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Advances in Oncolytic Adenovirus Therapy for Pancreatic Cancer

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An emerging alternative to current therapeutics is oncolytic adenoviruses; these engineered biological agents have proven efficacy and tumour-selectivity in preclinical pancreatic cancer models, including models of drug-resistant cancer. Safety of oncolytic adenoviral mutants has been extensively assessed in clinical trials with only limited toxicity to normal healthy tissue being reported. Promising efficacy in combination with gemcitabine was demonstrated in preclinical and clinical studies. A recent surge in novel adenoviral mutants entering clinical trials for pancreatic cancer indicates improved efficacy through activation of the host anti-tumour responses. The potential for adenoviruses to synergise with chemotherapeutics, activate anti-tumour immune responses, and contribute to stromal dissemination render these mutants highly attractive candidates for improved patient outcomes.

Currently, momentum is gathering towards the development of systemically-deliverable mutants that are able to overcome anti-viral host immune responses, erythrocyte binding and hepatic uptake, to promote elimination of primary and metastatic lesions. This review will cover the key components of pancreatic cancer oncogenesis; novel oncolytic adenoviruses; clinical trials; and the current progress in overcoming the challenges of systemic delivery.

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Abstract

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Introduction

Pancreatic cancer is the fourth leading cause of cancer-related deaths globally, even though it accounts for only 3% of all cancer diagnoses in the Western world [1]. Survival rates for the most aggressive form of pancreatic cancer, pancreatic ductal adenocarcinoma (PDAC), have not improved significantly since the 1980s [2]. The prognosis for PDAC patients remains unacceptably poor, owing to the fact that around 80% of patients have evidence of distant metastases at the time of diagnosis. Evidence of locally advanced or metastatic disease preclude potentially curative surgical resection resulting in treatment with chemotherapy and the rapid development of resistance to all current therapeutics [3]. In patients with resectable tumours, the 5-year overall survival (OS) rate is still remarkably low at 15-20%; for non-resectable disease, it is <5%. The low OS rates in patients undergoing surgical resection is most likely due to undetectable distant metastases and incomplete removal of the tumour [4].

The incidence of pancreatic cancer is highest within the 65-75-year-old age group. Current observations suggest that 5-10% of all cases have autosomal dominant hereditary components with reduced penetrance; the remainder of cases arise sporadically [5]. Some indicated risk factors are smoking, high alcohol intake, obesity, pancreatitis and diabetes mellitus [5-7]. The majority (65%) of tumours are located in the head of the pancreas, with 15% in the body, 10% in the tail and 10% within multiple sites (**Figure 1)**. Patients with tumours originating in the head of the pancreas tend to present earlier with obstructive jaundice and pancreatitis, while patients with tumours of the tail and body present later and have worse prognoses [7].

Pancreatic cancer is histologically characterised into adenocarcinomas (>90%), mucinous tumours, neuroendocrine tumours and cystadenocarcinomas [8, 9]. The survival rates vary significantly depending on the histological type; the best prognosis is for patients with neuroendocrine tumours and the worst for patients with adenocarcinomas and mucinous tumours [9]. More than 80% of patients present with metastasis at the time of diagnosis, with lesions predominantly detected within the abdominal viscera and peritoneum [10]. The liver is a common site for metastasis due to the complete hepatoportal venous drainage of the pancreas [11]. Metastases originating from the body and tail of the pancreas can bypass lymphatic, hepatic and pulmonary filters, resulting in spread to sites throughout the entire body including lungs, bones and adrenal glands [10-13].

Despite recent advancements in understanding the underlying molecular causes of pancreatic cancer, current therapeutics neither significantly prolong survival nor alleviate morbidity. Treatments are rarely curative outside surgical resection, with first line palliative chemotherapy remaining relatively unchanged since the evaluation of gemcitabine monotherapy in 1997 [14]. In 2011, it was

demonstrated that a combination regime of leucovorin, fluorouracil, irinotecan and oxaliplatin (FOLFIRINOX) generated a slight increase in survival of 4.3 months when compared to gemcitabine monotherapy; 11.1 and 6.8 months, respectively [15]. Unfortunately, prolonged survival came at a cost, with hugely increased toxicity profiles compared to gemcitabine monotherapy, including multiple grade 3/4 adverse events. After FDA approval in 2013, gemcitabine can now be given in combination with nab-paclitaxel, following reports that the combination granted prolonged survival compared to gemcitabine alone [16]. Although only a slight improvement (< 2 months) in survival, the toxicity profile was more tolerable than with FOLFIRINOX, and grade 3/4 adverse events were less common.

Presently, clinical data indicate that both FOLFIRINOX and gemcitabine with nab-paclitaxel are acceptable first-line treatment options for patients with a good performance status and non-resectable disease [17, 18], however, a significant proportion of patients do not present with a good performance status and may not tolerate these combination therapies. For these patients, the only option is gemcitabine monotherapy [19].

The limitations of current treatments for advanced and metastatic pancreatic cancer highlight the need for innovative and novel therapeutics with different mechanisms of action. The dismal prognosis for patients afflicted with PDAC and the lack of significant improvements in survival during recent decades signifies that the sole use of chemotherapeutics may never be enough. This review will discuss the exciting therapeutic potential that oncolytic adenoviruses pose for future treatment of pancreatic cancer.

Molecular alterations during the oncogenesis of pancreatic cancer

Our understanding of how the combination of complex malignant transformations and precursor lesions ultimately develop into PDAC has significantly progressed during the last 20 years [20-22]. The evolution of a pancreatic ductal epithelial cell into a PDAC cell occurs in several stages: driver gene mutations within precursor cells followed by clonal expansion into multicellular neoplasms leads to the development of cellular heterogeneity and alterations in the surrounding microenvironment. Formation of PDAC precursor lesions is, in the majority of cases, induced via oncogenic *KRAS* activation. The two most noted precursor lesions are pancreatic intraepithelial neoplasias (PanIN) and intraductal papillary mucin-producing neoplasm (IPMN) [21, 23, 24]. PanINs are microscopically visible, non-invasive proliferations and metaplasia of once healthy ductal epithelium, and are the most common precursor lesions; IPMNs are grossly visible, non-invasive, mucin-producing neoplasias from the pancreatic ducts and branches. The progression of PanINs to dysplastic lesions occurs

through three distinct stages: PanIN-1, PanIN-2 and PanIN-3. A PanIN-3 lesion is described as carcinoma in situ within the TNM Classification for Pancreatic Cancer [25]. Cells are characterised by both morphological and genetic-alteration signposts for each of the PanIN stages, with only stages 2 and 3 showing cellular dysplasia. It is important to note that PanINs will not definitely progress to an infiltrating PDAC, although they have the full potential to do so [26]. The genetics of pancreatic cancer is dominated by the presence of three to four known alterations in *KRAS*, *CDKN2A*, *TP53* and *SMAD4* [20, 22, 26, 27]. Accumulation of these somatic alterations have been identified within the various PanIN stages and PDAC itself **(Table 1).** The key mutations of pathway and regulatory proteins, alongside their usual functions and rate of occurrence, are outlined in **Table 2.** The consistently high prevalence of these specific genetic alterations suggests that a main evolutionary pathway plays a role in the development of PDAC. However, low-frequency genetic alterations have been recently identified, which may provide scope for a more personalised treatment with continued extensive research [28, 29].

