett; 2011.

44. Liu G, Ji L, Ke M, Ou Z, Tang N, Li Y. miR-125a-3p is responsible for chemosensitivity in PDAC by inhibiting epithelial-mesenchymal transition via Fyn. Biomed Pharmacother 2018;106:523-31.

45. Zhang R, Li M, Zang W, Chen X, Wang Y, Li P, et al. MiR-148a regulates the growth and apoptosis in pancreatic cancer by targeting CCKBR and Bcl-2. Tumor Biol 2014;35:837-44.

46. Thompson CB, Shepard HM, O'Connor PM, Kadhim S, Jiang P, Osgood RJ, et al. Enzymatic depletion of tumor hyaluronan induces antitumor responses in preclinical animal models. Mol Cancer Ther 2010;9:3052-64.

47. Provenzano PP, Cuevas C, Chang AE, Goel VK, Von Hoff DD, Hingorani SR. Enzymatic targeting of the stroma ablates physical barriers to treatment of pancreatic ductal adenocarcinoma. Cancer cell 2012;21:418-29.

48. Angelova AL, Aprahamian M, Grekova SP, Hajri A, Leuchs B, Giese NA, et al. Improvement of gemcitabine-based therapy of pancreatic carcinoma by means of oncolytic parvovirus H-1PV. Clin Cancer Res 2009;15:511-9.

49. Bailey JM, Swanson BJ, Hamada T, Eggers JP, Singh PK, Caffery T, et al. Sonic hedgehog promotes desmoplasia in pancreatic cancer. Clin Cancer Res 2008;14:5995-6004.

50. Erkan M, Adler G, Apte MV, Bachem MG, Buchholz M, Detlefsen S, et al. StellaTUM: current consensus and discussion on pancreatic stellate cell research. Gut 2012;61:172-8.

51. Vonlaufen A, Joshi S, Qu C, Phillips PA, Xu Z, Parker NR, et al. Pancreatic stellate cells: partners in crime with pancreatic cancer cells. Cancer Res 2008;68:2085-93.

52. Lonardo E, Frias-Aldeguer J, Hermann PC, Heeschen C. Pancreatic stellate cells form a niche for cancer stem cells and promote their self-renewal and invasiveness. Cell Cycle 2012;11:1282-90.

53. Clark CE, Hingorani SR, Mick R, Combs C, Tuveson DA, Vonderheide RH. Dynamics of the immune reaction to pancreatic cancer from inception to invasion. Cancer Res 2007;67:9518-27.

54. Momi N, Kaur S, Krishn S, Batra SK. Discovering the route from inflammation to pancreatic cancer. Minerva gastroenterol Dietol 2012;58:283.

55. Shah AN, Summy JM, Zhang J, Park SI, Parikh NU, Gallick GE. Development and characterization of gemcitabine-resistant pancreatic tumor cells. Ann Surg Oncol 2007;14:3629-37.

56. Wang Z, Li Y, Kong D, Banerjee S, Ahmad A, Azmi AS, et al. Acquisition of epithelial-mesenchymal transition phenotype of gemcitabine-resistant pancreatic cancer cells is linked with activation of the notch signaling pathway. Cancer Res 2009;69:2400-7.

57. Thiery JP, Acloque H, Huang RY, Nieto MA. Epithelial-mesenchymal transitions in development and disease. Cell 2009;139:871-90.

58. Yang AD, Camp ER, Fan F, Shen L, Gray MJ, Liu W, et al. Vascular endothelial growth factor receptor-1 activation mediates epithelial to mesenchymal transition in human pancreatic carcinoma cells. Cancer Res 2006;66:46-51.

59. Yang AD, Fan F, Camp ER, van Buren G, Liu W, Somcio R, et al. Chronic oxaliplatin resistance induces epithelial-to-mesenchymal transition in colorectal cancer cell lines. Clinical Cancer Res 2006;12:4147-53.

60. Buck E, Eyzaguirre A, Barr S, Thompson S, Sennello R, Young D, et al. Loss of homotypic cell adhesion by epithelial-mesenchymal transition or mutation limits sensitivity to epidermal growth factor receptor inhibition. Mol Cancer Ther 2007;6:532-41.

61. Bilimoria KY, Bentrem DJ, Ko CY, Ritchey J, Stewart AK, Winchester DP, et al. Validation of the 6th edition AJCC pancreatic cancer staging system: report from the national cancer database. Cancer 2007;110:738-44.

62. Allison DC, Piantadosi S, Hruban RH, Dooley WC, Fishman EK, Yeo CJ, et al. DNA content and other factors associated with ten-year survival after resection of pancreatic carcinoma. J Surg Oncol 1998;67:151-9.

