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ABOUT COVER

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AIMS AND SCOPE

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The *WJG* is now indexed in Current Contents®/Clinical Medicine, Science Citation Index Expanded (also known as SciSearch®), Journal Citation Reports®, Index Medicus, MEDLINE, PubMed, PubMed Central, and Scopus. The 2021 edition of Journal Citation Report® cites the 2020 impact factor (IF) for *WJG* as 5.742; Journal Citation Indicator: 0.79; IF without journal self cites: 5.590; 5-year IF: 5.044; Ranking: 28 among 92 journals in gastroenterology and hepatology; and Quartile category: Q2. The *WJG*'s CiteScore for 2020 is 6.9 and Scopus CiteScore rank 2020: Gastroenterology is 19/136.

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SYSTEMATIC REVIEWS

Endoscopic ultrasound-guided injectable therapy for pancreatic cancer: A systematic review

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Abstract

BACKGROUND
Given the low survival rate in pancreatic cancer, new therapeutic techniques have been explored, especially for unresectable or borderline resectable disease. Endoscopic ultrasound (EUS) provides real-time imaging and minimally invasive access for local and targeted injection of anti-tumor agents directly into the pancreatic tumor. Limited studies have been reported using this technique for the treatment of pancreatic ductal adenocarcinoma (PDAC).

AIM
To evaluate the progress made with EUS-guided injectable therapies in the treatment of PDAC.

METHODS
All original articles published in English until July 15, 2021, were retrieved *via* a library-assisted literature search from Ovid Evidence-Based Medicine Reviews and Scopus databases. Reference lists were reviewed to identify additional relevant articles. Prospective clinical studies evaluating the use of EUS-guided injectable therapies in PDAC were included. Studies primarily directed at non-EUS injectable therapies and other malignancies were excluded. Retrieved manuscripts were reviewed descriptively with on critical appraisal of published studies based on their methods and outcome measures such as safety, feasibility, and effectiveness in terms of tumor response and survival. Heterogeneity in data outcomes and therapeutic techniques limited the ability to perform comparative statistical analysis.

RESULTS
A total of thirteen articles (503 patients) were found eligible for inclusion. The EUS-injectable therapies used were heterogeneous among the studies consisting of immunotherapy (n = 5) in 59 patients, chemotherapy (n = 1) in 36 patients, and viral and other biological therapies (n = 7) in 408 patients. Eleven of the studies reviewed were single armed while two were double armed with one randomized

trial and one non-randomized comparative study. Overall, the included studies demonstrated EUS-guided injectable therapies to be safe and feasible with different agents as monotherapy or in conjunction with other modalities. Promising results were also observed regarding their efficacy and survival parameters in patients with PDAC.

CONCLUSION
EUS-guided injectable therapies, including immunotherapy, chemotherapy, and viral or other biological therapies have shown minimal adverse events and potential efficacy in the treatment of PDAC. Comparative studies, including controlled trials, are required to confirm these results in order to offer novel EUS-based treatment options for patients with PDAC.

Key Words: Pancreatic ductal adenocarcinoma; Endoscopic ultrasound-guided fine-needle injection; Local injectable therapy; Immunotherapy; Chemotherapy; Oncolytic viral therapy

Core Tip: Pancreatic ductal adenocarcinoma (PDAC) is a highly lethal malignancy. Resistance to systemic therapies may be attributable to the dense stromal matrix in the pancreatic tumor mass. Endoscopic ultrasound-guided fine-needle injection (EUS-FNI) is a novel technique to deliver various anti-tumor agents locally in real-time and may overcome this limitation. This review examines the EUS-FNI therapies used to treat PDAC.

INTRODUCTION

Pancreatic cancer is associated with a 5-year survival rate of approximately 10% at diagnosis and is the seventh leading cause of cancer-related deaths worldwide[1]. Pancreatic ductal adenocarcinoma (PDAC) accounts for more than 90% of cases of pancreatic cancer. The very low survival rate is partly due to the lack of early diagnosis and limited response to systemic therapies[2]. Only 15%-20% of patients present with surgically resectable disease and less than 10% undergo complete resection, which is the only curative intervention[3,4]. The majority of patients who present with unresectable locally advanced pancreatic cancer (LAPC) or metastatic disease are managed with systemic chemotherapy and/or radiotherapy with a very limited prognosis. The introduction of newer chemotherapy combinations and regimens have shown some promising results but still the overall survival (OS) remains dismal[5,6]. One of the many reasons for failure of systemic chemotherapy has been hypothesized as poor delivery of these agents due to abundant stromal matrix and deficient vasculature[7]. This justifies the rationale to explore the use of direct intratumoral injection for targeted delivery of an anti-tumor agent into the tumor mass while minimizing systemic complications. Percutaneous injection of direct intratumoral agents under ultrasound or computed tomography (CT) guidance has been demonstrated to be safe and feasible in phase I trials but this is technically cumbersome and difficult for administering multiple doses[8,9]. Endoscopic ultrasound (EUS) provides the opportunity for real-time visualization of the pancreatic mass and allows minimally invasive access for injectable therapies. This systematic review focuses on the methodology and outcomes of previously published clinical studies on EUS-guided fine needle injection (EUS-FNI) of anti-tumor agents in patients with PDAC.