Activation of KRAS

Mutations leading to the activation of the *KRAS* oncogene are already present during the early stages of PanIN lesions and in more than 95% of PDAC cases [22, 30, 31]. KRAS is a small GTPase involved in a plethora of cellular functions, including cell survival, proliferation and cytoskeleton remodelling. It functions as a transducer between cell surface receptors and downstream intracellular pathways, existing in 'on' and 'off' conformations which is conferred by binding of GTP and GDP, respectively [32]. The majority of mutations, including $KRAS^{G12D}$ and $KRAS^{G12V}$, cause constitutive activation of KRAS by preventing hydrolysis of GTP to GDP [33, 34]. This locks the protein into an active conformation, leading to persistent stimulation of signalling pathways that drive the early oncogenesis of pancreatic cancer including the deregulation of cell cycle progression. The three major affected pathways are Raf-Mek-Erk, PI3K-Pdk1-Akt, and the Ral-guanine nucleotide exchange factor pathway [35, 36].

Inactivation of CDKN2A

The human *CDKN2A* gene encodes the tumour suppressor genes $p16^{INK4A}$ and $p14^{ARF}$ [37]. In PDAC, loss of both proteins via larger gene deletions may contribute to oncogenesis by varying mechanisms, although evidence from humans and mice allude to $p16^{INK4A}$ as the primary deleterious factor. For example, $p16^{INK4A}$ inhibits cell cycle progression at the G1/S checkpoint, which is mediated primarily by CDK4 and CDK6; consequently, $p16^{INK4A}$ loss through CDKN2A inactivation will abrogate this vital checkpoint enabling continuous cell cycle progression [38]. The $p14^{ARF}$ protein also activates the G1/S checkpoint, through a CDK-independent mechanism, by preventing p53-degradation [37, 39]. Loss of $p14^{ARF}$ prevents cell cycle arrest and p53-induced apoptosis.

Inactivation of p53

The *p53* gene encodes the tumour suppressor transcription factor p53, which is activated in the presence of DNA stress or damage [40, 41]. Expression of p53 is fundamental in the regulation of both G1/S and G2M checkpoints, which enable DNA repair or apoptosis to occur when necessary. In addition, p53 increases expression of the cyclin-dependent kinase inhibitor CDKN1A, thus preventing cell cycle progression [42]. In pancreatic cancer, p53 is either completely inactivated by genetic mutations, or by alterations in the DNA binding domain, preventing p53-mediated transcriptional activity [20, 43]. The mutations result in continuous cell cycle progression, even in the presence of severe DNA damage, promoting further accumulation of genetic abnormalities in the cells [44].

Inactivation of SMAD4

SMAD4 is a major tumour suppressor gene that is specifically altered in 45% of PDAC cases either through homozygous deletions (30%) or direct mutation with loss of the second gene copy (25%) [45, 46]. *SMAD4* encodes a transcription factor that acts as a crucial central mediator in the TGF-β signalling pathway for cellular differentiation, growth and maintenance of homeostasis [47]. During PanIN stages 1 and 2, the TGF-β pathway remains functional as a tumour suppressor. In contrast, during PanIN stage 3, *SMAD4* is inactivated and the TGF-β pathway promotes growth.

Induction of Epithelial to Mesenchymal Transition (EMT)

The gain of infiltrative capacity of cancer cells via the epithelial to mesenchymal transition (EMT) is considered a pivotal step in the progression of primary tumours to invasive and metastatic cancer, including PDAC. During the process of EMT, cells undergo a developmental shift from an epithelial to highly motile mesenchymal or fibroblastoid phenotype; this shift facilitates invasion of malignant cells into surrounding tissues, the circulation and, ultimately, dissemination to distant sites [48]. EMT is strongly associated with decreasing expression levels of E-cadherin and increasing N-cadherin levels [49]. The low levels of E-cadherin result in reduced formation of adherens-junctions, thus leading to the loss of maintenance of the epithelial phenotype [50]. Furthermore, higher levels of N-cadherin expression contribute to the increased invasive potential of the cells [51].

Treatment-resistance in PDAC

In addition to the late presentation of symptoms, the rapid development of resistance to chemotherapy remains one of the greatest barriers to curative treatments in pancreatic cancer [52]. Treatment of PDAC is in most cases a losing battle because of the numerous underlying genetic reprogramming events, including altered apoptosis, metabolic and cellular pathways, and increased EMT. In addition,

acquired drug-dependent alterations such as upregulation of drug efflux pumps and deregulation of miRNAs counteract the effects of cytotoxic drugs [53]. Cancer cells generated from the predominant oncogenic drivers mentioned above may also have innate resistance to chemotherapeutic drugs [54]. However, many PDAC patients show some extent of gemcitabine susceptibility before the first round of treatment. This initial susceptibility with rapid subsequent development of resistance suggests that there are pre-existing resistant cell populations within the stroma or the heterogeneous tumour itself, with the initial treatment selecting for these resistant populations [55]. For example, elimination of gemcitabine-sensitive cell populations enables resistant cell populations to expand and repopulate the tumour microenvironment within weeks of treatment [54].

Interestingly, the tumour microenvironment has been increasingly recognised as a major contributor to the development of chemoresistance in pancreatic cancer [56-58]. Desmoplasia is a well-established characteristic of PDAC that involves a fibro-inflammatory process leading to cancer-associated fibrosis of the stroma which restricts access of chemodrugs. The desmoplastic stroma is comprised of both cellular and non-cellular components, with the main constituents being non-quiescent pancreatic stellate cells (PSCs), immune cells, endothelial cells and fibroblasts, that promote tumour growth [59]. Activated PSCs are known to be the main regulators of pancreatic cancer-associated desmoplasia, and may promote further progression and metastasis [60, 61]. The non-cellular component is comprised of an extracellular matrix (ECM), which contains proteins, glycosaminoglycans and proteoglycans, forming the architectural foundations for cancer growth and maintenance. A key glycosaminoglycan, hyaluronic acid, is markedly overexpressed in PDAC, making it a structural hallmark and potential therapeutic target of the pancreatic cancer stroma [62]. In summary, the roles of the microenvironment in the development of chemoresistance are: i) Dysfunctional vasculature resulting in raised stromal interstitial pressure that prevents sufficient deposition of drugs within the tumour. ii) Stromal cells promote resistance by generation of the desmoplastic microenvironment, promoting EMT and, therefore, metastasis. iii) Components of the ECM promote chemoresistance by affecting gene expression in cancer cells.

It is clear that the resistance to cytotoxic drugs in PDAC cannot be conquered by currently available anti-cancer drugs but could, however, be combatted by directly altering the genetic landscape of the cancer cells. Oncolytic adenoviral mutants may pave the way as genetic vectors, supplying the cancer cells with new ways of dying with no or limited toxicity to healthy cells.

Oncolytic Adenoviruses

Adenoviruses are small non-enveloped double-stranded DNA viruses with a 30-38 kb linear double-stranded genome (**Figure 2**) [63]. The family includes >57 serotypes that are classified in subtypes A-G, based on their respective agglutination properties. Adenovirus-infection is facilitated by binding of viral fibre-proteins to the epithelial-specific cellular Coxsackie virus and Adenovirus Receptor (CAR), followed by internalisation through viral penton-binding to integrins; mainly αvß3 and αvß5 (**Figure 2**). Once internalised, the viral protein coat is degraded, and the genome is transported to the host cell nucleus for transcription. The first step in the viral life-cycle is expression of the early viral E1A proteins, required for initiation of S-phase that is fundamental for viral propagation [63]. The E1ACR2 domain in the E1A protein binds to the retinoblastoma protein (pRb), which in turn releases E2F and forces the infected cell to enter S-phase. Following E1A translation and S-phase entry, expression of the viral anti-apoptotic E1B55K and E1B19K proteins protect the infected cells from premature death by inhibiting the G1/S checkpoint and inactivating both p53 and mitochondrial depolarisation. In addition, the viral E3- and E4-genes protect the infected cell from immune-mediated cell killing and prevent activation of DNA-damage repair, respectively.