63. Conroy T, Desseigne F, Ychou M, Bouché O, Guimbaud R, Bécouarn Y, et al. FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. N Eng J Med 2011;364:1817-25.

64. Neoptolemos JP, Stocken DD, Bassi C, Ghaneh P, Cunningham D, Goldstein D, et al. Adjuvant chemotherapy with fluorouracil plus folinic acid vs gemcitabine following pancreatic cancer resection: a randomized controlled trial. JAMA 2010;304:1073-81.

65. Zygogianni G, Kyrgias G, Kouvaris J, Antypas C, Skarlatos J, Armpilia C, et al. Intraoperative radiation therapy on pancreatic cancer patients: a review of the literature. Minerva Chir 2011;66:361-9.

66. Ruano-Ravina A, Ortega RA, Guedea F. Intraoperative radiotherapy in pancreatic cancer: a systematic review. Radiother Oncol 2008;87:318-25.

67. Rhim AD, Mirek ET, Aiello NM, Maitra A, Bailey JM, McAllister F, et al. EMT and dissemination precede pancreatic tumor formation. Cell 2012;148:349-61.

68. Burris Hr, Moore MJ, Andersen J, Green MR, Rothenberg ML, Modiano MR, et al. Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: a randomized trial. J Clin Oncol 1997;15:2403-13.

69. Zhu C-P, Shi J, Chen Y-X, Xie W-F, Lin Y. Gemcitabine in the chemoradiotherapy for locally advanced pancreatic cancer: a meta-analysis. Radiother Oncol 2011;99:108-13.

70. Moore MJ, Goldstein D, Hamm J, Figer A, Hecht JR, Gallinger S, et al. Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group. J Clin Oncol 2007;25:1960-6.

71. Mizrahi JD, Surana R, Valle JW, Shroff RT. Pancreatic cancer. Lancet 2020;395:2008-20.

72. Ferrone CR, Marchegiani G, Hong TS, Ryan DP, Deshpande V, McDonnell EI, et al. Radiological and surgical implications of neoadjuvant treatment with FOLFIRINOX for locally advanced and borderline resectable pancreatic cancer. Ann Surg 2015;261:12-7.

73. Seufferlein T, Hammel P, Delpero JR, Macarulla T, Pfeiffer P, Prager GW, et al. Optimizing the management of locally advanced pancreatic cancer with a focus on induction chemotherapy: expert opinion based on a review of current evidence. Cancer Treat Rev 2019;77:1-10.

74. Chin V, Nagrial A, Sjoquist K, O'Connor CA, Chantrill L, Biankin AV, et al. Chemotherapy and radiotherapy for advanced pancreatic cancer. Cochrane Database Syst Rev 2018;3:CD011044.

75. Zhen DB, Coveler A, Zanon S, Reni M, Chiorean EG, editors. Biomarker-driven and molecularly targeted therapies for pancreatic adenocarcinoma. Semin Oncol; 2018: Elsevier.

76. Haanen J, Carbonnel F, Robert C, Kerr K, Peters S, Larkin J, et al. Corrections to “Management of toxicities from immunotherapy: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2018;29:iv264-iv6.

77. Vacchelli E, Eggermont A, Sautes-Fridman C, Galon J, Zitvogel L, Kroemer G, et al. Trial watch: Oncolytic viruses for cancer therapy. Oncoimmunology 2013;2:24612.

78. Zeyaullah M, Patro M, Ahmad I, Ibraheem K, Sultan P, Nehal M, et al. Oncolytic viruses in the treatment of cancer: a review of current strategies. Pathol Oncol Res 2012;18:771-81.

79. Cerullo V, Koski A, Vähä-Koskela M, Hemminki A. Oncolytic adenoviruses for cancer immunotherapy: data from mice, hamsters, and humans. Adv Cancer Res 2012;115:265-318.

80. Hecht JR, Bedford R, Abbruzzese JL, Lahoti S, Reid TR, Soetikno RM, et al. A phase I/II trial of intratumoral endoscopic ultrasound injection of ONYX-015 with intravenous gemcitabine in unresectable pancreatic carcinoma. Clin Cancer Res 2003;9:555-61.

81. Bischoff JR, Kirn DH, Williams A, Heise C, Horn S, Muna M, et al. An adenovirus mutant that replicates selectively in p53-deficient human tumor cells. Science 1996;274:373-6.