MATERIALS AND METHODS

Literature search

An expert librarian conducted searches of the Ovid Evidence-Based Medicine Reviews (Embase, MEDLINE, Cochrane Central Register of Controlled Trials, Cochrane Database of Systematic Reviews) and Scopus databases to identify studies published between database inception until July 15, 2021, using the search strategy in Supplementary Table 1. The search was limited to full reports and articles published in English. The titles and abstracts were screened by two independent reviewers (JK and VC) and were assessed for eligibility based on the evaluation of the full manuscript. Disagreements between

the two reviewers were resolved by discussion. Additional studies were identified from searching through references and were screened similarly.

Inclusion criteria

The review is restricted to published prospective studies reporting the effects of injectable interventions primarily using the EUS-FNI technique in patients with PDAC, irrespective of the stage. Therapeutic interventions may include any form of immunotherapy, chemotherapy, or biological agents. Studies that utilized co-interventions and other modes of therapy delivery along with EUS-FNI were included. Studies were eligible if they assessed at least one of the outcomes of interest: Safety, feasibility, and efficacy in terms of tumor response and/or survival.

Exclusion criteria

Benchtop and animal models were excluded as were studies using non-EUS directed therapies. Studies investigating other pancreatic tumors and multiple gastrointestinal cancers where data for the PDAC group was not separately reported were also excluded. Studies were not considered eligible for inclusion if they did not focus on the treatment of PDAC and rather explored the effects of the interventions on palliation and symptom control.

Data extraction

Data was extracted on studies' characteristics of interest- participants, study design, interventions (*e.g.*, therapeutic agent, dosage, and EUS-FNI technique), prior therapies and co-interventions, outcome measures, and results. Relevant data from the included articles were recorded in itemized tables using Microsoft Excel for Microsoft 365 (MSO 16.0.13801.21002) 64 bit.

Outcomes

Outcome parameters of toxicity and clinical efficacy (tumor response and/or survival parameters) were reported as defined by the individual studies. Grade 3-4 AEs included those with severe or life-threatening toxicity.

Statistical analysis

Heterogeneity in data outcomes and therapeutic techniques limited the ability to perform comparative statistical analysis.

Quality assessment

The risk of bias in the included studies was assessed by two independent reviewers (JK, VJ) using the NIH Study Quality Assessment tools for controlled intervention studies and the before-after (pre-post) studies with no control group[10]. These guidelines help to rate the studies as good, fair, or poor based on a set of quality criteria questions. The tools were adapted keeping in mind the nature of the study being reviewed by identifying and reporting some questions as non-applicable as deemed by the reviewers. The results were compared, and any differences were resolved by discussion.

RESULTS

The literature search yielded 101 publications. Title and abstract screening further yielded 30 potentially eligible publications. A full review of manuscripts identified 9 eligible reports along with 4 eligible reports found after backward reference searching leading to a total of 13 full-text articles with 503 patients that were included in the systematic review. The baseline characteristics of these studies are included in Table 1. All were single arm studies except 2, one of which was a randomized controlled trial (RCT) while the other was a non-randomized study. The EUS-injectable therapy administered was heterogeneous among the studies and consisted of immunotherapy (*n* = 5) in 59 patients, chemotherapy (*n* = 1) in 36 patients, and viral and other biological therapies (*n* = 7) in 408 patients.

The quality assessment process identified 11 studies as good and 2 as fair, the details of which are attached in Supplementary Tables 2 and 3.

EUS-guided fine needle injection

Linear array echoendoscopes have facilitated the simultaneous visualization of a target lesion and advancement of a needle from the distal tip of the echoendoscope under precise control to aspirate, inject, or gain access to the organ[11]. This has expanded the role of EUS into the realm of therapeutic interventions with a wide range of applications. EUS-FNI has demonstrated safety and feasibility in applications such as celiac plexus block/neurolysis for the management of pancreas-related pain or pancreatic cyst ablation[12,13]. More recently, its use has been explored for the injection of anti-tumor agents in patients with pancreatic cancer as an attractive method of delivery of such agents considering its minimal invasiveness and low rate of adverse events (AEs)[14].