To date, multiple oncolytic viruses have been generated, with the majority based on genomic alterations of serotype 5 species C (Ad5) [64-66]. The advantages of Ad5-based mutants include the ease of genetic engineering of the small genome with all gene functions established; high-titre production under good manufacturing production (GMP); and specific targeting of both dividing and non-dividing epithelial cells, including adenocarcinomas. Furthermore, even the wild type Ad5 virus causes only mild upper respiratory tract infections that resolve spontaneously in otherwise healthy and immunocompetent individuals [67]. Extensive data from clinical trials using replication-selective oncolytic Ad5-based mutants have proved that these mutants are safe in cancer patients and specifically eliminate tumour cells with limited toxicity to healthy cells [64, 68-72].

Viral activation of the anti-tumour immune response

In contrast to current chemotherapeutic and targeted anticancer drugs, oncolytic adenoviruses act through multiple mechanisms to eliminate cancer cells and prevent recurrence. In addition to local amplification of viral dose within the tumour microenvironment and activation/inhibition of numerous cellular pathways, adenoviruses also activate the host anti-tumour immune responses [65, 73, 74]. Generation of a potent immune response is critical for maximum therapeutic efficacy in response to oncolytic viruses.

One of the emerging hallmarks of cancer is the ability to evade the host immune system and, thus, avoid immunologically-mediated destruction [75]. During the process of early immune editing, cancer

cells that present tumour antigens are highly susceptible to detection and removal by the immune system, resulting in a selection-based elimination [76]. Following immune destruction of the initial immunogenic cancer cells, continued aberrant cell division of the remaining populations results in accumulation of mutations and reduction in cancer-cell immunogenicity. Eventually, the tumour will fully escape recognition by the immune system [76, 77]. The main mechanisms underlying tumour evasion are defective tumour-associated antigen presentation and over-production of immune-suppressive factors [78]. Adenovirus-induced cancer cell lysis causes activation of the innate immune defence and exposure of novel tumour antigens that facilitates the generation of an anti-tumour immune response that counteracts the immune suppressive tumour microenvironment. Importantly, adenoviral infection induces immunogenic cell death (ICD) through direct cell lysis and release of tumour-associated antigens (TAAs), as well as damage- and pathogen-associated molecular pattern molecules (DAMPs and PAMPs) [73, 74, 79, 80]. Activation of the adaptive immune responses occurs after presentation of these molecules, particularly DAMPs, to antigen-presenting cells (APCs) such as the dendritic cell (DC) [81, 82]. Activated DCs migrate to draining lymph nodes for cross-presentation to $CD8^+$ T-cells, the primary anti-tumour effector cells [83]. DCs also activate $CD4^+$ T-helper cells, which are fundamental for the development of long-term anti-tumour immunity [84]. Viral infection induces the release of pro-inflammatory cytokines and chemokines from both infiltrating and resident immune cells as well as from the infected cells. As a result, this localised inflammation may augment the function of infiltrating lymphocytes while contributing towards the generation of anti-tumour immunity [84]. Therefore, oncolytic adenoviruses may play a potential role in reversing the profound immunosuppressed state of the PDAC microenvironment, which ordinarily prevents the successful infiltration/activation of $CD4^+$ and $CD8^+$ T-cells and release of anti-tumour chemokines and cytokines [85]. In addition, adenoviruses are often armed with therapeutic transgenes that promote local cytokine-activation to exploit inflammatory-induced infiltration of lymphocytes, including IL-12, IL-18 and IFN-α that stimulate both innate and adaptive immune responses [73, 86-88].

Clinical trials with oncolytic adenoviruses may be the only current way of demonstrating the induction of potent long-term anti-tumour immunity in response to adenoviral infection due to the limitations with preclinical *in vivo* models. The species-specificity of adenovirus precludes the generation of relevant immune mechanisms in other species in response to potent viral replication and cell lysis; as a result, murine models are inadequate for investigating these anti-tumour immune responses.

Generation of PDAC-selective potent oncolytic adenoviral mutants

The first oncolytic adenoviruses were developed via the deletion of viral genes, the so-called complementation deletions, which are fundamental for viral replication within normal cells but not in cancer cells due to their altered cell cycle and apoptosis pathways. The first oncolytic adenovirus to be evaluated in cancer patients, including PDAC patients, was Onyx-015 (*dl*1520) with the anti-apoptotic *E1B55K*-gene deleted (**Figure 2**) [71, 89, 90]. The E1B55K protein binds and inactivates p53, which is already non-functional in the majority of cancers including pancreatic cancer. Thus, Onyx-015 propagation could proceed in cancer cells but not in normal cells with functional p53. In the initial Phase I trial targeting patients with unresectable, locally advanced pancreatic cancer, Onyx-015 was delivered directly into the tumours by CT-guided or intraoperative administration [89]. While safety was clearly demonstrated, no significant responses were reported. In a follow-up Phase I/II trial, the virus was delivered intra-tumourally by ultrasound-guided administration in combination with intravenous gemcitabine [68]. Despite improved efficacy compared to each agent alone (partial regression, 2/21; minor responses, 2/21), the overall outcomes were disappointing and no further trials in PDAC patients were undertaken with Onyx-015. The poor efficacy of Onyx-015 was attributed to the lack of viral mRNA nuclear export, a function mediated by E1B55K in addition to p53 inactivation, that was later demonstrated to be essential for viral replication [70, 91]. Without a functional E1B55K-protein, viral replication and spread within the tumour were severely attenuated. Furthermore, the deletion of the immune-regulatory *E3B*-genes, that had been included for safety reasons, contributed to premature elimination of virus-infected cells by the host macrophages prior to maximal viral replication [92].

To date, several oncolytic adenoviral deletion-mutants have been developed with highly specific genetic alterations and retained activity, resulting in promising improvements in efficacy within both preclinical studies and early-phase clinical trials with PDAC patients (**Table 3**). The most common alteration is deletion of the pRb-binding E1ACR2-region, generating mutants with proven efficacy in the majority of cancers, including pancreatic cancer. Deletion of the E1ACR2-domain prevents viral replication in normal cells while propagation readily proceeds in cancer cells with deregulated cell cycle, such as in PDAC cells with activating KRAS mutations. Mutants with the E1ACR2-deletion include *dl*922-947 [93], AdΔ24RGD [94], AdΔΔ and Ad5-3Δ-A20T [95, 96], and have been demonstrated to potently replicate and spread within PDAC cell models in both *in vitro* and *in vivo* preclinical studies. Additional modifications include insertion of E2F-binding domains to drive viral replication (*e.g.* VCN-01 and LOAd703) and transgene expression to activate the host anti-tumour immune responses (*e.g.* LOAd703 and Ad5-yCD/mutTK$_{SR39}$rep-hIL12), which will be discussed below.

Clinical trials targeting pancreatic cancer with adenoviral mutants

Despite the poor efficacy of Onyx-015, the trials convincingly demonstrated feasibility, tolerance and safety of adenoviral delivery, paving the way for future clinical evaluation with improved oncolytic viruses. Interestingly, the Chinese FDA (SFDA) approved the clinical application of an almost identical mutant, H101 (*E1B55K*- and *E3B*-deleted) for head and neck cancers in combination with cisplatin and/or 5-fluorouracil (5-FU) in 2005 [97, 98]. Currently, thousands of patients have been treated with H101 with no reported side-effects. To date, there are a great number of published clinical trials involving PDAC patients that utilise modified adenoviral mutants, both replicating and non-replicating (outlined in **Table 3**).