82. Heise C, Sampson-Johannes A, Williams A, Mccormick F, Von Hoff DD, Kirn DH. ONYX-015, an E1B gene-attenuated adenovirus, causes tumor-specific cytolysis and antitumoral efficacy that can be augmented by standard chemotherapeutic agents. Nat Med 1997;3:639-45.

83. Heise C, Hermiston T, Johnson L, Brooks G, Sampson-Johannes A, Williams A, et al. An adenovirus E1A mutant that demonstrates potent and selective systemic anti-tumoral efficacy. Nat Med 2000;6:1134-9.

84. Oberg D, Yanover E, Adam V, Sweeney K, Costas C, Lemoine NR, et al. Improved potency and selectivity of an oncolytic E1ACR2 and E1B19K deleted adenoviral mutant in prostate and pancreatic cancers. Clin Cancer Res 2010;16:541-53.

85. Cherubini G, Kallin C, Mozetic A, Hammaren-Busch K, Müller H, Lemoine N, et al. The oncolytic adenovirus AdΔΔ enhances selective cancer cell killing in combination with DNA-damaging drugs in pancreatic cancer models. Gene Ther 2011;18:1157-65.

86. Xu C, Sun Y, Wang Y, Yan Y, Shi Z, Chen L, et al. CEA promoter-regulated oncolytic adenovirus-mediated Hsp70 expression in immune gene therapy for pancreatic cancer. Cancer Lett 2012;319:154-63.

87. Mihaljevic AL, Michalski CW, Friess H, Kleeff J. Molecular mechanism of pancreatic cancer—understanding proliferation, invasion, and metastasis. Langenbecks Arch Surg 2010;395:295-308.

88. Bortolanza S, Bunuales M, Otano I, Gonzalez-Aseguinolaza G, Ortiz-de-Solorzano C, Perez D, et al. Treatment of pancreatic cancer with an oncolytic adenovirus expressing interleukin-12 in Syrian hamsters. Mol Ther 2009;17:614-22.

89. Hu Y, Ou Y, Wu K, Chen Y, Sun W. miR-143 inhibits the metastasis of pancreatic cancer and an associated signaling pathway. Tumor Biol 2012;33:1863-70.

90. Ramírez PJ, Vickers SM, Ono HA, Davydova J, Takayama K, Thompson TC, et al. Optimization of conditionally replicative adenovirus for pancreatic cancer and its evaluation in an orthotopic murine xenograft model. Am J Surg 2008;195:481-90.

91. Nelson AR, Davydova J, Curiel DT, Yamamoto M. Combination of conditionally replicative adenovirus and standard chemotherapies shows synergistic antitumor effect in pancreatic cancer. Cancer Sci 2009;100:2181-7.

92. Huch M, Gros A, José A, González JR, Alemany R, Fillat C. Urokinase-type plasminogen activator receptor transcriptionally controlled adenoviruses eradicate pancreatic tumors and liver metastasis in mouse models. Neoplasia 2009;11:518-IN6.

93. Onimaru M, Ohuchida K, Nagai E, Mizumoto K, Egami T, Cui L, et al. Combination with low-dose gemcitabine and hTERT-promoter-dependent conditionally replicative adenovirus enhances cytotoxicity through their crosstalk mechanisms in pancreatic cancer. Cancer Lett 2010;294:178-86.

94. Onimaru M, Ohuchida K, Mizumoto K, Nagai E, Cui L, Toma H, et al. hTERT-promoter-dependent oncolytic adenovirus enhances the transduction and therapeutic efficacy of replication-defective adenovirus vectors in pancreatic cancer cells. Cancer Sci 2010;101:735-42.

95. Wang H, Li Z-Y, Liu Y, Persson J, Beyer I, Möller T, et al. Desmoglein 2 is a receptor for adenovirus serotypes 3, 7, 11 and 14. Nat Med 2011;17:96-104.

96. Chu QD, Sun G, Pope M, Luraguiz N, Curiel DT, Kim R, et al. Virotherapy using a novel chimeric oncolytic adenovirus prolongs survival in a human pancreatic cancer xenograft model. Surgery 2012;152:441-8.

97. Abate-Daga D, Andreu N, Camacho-Sanchez J, Alemany R, Herance R, Millan O, et al. Oncolytic adenoviruses armed with thymidine kinase can be traced by PET imaging and show potent antitumoural effects by ganciclovir dosing. PLoS One 2011;6:26142.

98. Eisenberg DP, Carpenter SG, Adusumilli PS, Chan M-K, Hendershott KJ, Yu Z, et al. Hyperthermia potentiates oncolytic herpes viral killing of pancreatic cancer through a heat shock protein pathway. Surgery 2010;148:325-34.