Currently, patients are being recruited in two phase I trials aimed at evaluating mutants based on the Onyx-015 backbone with additional modifications, which were reported to improve efficacy in preclinical studies; Ad5-yCD/*mutTK*(sr39)*rep*-ADP and Ad5-yCD/*mutTK*(sr39)*rep*-IL12 [99, 100]. Both mutants express the chimeric prodrug converting enzyme yeast cytosine deaminase (yCD)/mutant sr39 herpes simplex virus thymidine kinase (yCD/mutTKsr39) that potently converts the prodrugs 5-FC and ganciclovir to their respective metabolites 5-fluorouracil (5-FU) and ganciclovir-5-monophosphate (ganciclovir-MP). Enzyme expression is regulated by the CMV-promoter that also controls expression of the Adenoviral Death Protein (ADP) or IL-12 to further boost anti-tumour activity in PDAC patients. In contrast, the replicating AdVince virus targets liver metastases originating from pancreatic neuroendocrine tumours (NET) [101]. Although, pancreatic cancer is dominated by adenocarcinomas (PDAC), approximately 10% of the pancreatic patient population suffer from metastatic NETs, creating a distinct group in need of a novel therapeutic option. NETs typically produce the secretory protein chromogranin A (CgA), which presents the opportunity to exclusively target viral replication to NET cells. In AdVince, E1A expression is controlled by the CgA-promoter region and to minimise hepatocyte toxicity, target sequences for the hepatocyte-specific microRNA miR122 were incorporated into the 3'-untranslated region of the E1A gene to prevent viral replication in hepatocytes [101]. In order to enhance the transduction of tumour cells, the protein transduction domain (PTD) from the Trans-Activator of Transcription (Tat) protein of human immunodeficiency virus (HIV)-1 was inserted into the hypervariable region (HVR)-5 of the viral hexon protein. This PTD insertion achieves a CAR-independent route of infection and dissemination throughout the NETs. Although only tested pre-clinically on surgically-resected metastatic NETs originating from the small intestine, AdVince displayed NET-cell specificity with high lytic activity, as well as limited toxicity in isolated human hepatocytes compared to wild-type virus. AdVince is currently being evaluated in a phase I/IIa clinical trial for patients with multiple metastatic liver lesions of pancreatic NET origin. Importantly, CgA is expressed in healthy tissues including pancreatic β-cells, the pituitary gland, and adrenal medulla [101] and is therefore administered in close proximity to the target tumours via intrahepatic artery infusion.

Interestingly, a number of trials are currently evaluating non-replicating adenoviral mutants that cannot spread within the tumour and require expression of prodrug converting enzymes or cytotoxic factors to induce tumour cell killing (Table 3). By definition, non-replicating mutants have the viral E1A-gene deleted that prevents replication in any cell. In AdV-tk, the immunoregulatory E3-genes were deleted in addition to E1A to enable insertion of the HSV-tk enzyme expressed from the CMV-enhancer/promoter [102, 103]. Favourable responses were reported in a Phase I trial after intra-tumoural injections. In the TNFerade mutant, the entire E1-gene (E1A and E1B) was deleted in addition to the E4-genes and a partial E3-deletion to enable insertion of TNFα regulated by the radiation activated early growth response element (Egr-1) [104]. TNFerade was reported to be safe but had no significant advantage compared to current standard of care in a small randomised PhaseII/III trial. ETBX-011 was deleted in the E1-genes (E1A and E1B), the E2B- (the viral polymerase) and the E3-genes [105, 106]. The rationale for deleting the major early viral genes was to enable insertion of the tumour associated carcinoembryonic antigen (CEA) regulated by the CMV promoter. Potent expression of CEA elicited anti-tumour activity as a result of cell-mediated immunological responses. Furthermore, CEA was modified to contain the highly immunogenic epitope, CAP-1-6D. Promising preclinical results were reported [105], which explains why multiple phase I/II trials with ETBX-011 in PDAC patients are currently underway (outlined in **Table 3**).

To date, the most promising results for treatment of PDAC, have been reported for the replication-selective mutants LOAd703 and VCN-01, and these mutants will be discussed in detail below.

LOAd703

Deletion of the E1ACR2-region has been established to produce highly tumour-selective and efficacious oncolytic adenoviral mutants and was therefore included in LOAd703. In addition, E2F-binding sites were inserted upstream of E1A to control its expression and viral replication, and the immune regulatory *E36.7K* and *E3gp19K* genes were deleted for increased immunogenicity [107-109]. The novelty in LOAd703 is the insertion of a trimerized, membrane-bound human CD40 ligand (TMZ-CD40L) and the full-length human 4-1BB ligand (4-1BBL), both under control of the CMV promoter.

CD40 belongs to the tumour necrosis factor receptor (TNFR) family and is expressed by B-cells, professional antigen presenting cells (APCs), non-immune cells and tumour cells [110]. The ligand, CD40L (a.k.a. CD154) is expressed primarily by activated T-cells and B-cells, and by natural killer cells (NK), mast cells, monocytes and basophils[110]. CD40/CD40L interactions play pivotal roles in governing humoral and cell-mediated immunity, particularly for 'licensing' DCs to undergo maturation and effectively trigger cytotoxic T-cell activation/differentiation[110-112]. It was hypothesised that CD40/CD40L interactions in PDAC may provide a key regulatory step in the

generation of a T-cell-dependent anti-tumour immune response [113] by mechanisms including activation of the adaptive immune system and CD40-mediated apoptosis of cancer cells [108]. However, this hypothesis was contested when analysing data from a clinical trial evaluating a CD40-activating monoclonal antibody (mAB) in combination with gemcitabine in PDAC patients, resulting in partial responses in primary and metastatic lesions; analysis of tumour biopsies revealed infiltration by macrophages rather than T-cells [113]. Nonetheless, there is evidence that therapies involving CD40 agonists may produce an anti-tumour immune response via myeloid-cell activation, albeit potentially without T-cell involvement due to poor tumour infiltration.[108] The TMZ-CD40L in LOAd703 may increase myeloid and T-cell infiltration of PDAC tumours, and promote lymphocyte transmigration across CD40L-stimulated endothelial cells [108].

The 4-1BB protein is also a member of the TNFR family and is typically expressed on activated T-cells and natural killer cells, monocytes, neutrophils and macrophages [114]. Its natural ligand, 4-1BBL (a.k.a. CD137L), is expressed on B-cells, activated T-cells, dendritic cells and macrophages [115]. Binding of 4-1BBL to 4-1BB is associated with the expansion of innate immune cells, including NK cells, and potentiation of immunological memory [116, 117]. In one study, cultured surgical PDAC specimens were treated with an activating 4-1BB mAb, resulting in increased numbers of tumour-infiltration lymphocytes (TIL) [118]. The 4-1BBL in LOAd703 may potentiate the infiltration of lymphocytes into the tumour and increase the efficacy of CD40/CD40L orchestrated anti-tumour immune responses.

Pre-clinical studies using LOAd703 demonstrated that the mutant successfully replicated and lysed cultured human PDAC cells (Panc01, BxPc3, MiaPaca2, PaCa3) and subcutaneous Panc01 xenografts in immune deficient mice [107]. In these models, intratumoural administration of LOAd703 in combination with gemcitabine significantly reduced tumour growth compared to single agent treatments. Due to the lack of immune competent murine models, further evaluation was conducted in isolated human immune cells and infected DCs, which led to NK cell expansions in response to the replication-independent expression of TMZ-CD40L and 4-1BBL, resulting in cytokine production [108]. Due to the poor infiltrative capacity of leukocytes into the desmoplastic stroma in PDAC tumours, viral replication and upregulation of cytokines may aid in producing a significant anti-tumour immune response in patients. Safety and preliminary anti-tumour activity of LOAd703 is currently being evaluated in Phase I/II and I/IIa trials including PDAC patients with unresectable cancers **(Table 3)**. Patients in the phase I/II trial will receive eight intratumoural LOAd703 injections in combination with gemcitabine and/or nab-paclitaxel; in the phase I/IIa trial, six intratumoral LOAd703 injections every other week together will be performed alongside standard of care (SOC) gemcitabine and nab-paclitaxel.