99. Liu B, Robinson M, Han Z, Branston R, English C, Reay P, et al. ICP34. 5 deleted herpes simplex virus with enhanced oncolytic, immune stimulating, and anti-tumour properties. Gene Ther 2003;10:292-303.

100. Varghese S, Rabkin SD, Nielsen GP, MacGarvey U, Liu R, Martuza RL. Systemic therapy of spontaneous prostate cancer in transgenic mice with oncolytic herpes simplex viruses. Cancer Res 2007;67:9371-9.

101. Sarinella F, Calistri A, Sette P, Palu G, Parolin C. Oncolysis of pancreatic tumour cells by a γ34. 5-deleted HSV-1 does not rely upon Ras-activation, but on the PI 3-kinase pathway. Gene Ther 2006;13:1080-7.

102. Kasuya H, Nishiyama Y, Nomoto S, Hosono J, Takeda S, Nakao A. Intraperitoneal delivery of hrR3 and ganciclovir prolongs survival in mice with disseminated pancreatic cancer. J Surg Oncol 1999;72:136-41.

103. Watanabe I, Kasuya H, Nomura N, Shikano T, Shirota T, Kanazumi N, et al. Effects of tumor selective replication-competent herpes viruses in combination with gemcitabine on

pancreatic cancer. Cancer chemother Pharmacol 2008;61:875-82.

104. Kasuya H, Nishiyama Y, Nomoto S, Goshima F, Takeda S, Watanabe I, et al. Suitability of a US3-inactivated HSV mutant (L1BR1) as an oncolytic virus for pancreatic cancer therapy. Cancer Gene Ther 2007;14:533-42.

105. Dai M, Zamarin D, Gao S, Chou T, Gonzalez L, Lin S, et al. Synergistic action of oncolytic herpes simplex virus and radiotherapy in pancreatic cancer cell lines. Br J Surg 2010;97:1385-94.

106. Fu X, Tao L, Li M, Fisher WE, Zhang X. Effective treatment of pancreatic cancer xenografts with a conditionally replicating virus derived from type 2 herpes simplex virus. Clin Cancer Res 2006;12:3152-7.

107. Nawa A, Luo C, Zhang L, Ushjima Y, Ishida D, Kamakura M, et al. Non-engineered, naturally oncolytic herpes simplex virus HSV1 HF-10: applications for cancer gene therapy. Curr Gene Ther 2008;8:208-21.

108. Nakao A, Kasuya H, Sahin T, Nomura N, Kanzaki A, Misawa M, et al. A phase I dose-escalation clinical trial of intraoperative direct intratumoral injection of HF10 oncolytic virus in non-resectable patients with advanced pancreatic cancer. Cancer Gene Ther 2011;18:167-75.

109. Melcher A, Parato K, Rooney CM, Bell JC. Thunder and lightning: immunotherapy and oncolytic viruses collide. Mol Ther 2011;19:1008-16.

110. Campadelli-Fiume G, De Giovanni C, Gatta V, Nanni P, Lollini PL, Menotti L. Rethinking herpes simplex virus: the way to oncolytic agents. Rev Med Virol 2011;21:213-26.

111. Hu JC, Coffin RS, Davis CJ, Graham NJ, Groves N, Guest PJ, et al. A phase I study of OncoVEXGM-CSF, a second-generation oncolytic herpes simplex virus expressing granulocyte macrophage colony-stimulating factor. Clin Cancer Res 2006;12:6737-47.

112. Harrington KJ, Hingorani M, Tanay MA, Hickey J, Bhide SA, Clarke PM, et al. Phase I/II study of oncolytic HSVGM-CSF in combination with radiotherapy and cisplatin in untreated stage III/IV squamous cell cancer of the head and neck. Clin Cancer Res 2010;16:4005-15.

113. Kirn DH, Thorne SH. Targeted and armed oncolytic poxviruses: a novel multi-mechanistic therapeutic class for cancer. Nat Rev Cancer. 2009;9:64-71.

114. Tysome JR, Briat A, Alusi G, Cao F, Gao D, Yu J, et al. Lister strain of vaccinia virus armed with endostatin–angiostatin fusion gene as a novel therapeutic agent for human pancreatic cancer. Gene Ther 2009;16:1223-33.

115. Ishizaki H, Manuel ER, Song G-Y, Srivastava T, Sun S, Diamond DJ, et al. Modified vaccinia Ankara expressing survivin combined with gemcitabine generates specific antitumor effects in a murine pancreatic carcinoma model. Cancer Immunol Immunother 2011;60:99-109.