VCN-01

Replication-selectivity of VCN-01 was achieved via deletion of the E1ACR2-region and insertion of E2F-binding sites to control E1A-expression and viral replication [119, 120]. To circumvent adenoviral hepatocyte transduction, the heparin sulfate glycosaminoglycans (HSG) putative-binding site KKTK in the fibre shaft was replaced by an RGD-motif [121, 122]. This KKTK→RGDK mutation decreased liver transduction in murine models after intravenous injection and increased tumour-targeting [123, 124]. VCN-01 penetrates the dense desmoplastic stroma in PDAC tumours because of incorporation of the human glycosylphosphatidylinositol-anchored enzyme PH20 hyaluronidase in the viral genome, which breaks down the ECM [120]. Currently, two phase I trials are underway including PDAC patients with unresectable cancers **(Table 3)**. The use of the anchored PH20 hyaluronidase should theoretically allow for greater adenoviral dissemination throughout the desmoplastic stroma and ECM, facilitating both viral spread and immune cell infiltration. In one trial, three intratumoral injections of VCN-01 every 28 days in combination with intravenous Gemcitabine and Abraxane® is assessed, while in the second trial, a single intravenous injection of VCN-01 in combination with intravenous Gemcitabine and Abraxane® is evaluated.

Challenges with systemic delivery of adenoviral mutants in PDAC patients

In the vast majority of clinical trials with oncolytic adenoviruses the mutants have been administered intra-tumorally. Unless potent anti-tumour immune responses are initiated, many of the current mutants are not suitable for the 80% of patients that present with already active metastatic disease because of the inability to reach distant tumour lesions in sufficiently high doses after systemic delivery [125]. The ultimate aim is to generate mutants that have prolonged half-life in blood and can reach and eliminate all tumour lesions after systemic delivery, and concurrently activate anti-tumour innate and adaptive immune responses. The barriers for successful systemic delivery will be discussed below.

Pre-existing antibodies neutralise Ad5

One challenge with systemic delivery is the presence of pre-existing humoral immunity. Anti-Ad5 neutralising antibodies (NABs) are detectable in up to 85% of the population indicating prior exposure to Ad5 at some point in their lives [126]. For the remaining few lacking acquired immunity, primary exposure to Ad5 will initiate innate and adaptive immune responses, rendering the individual immune to future administrations within two weeks [127]. One strategy to evade NABs is the chemical conjugation of polymers such as N-(2-hydroxypropyl)methacrylamide (HPMA) or polyethyleneglycol (PEG) to the viral capsid. However, it was reported that viral infectivity was decreased and a strong humoral immune response still occurred suggesting that shielding may be

more of a hindrance than a benefit [127-129]. Recent work using an albumin-binding strategy protected adenoviruses from NABs and reduced blood clearance in mice [130]. An albumin-binding domain (ABD) was inserted in the HVR1 region of the hexon protein, thus allowing viral binding to albumin, which prevented binding by NABs. However, *in* vitro studies demonstrated that infectivity of adenoviruses with the inserted ABD was decreased in the presence of human serum albumin. This method may be of future interest when used in combination with tumour targeting modifications.

Non-tumour uptake of Ad5

The predominant sites for Ad5 uptake after systemic delivery are liver hepatocytes and the hepatic reticuloendothelial system, which is composed of resident macrophages called Kupffer cells (KC), and liver sinusoidal endothelial cells (LSEC). KCs are responsible for sequestration of up to 90% of intravenously administered Ad5 within minutes, posing a monumental barrier to systemic delivery [131, 132]. Ad5 uptake by KCs is independent of CAR but may involve αvβ5-integrins [122, 133, 134]. Ad5 also binds heparan sulphate glucosaminoglycans (HSPG), expressed on cell membranes, via a KKTK motif in the viral fibre shaft and mediates binding to numerous tissues including hepatic cells. By substituting the four amino acid KKTK domain with the integrin binding sequence RGDK in the VCN-01 mutant, systemic delivery to tumours was reported to be more efficient, ablating HSPG binding. Although, hepatocyte uptake and KC elimination were not prevented [123][[124]. A family of receptors known as scavenger receptors (SR) have been shown to facilitate KC-mediated uptake of Ad5, with SRA-II involved in the *in vitro* uptake through the hexon hypervariable regions (HVR) 1, 2, 5 and 7 [132]. PEGylation of the HVRs prevented KC uptake, with a subsequent 20-fold increase in hepatocyte transduction. Furthermore, depletion of platelets with either anti-CD42b, neuraminidase or anti-CD41, suggested that KC-uptake was platelet-independent contesting a previous hypothesis that platelets assist the sequestration [135, 136]. *In vivo* studies demonstrated an increase in adenoviral transduction of hepatocytes in the presence of the vitamin K-dependent coagulation factors VII, IX, X and protein C [137, 138]. Factor X binding to Ad5 HVR 5 and 7 promotes KC uptake and fibre knob binding to FIX/complement-4 binding protein (C4BP) increases hepatocyte uptake. However, it was reported that in mice, these coagulation factors were only required for hepatocyte-uptake but not for KC-uptake [135]. Additionally, pre-existing IgM antibodies neutralise Ad5, with opsonisation orchestrated via the co-interaction with various complement components including complement receptor 3 (CR-3) or the Fc receptor, that facilitate subsequent KC-uptake [132, 135, 139].

The reticuloendothelial system has been evaded by different strategies including pre-administration of virus to saturate receptors and uptake mechanisms [127, 140]; modification of the virus (*e.g.* HVR PEGylation) [127, 132]; and by detargeting and retargeting of the fibre (*e.g.* KKTK to RGDK mutation) [123]. Saturation of KCs with an oncolytic adenovirus in pre-clinical *in vivo* models increased the anti-tumour responses after a subsequent single intravenous injection of the same virus [141]. When KC saturation was combined with warfarin pre-treatment, hepatotoxicity was reduced,

and anti-tumour efficacy enhanced. These findings indicate that a two-pronged attack is superior in order to prevent both reticuloendothelial sequestration and hepatic parenchyma uptake when delivering adenoviruses systemically.

Erythrocyte binding

Ad5 binds with high affinity to human erythrocytes via both CAR and complement receptor-1 (CR1), but is not internalised [142]. Thus, the erythrocytes act as a viral sink, dramatically reducing the levels of free circulating virus and the bioavailability. A strategy to partly overcome this is polymer 'stealthing', for example, by using a new generation of an epidermal growth factor (EGF)-targeted, positively charged, HPMA polymer (EGF-P) [142]. However, early data from an ongoing clinical trial of ICOVIR-5 (NCT01864759), suggests that erythrocyte-binding may not pose a major obstacle to systemic viral delivery and that focus should be diverted to other major obstacles [143]. Murine erythrocytes do not express CAR which means that, as a consequence, murine models are not representative of the situation in humans after systemic delivery of Ad5-mutants when assessing the erythrocyte viral sink [144].

To move towards systemically deliverable oncolytic adenoviruses for targeting of metastatic PDAC, strategies to overcome the hepatic and blood factor barriers need to be developed for example by combining coat modifications, de- and re-targeting, and chimeric serotypes with different receptor and binding preferences.