116. Hiley CT, Yuan M, Lemoine NR, Wang Y. Lister strain vaccinia virus, a potential therapeutic vector targeting hypoxic tumours. Gene Ther 2010;17:281-7.

117. Wennier ST, Liu J, Li S, Rahman MM, Mona M, McFadden G. Myxoma virus sensitizes cancer cells to gemcitabine and is an effective oncolytic virotherapeutic in models of disseminated pancreatic cancer. Mol Ther 2012;20:759-68.

118. Woo Y, Kelly KJ, Stanford MM, Galanis C, Shin Chun Y, Fong Y, et al. Myxoma virus is oncolytic for human pancreatic adenocarcinoma cells. Ann Surg Oncol 2008;15:2329-35.

119. Petrulio CA, Kaufman HL. Development of the PANVAC™-VF vaccine for pancreatic cancer. Expert Rev Vaccines 2006;5:9-19.

120. Kaufman HL, Kim-Schulze S, Manson K, DeRaffele G, Mitcham J, Seo KS, et al. Poxvirus-based vaccine therapy for patients with advanced pancreatic cancer. J Transl Med 2007;5:1-10.

121. Rommelaere J, Geletneky K, Angelova AL, Daeffler L, Dinsart C, Kiprianova I, et al. Oncolytic parvoviruses as cancer therapeutics. Cytokine Growth Factor Rev 2010;21:185-95.

122. Grekova SP, Aprahamian M, Giese N, Schmitt S, Giese T, Falk CS, et al. Immune cells participate in the oncosuppressive activity of parvovirus H-1PV and are activated as a result of their abortive infection with this agent. Cancer Biol Ther 2010;10:1280-9.

123. Bhat R, Dempe S, Dinsart C, Rommelaere J. Enhancement of NK cell antitumor responses using an oncolytic parvovirus. Int J Cancer 2011;128:908-19.

124. Dempe S, Lavie M, Struyf S, Bhat R, Verbeke H, Paschek S, et al. Antitumoral activity of parvovirus-mediated IL-2 and MCP-3/CCL7 delivery into human pancreatic cancer: implication of leucocyte recruitment. Cancer Immunol Immunother 2012;61:2113-23.

125. Galanis E. Therapeutic potential of oncolytic measles virus: promises and challenges. Clin Pharmacol Ther 2010;88:620-5.

126. Penheiter AR, Wegman TR, Classic KL, Dingli D, Bender CE, Russell SJ, et al. Sodium iodide symporter (NIS)-mediated radiovirotherapy for pancreatic cancer. Am J Roentgenol 2010;195:341.

127. Penheiter AR, Dingli D, Bender CE, Russell SJ, Carlson SK. Monitoring the initial delivery of an oncolytic measles virus encoding the human sodium iodide symporter to solid tumors using contrast-enhanced computed tomography. J Gene Med 2012;14:590-7.

128. Carlson SK, Classic KL, Hadac EM, Dingli D, Bender CE, Kemp BJ, et al. Quantitative molecular imaging of viral therapy for pancreatic cancer using an engineered measles virus expressing the sodium-iodide symporter reporter gene. Am J Roentgenol 2009;192:279.

129. Bossow S, Grossardt C, Temme A, Leber M, Sawall S, Rieber E, et al. Armed and targeted measles virus for chemovirotherapy of pancreatic cancer. Cancer Gene Ther 2011;18:598-608.

130. Himeno Y, Etoh T, Matsumoto T, Ohta M, Nishizono A, Kitano S. Efficacy of oncolytic reovirus against liver metastasis from pancreatic cancer in immunocompetent models. Int J Oncol 2005;27:901-6.

131. Etoh T, Himeno Y, Matsumoto T, Aramaki M, Kawano K, Nishizono A, et al. Oncolytic viral therapy for

human pancreatic cancer cells by reovirus. Clin Cancer Res 2003;9:1218-23.

132. Maitra R, Ghalib MH, Goel S. Reovirus: a targeted therapeutic—progress and potential. Mol Cancer Res 2012;10:1514-25.

133. Galanis E, Markovic SN, Suman VJ, Nuovo GJ, Vile RG, Kottke TJ, et al. Phase II trial of intravenous administration of Reolysin®(Reovirus Serotype-3-dearing Strain) in patients with metastatic melanoma. Mol Ther 2012;20:1998-2003.

134. Morris DG, Feng X, DiFrancesco LM, Fonseca K, Forsyth PA, Paterson AH, et al. REO-001: A phase I trial of percutaneous intralesional administration of reovirus type 3 dearing (Reolysin®) in patients with advanced solid tumors. Invest New Drugs 2013;31:696-706.