Promising Developments

We recently developed a novel adenoviral mutant with multiple gene alterations to target PDAC through systemic administration (Ad5-3Δ-A20T) [96]. The mutant was generated from AdΔΔ, which is tumour selective because of the E1ACR2-deletion, and enhances chemodrug-induced apoptosis through deletion of the viral anti-apoptotic Bcl-2 functional homologue E1B19K [95, 145-147]. The absence of a functional anti-apoptotic *E1B19K*-gene allowed for greater pancreatic cancer cell death through enhancement of drug-induced apoptosis and synergistic cell killing in combination with chemotherapeutics [95, 146, 148, 149]. In pre-clinical studies, the *E1B19K*-gene-deleted mutants potently synergised with apoptosis-inducing cytotoxic drugs including gemcitabine, which allows for highly efficient tumour elimination while utilising significantly lower doses of chemotherapeutics. The double-deletion of E1ACR2 and E1B19K has the greatest synergistic effects on cancer cell killing and is significantly more potent than wild type Ad5. Additionally, in Ad5-3Δ-A20T, the *E3gp19K*-gene is deleted to promote MHC class I expression on the infected cancer cells and promote reactivation of the host anti-tumour immune responses [96]. Furthermore, Ad5-3Δ-A20T is targeted to

αvß6-integrins that are frequently expressed by PDAC tumours but not by healthy tissues [150]. Additional fibre-modifications improved specific uptake in tumour cells by reducing blood-factor binding and improved the tumour-to-liver viral genome ratios in murine models [140, 142, 151]. These preclinical findings suggest that Ad5-3Δ-A20T may be suitable for clinical translation targeting late stage PDAC lesions in combination with current standard of care. Additional preclinical toxicity and efficacy studies are underway. However, translation of any promising oncolytic viral mutant from preclinical studies to the clinic is considerably hampered by the limitations of *in vivo* models. Human adenoviruses do not replicate and spread within the murine components of xenografted human tumour cells. Furthermore, the absence of an adaptive immune system within these models prevents exploration of relevant cytokines, immune cells and development of long-term immunity.

Currently, several *in vitro* model systems have been explored to bridge the gap that currently exists between cells grown on plastic or xenografted into immune-deficient mice and clinical translation. Promising developments involve PDAC cells co-cultured with various stromal cells including activated stellate cells in 3-dimensional (3D) collagen/Matrigels (*e.g.* organotypic models) [152, 153] and in organoids (without supporting gels) [154, 155]. In the organotypic 3D models, PDAC cells invaded the gels only in the presence of activated stellate cells (non-transformed PS1 cells), mimicking tumour invasion in patients [153, 156]. Importantly, it was demonstrated that Ad5, AdΔΔ and the novel mutant Ad5-3Δ-A20T infected and spread within the 3D structures. Furthermore, they potently eliminated PDAC cells and also infected the activated PS1 cells that likely contributed to the higher cell killing efficiency compared to monocultures of PDAC cells [96]. Interestingly, PDAC and PS1 cells grown in organotypic cultures have been co-cultured with isolated human immune cells to study migration and interactions with T-cells and macrophages [157]. Further optimisation of these organotypic or organoid cultures may enable more accurate predictions of viral interactions with host immune factors within the human tumour microenvironment and better predict which viral mutant should be pursued for clinical development.

Concluding remarks

Oncolytic adenoviruses are at the forefront of clinical utilisation, however, the future success of providing systemically-delivered therapy for pancreatic cancer depends hugely on the strategies used to overcome a plethora of limitations. Achieving sufficient bioavailable doses of adenovirus after intravenous injection in an immune-competent host will likely be a major challenge that may require a combination of strategies such as those describe for Ad5-3Δ-A20T, VCN-01 and LOAd703. Arming the viruses with immune stimulatory factors and cytotoxic transgenes is the most promising way forward to target both early- and late-stage cancers, and with additional capsid-modifications these

mutants may also be delivered systemically to distant metastasis. Although the number of novel mutants currently entering clinical trials for pancreatic cancer is a monumental achievement, the vast majority of trials still fail to target metastatic lesions that are present in 80% of patients at the initial treatment. However, the burst of novel mutants that are clinically evaluated is exciting as they will provide fundamental information that cannot currently be obtained in pre-clinical studies. Results on efficacy and the effects of viral replication on the host anti-tumour immune responses and development of tumour immunity are eagerly awaited from these trials.

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Conflict of Interest

The authors claim no conflict of interest.

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[179] QUILT-3.040: ETBX-011 (Ad5 E1-, E2b- -CEA(6D)) Vaccine in Combination With ALT-803 (Super-agonist IL-15) in Subjects Having CEA-Expressing Cancer, https://ClinicalTrials.gov/show/NCT03127098.

Tables

Table 1: The morphological features and key driver gene alterations from normal pancreas, through the various PanIN stages to PDAC.

Stage	Morphological Features	Key Cumulative Driver Gene Alterations at Various Stages	Refs
Normal	Cuboidal epithelium with ducts; islet cells with surrounding acinar tissue.	None	[22, 158]
PanIN-1	Columnar or cuboidal cells which are flat or papillary. Cells have complete nuclear polarity and no nuclear atypia.	*KRAS* activation	[22, 158, 159]
PanIN-2	Multiple nuclear abnormalities: pleomorphism; hyperchromasia; loss of polarity; crowding; and nuclear pseudostratification.	*CDKN2A* inactivation	[22, 158, 159]
PanIN-3	Widespread loss of nuclear polarity; marked atypia of nuclei; and prevalent mitosis within the basement membrane.	Inactivation of *TP53* and *SMAD4*	[22, 158, 159]
PDAC	Similar to PanIN-3 but with infiltration through the basement membrane. Neoplastic glands undergo perineural and vascular invasion in a completely haphazard manner.		[158, 160]

Table 2: The proportions and downstream consequences of the key PanIN stage and PDAC gene alterations.

Pathway or Regulatory Process	PanIN Driver Gene Alterations	Protein Function	Downstream Consequences of Genetic Alteration	Proportion of Tumours with PanIN Driver Gene Alterations	Refs
KRAS Signalling	*KRAS* activation	Oncogene; GTPase; activation of MAPK activity.	Growth independent of ligand-bound RTK. Immunosupression Metabolic reprogramming Protein scavenging	95%	[20, 30, 161]
TGF-β Signalling	*TGFBR2* inactivation *SMAD4* inactivation	Transforming growth factor-β receptor type II; regulation of growth. Mothers against decapentaplegic homologue 4; BMP signalling pathway.	Loss of homeostatic mechanisms. Loss of gene expression that is co-regulated by p53 and TGF- β	5 - 10% 55%	[20, 30, 161]
G1/S Phase Transition Regulation	*CDKN2A* inactivation	Cyclin-dependent kinase inhibitor 2A; G1/S transition of mitotic cell cycle; tumour suppressor.	Loss of G1/S checkpoint.	90%	[20, 30, 161]
DNA Damage Control	*TP53* gain of function	Tumour suppressor p53; DNA damage response.	Loss of G1/S and G2/M checkpoints. Resistance to apoptotic signalling.	80 - 85%	[20, 30, 161]

Usual protein function from: [161] Downstream consequences from: [20]
Proportion of tumours data from: [30]

Table 3: Pancreatic cancer clinical trials with adenoviral mutants.

Adenovirus Mutant	Genetic Modifications	Trial Phase; Number of Patients	Route of Administration	Trial Outcomes	Ref
ONYX-015 (*dl*1520)	E1B55K- and E3B-deleted.	Phase I; 23	Intratumoral injection via CT-guided or intraoperative injection.	Feasible and well-tolerated. Viral replication was not detectable. 6/23 minor responses; 11/23 stable disease; 5/23 progressive disease.	[162] [89]
ONYX-015 (*dl*1520)	E1B55K- and E3B-deleted.	Phase I/II; 21	Intratumoral injection via endoscopic ultrasound guidance.	Feasible and generally well-tolerated. 2/21 minor responses; 6/21 stable disease; 2/21 partial regression; 11/21 progressive disease or dropped out of study.	[162] [68]
Ad5-yCD/*mut*TK$_{SR39}$*rep*-ADP	E1B55K-deleted; expression of chimeric yCD/*mut*TK$_{SR39}$ and the 11.6K (ADP) genes regulated by the CMV promoter.	Phase I; 8	Endoscopic ultrasound guided intratumoral injection.	Terminated due to poor enrolment.	[99, 163]
Theragene®, Ad5-yCD/*mut*TK$_{SR39}$*rep*-ADP	E1B55K-deleted; expression of chimeric yCD/*mut*TK$_{SR39}$ and the 11.6K (ADP) genes regulated by the CMV promoter.	Phase I; 9	Endoscopic ultrasound guided intratumoral injection.	Currently recruiting patients.	[99, 164]
Ad5-yCD/*mut*TK$_{SR39}$*rep*-hIL12	E1B55K-deleted; expression of chimeric yCD/*mut*TK$_{SR39}$ and single-chain murine IL-12 genes in E3-region under control of the CMV promoter.	Phase I; 9	Endoscopic ultrasound guided intratumoral injection.	Currently recruiting patients.	[100, 165]
AdVince	Human chromogranin A promoter driving E1A expression in neuroendocrine cells; miR122 target sequences to reduce liver toxicity; expression of cell-penetrating peptide in capsid.	Phase I/IIa; 35	Intrahepatic artery infusion.	Currently recruiting patients.	[101, 166]

LOAd703	E1ACR2-, E3gp19K- and E36.7K-deleted; expression of TMZ-CD40L and 4-1BBL under control of the CMV promoter.	Phase I/IIa; 26	Image-guided intratumoral injection.	Currently recruiting patients.	[107, 108, 119, 167, 168]
LOAd703	E1ACR2-, E3gp19K- and E36.7K-deleted; expression of TMZ-CD40L and 4-1BBL under control of the CMV promoter.	Phase I/II; 50	Image-guided intratumoral injection.	Not yet open for patient recruitment.	[107, 108, 119, 167, 169]
VCN-01	E1ACR2-deleted; E1A expression regulated by 8x E2F- and 1x Sp1-binding sites; expression of human hyaluronidase; and retargeted by RGDK insertion in the fibre knob.	Phase I; 18	Intratumoral injection.	Ongoing.	[170, 171]
VCN-01	E1ACR2-deleted; E1A expression regulated by 8x E2F- and 1x Sp1-binding sites; expression of human hyaluronidase; and retargeted by RGDK insertion in the fibre knob.	Phase I; 36	Intravenous injection.	Currently recruiting patients.	[170, 172]
TNFerade ™	E1A-, E1B- and E4-genes deleted and partial E3-deletion. Tumour necrosis factor alpha (TNFα) expression regulated by radiation-sensitive promoter Early Growth Response (Egr-1).	Phase II/III; 304 Randomly assigned 2:1; SOC+TNFerade:SOC.	Intratumoral injection by percutaneous trans-abdominal or endoscopic ultrasound.	Median survival 10.0 months for both treatment arms, n=277. SOC + TNFerade was safe but not effective in patients with locally advanced pancreatic cancer.	[104, 173]

AdV-tk	E1-deleted and partial E3-deletion. The HSV-tk gene expressed from CMV enhancer/promoter with SV40 polyadenylation signal.	Phase I; 27	CT or endoscopic ultrasound guided intratumoral injection.	Safe without toxicity in combination with SOC, n=24. Favourable anti-tumour responses and increased immune responses.	[102, 103]
AdV-tk	E1-deleted and partial E3-deletion. The HSV-tk gene expressed from CMV enhancer/promoter with SV40 polyadenylation signal.	Phase I/II; 44	Intratumoral injection.	Currently recruiting patients.	[103, 174]
ETBX-011	E1-, E2B- and E3-deletions with epitopes of human CEA expressed under control of the CMV promoter.	Phase Ib/II; 80	N/A	Ongoing.	[105, 106, 175, 176]
ETBX-011	E1-, E2B- and E3-deletions with epitopes of human CEA expressed under control of the CMV promoter.	Phase Ib/II; 173	N/A	Currently recruiting patients.	[105, 106, 175, 177]
ETBX-011	E1-, E2B- and E3-deletions with epitopes of human CEA expressed under control of the CMV promoter.	Phase Ib/II; 3	N/A	Ongoing.	[105, 106, 175, 178]
ETBX-011	E1-, E2B- and E3-deletions with epitopes of human CEA expressed under control of the CMV promoter.	Phase Ib/II; 3	N/A	Ongoing.	[105, 106, 175, 179]

Figure Legends

Figure 1. Percentage distribution of pancreatic tumours by anatomical site. A diagram illustrating the structure of the pancreas as well as its anatomical relation to both the duodenum and hepatobiliary tree. Pancreatic tumours can arise at any site within the pancreas and are situated most commonly within the head; tumours originating here have the ability to disrupt hepatobiliary architecture and disturb the passage of duodenal contents.

Figure 2. Illustration of the 36kb adenovirus type 5 (Ad5) genome with selected genes at approximate locations. The first gene to be expressed after viral infection is E1A, which is required for viral genome amplification; protein synthesis; and viral replication. The E1A gene products force the infected cell into S-phase and drive the expression of other early viral genes including E1B, E3 and E4. These genes are essential for preventing premature apoptosis by directly inhibiting the G1/S-checkpoint (E1B); the host antiviral immune defense (E3); and DNA-damage repair mechanisms (E4). The E3 immunomodulatory domain codes for E3-12.5K; E3-6.7K; E3gp19K; the adenovirus death protein (ADP); the E3B proteins RIDα and β; and E3-14.7K — the main functions are indicated in the figure. The viral DNA polymerase (Pol) and the precursor terminal protein (pTP) are encoded by the E2B genes, and the viral DNA-binding protein (DBP) is transcribed from the E2A gene. Both E2A and E2B proteins are essential for viral DNA synthesis. VA-RNAs inhibit the cellular protein kinase R (PKR) that is activated in response to viral infection. Following viral genome synthesis, viral late gene expression is initiated from the major late promoter (MLP). The late genes 1-5 (L1–5) code for structural proteins essential for virion assembly, including penton (L2), hexon (L3), fiber (L5) and the viral protease (L3; Pr). The LITR and RITR indicate the left and right inverted terminal repeats, respectively, and Ψ is the packaging sequence. Transgenes are often inserted to replace one or both of the E1B19K- and E1B55K-genes. More frequently, transgenes are inserted to replace the entire E3-genome or specific E3-genes such as the E3B- or the E3gp19K-genes. * Indicates regions frequently deleted in the viral coding genes to generate oncolytic replication-selective adenoviral mutants (E1ACR2 and E1B55K) and/or enhance the potency (E1B19K and E3gp19K). # Indicates viral promoters frequently exchanged for cancer-specific promoters for selective viral replication in tumours. € Indicates sites used to change cellular tropism of the fiber protein, for example by de-targeting of CAR-binding and retargeting to cancer-specific receptors. See text for examples of viral modifications.
Abbreviations: MHC, major histocompatibility complex; EGFR, epidermal growth factor receptor; TRAIL, TNF-related apoptosis inducing ligand.

Figure 1

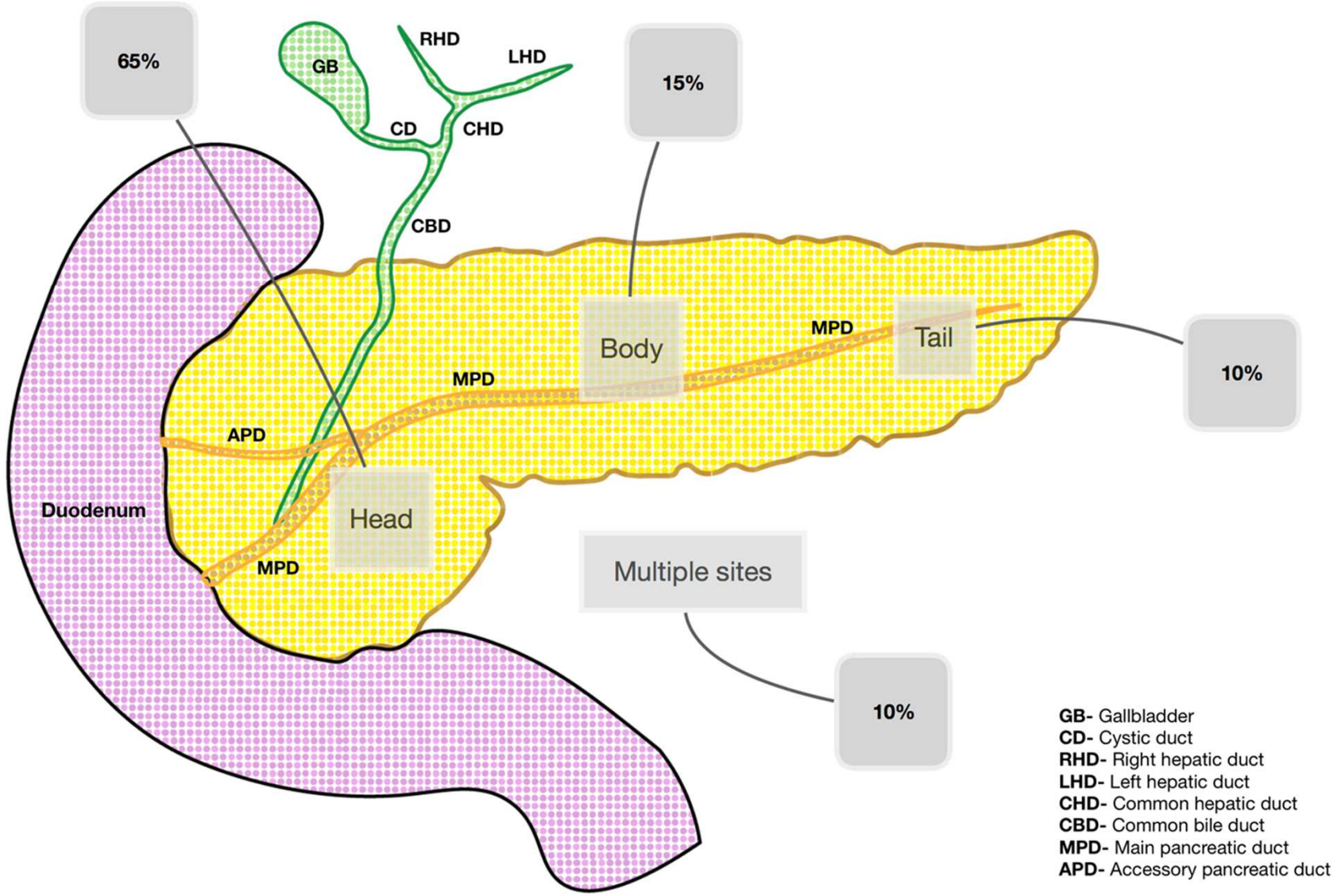
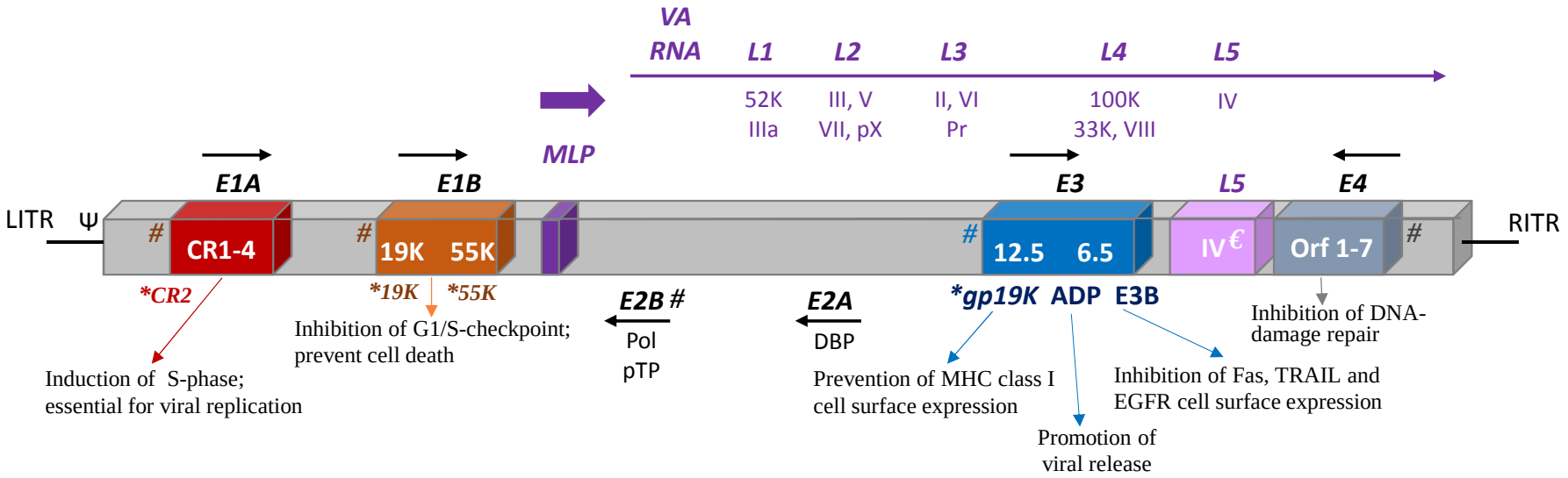

65%
GB
RHD
LHD
CD
CHD
CBD
15%
Body
MPD
Tail
10%
MPD
APD
Duodenum
Head
MPD
Multiple sites
10%
GB- Gallbladder
CD- Cystic duct
RHD- Right hepatic duct
LHD- Left hepatic duct
CHD- Common hepatic duct
CBD- Common bile duct
MPD- Main pancreatic duct
APD- Accessory pancreatic duct

Figure 2


VA RNA
L1
L2
L3
L4
L5
52K
IIIa
III, V
VII, pX
II, VI
Pr
100K
33K, VIII
IV
MLP
E1A
E1B
E3
L5
E4
LITR
Ψ
CR1-4
19K
55K
12.5
6.5
IV €
Orf 1-7
RITR
*CR2
*19K
*55K
E2B #
Pol
pTP
E2A
DBP
*gp19K
ADP
E3B
Induction of S-phase; essential for viral replication
Inhibition of G1/S-checkpoint; prevent cell death
Prevention of MHC class I cell surface expression
Promotion of viral release
Inhibition of Fas, TRAIL and EGFR cell surface expression
Inhibition of DNA-damage repair

Highlights

- Metastatic PDAC is incurable due to the late presentation of symptoms and rapid development of resistance to all current therapeutics. It is a cancer of unmet medical need with no significant improvements in survival over the last 40 years.
- PDAC is characterised by numerous genetic alterations resulting in deregulation of cell cycle and growth control and expression of cell surface proteins that are not present in normal cells. Adenoviral mutants have been engineered to complement and utilise the altered genetic programme in PDAC cells and have emerged as a promising new strategy to overcome drug resistance.
- Adenoviral mutants penetrate the dense tumour stroma that frequently contribute to tumour growth paving the way for drugs and tumour infiltrating immune cells.