Table 1 Characteristics of published clinical studies using endoscopic ultrasound-guided fine-needle injection for pancreatic ductal adenocarcinoma

Ref.	Disease	Country	No. of subjects, No. of groups	Study type	EUS-FNI injectable agent	Type of therapy	Aes	Tumor response	Median survival
Chang *et al* [17], 2000	Unresectable PDAC	United States	8, single arm	Phase I	Allogeneic mixed lymphocyte culture	Immunotherapy	DLT-0	Partial remission 25%, minor response 12.5%	13.2 mo (OS)
Irisawa *et al* [21], 2007	Unresectable PDAC refractory to gemcitabine	Japan	7, single arm	Pilot clinical study	Dcs	Immunotherapy	Aes-0	Mixed response 28.6%, stable disease 28.6%	9.9 mo (OS)
Hirooka *et al* [22], 2009	LAPC	Japan	5, single arm	Phase I	OK-432-pulsed dcs	Immunotherapy	Grade 3 or 4 aes-0	Effective response 60% (partial remission 20%, stable disease 40%)	15.9 mo (OS)
Endo *et al*[24], 2012	Resectable PDAC	Japan	24, two arms	Phase I	Idcs and OK-432	Immunotherapy	Grade 3 aes-1	NA	No difference
Hirooka *et al* [25], 2017	LAPC	Japan	15, single arm	Phase I/II	Zoledronate-pulsed dcs	Immunotherapy	DLT-0 (grade 3 aes-4)	Stable disease 46.7%	11.5 mo (OS)
Levy *et al*[27], 2017	Unresectable PDAC	United States	36, single arm	Prospective non-randomized	Gemcitabine	Chemotherapy	Aes-0	Partial response 25%, stable disease 57%	10.4 mo (OS)
Hecht *et al*[31], 2003	Unresectable PDAC without liver metastasis	United States	21, single arm	Phase I/II	ONYX-015	Viral therapy	Aes-8 (four related to the virus and four to the injection technique)	Partial response 10%, stable disease 38%	7.5 mo (OS)
Hecht *et al*[9], 2012	LAPC	United States	50, single arm	Phase I/II	Tnferade Biologic	Viral therapy	DLT-3	Complete response 2%, partial response 6%, minor response 8%, stable disease 24%	297 d (OS)
Herman *et al* [33], 2013	LAPC	United States	304, two arms	Randomized phase III	Tnferade Biologic	Viral therapy	No difference in grade 3 to 4 aes	No difference	10.0 mo (OS) for both arms
Hirooka *et al* [35], 2018	LAPC	Japan	12, single arm	Phase I	HF-10	Viral therapy	DLT-0, Serious aes-2, Grade 3 aes-5	Effective response 78%	5.5 mo (OS)
Lee *et al*[36], 2020	LAPC	South Korea	9, single arm	Phase I	Ad5-DS	Viral therapy	DLT-0	Overall response 11%, disease control rate 100%	11.4 mo (PFS)
Nishimura *et al* [40], 2018	Unresectable PDAC	Japan	6, single arm	Prospective non-randomized	STNM01	RNA oligonucleotide	Aes-0	NA	5.8 mo (OS)
Hanna *et al* [42], 2012	Unresectable PDAC	United States, Israel	6, single arm	Phase I/IIA	BC-819	DNA plasmid	DLT-1	Overall response 33.3% and 66.7% in the two dose cohorts respectively	100% and 66.7% (six-month survival) in the two dose cohorts

AEs: Adverse events; NA: Not available; PDAC: Pancreatic ductal adenocarcinoma; LAPC: Locally advanced pancreatic cancer; iDC: Immature dendritic cell; DLT: Dose-limiting toxicity; OS: Overall survival; PFS: Progression-free survival.

Immunotherapy

Cancer immunotherapy aims to harness the inherent ability of the host immune system to mount an effective anti-tumor response against cancer cells through multiple strategies. Therapeutic cancer vaccines stimulate the activation of cytotoxic T lymphocytes (CTLs) against unique immunogenic tumor antigens by enhancing the delivery of these antigens[15].Targeting immune checkpoint inhibitor molecules aids in disrupting the immune suppressive mechanisms developed by cancer cells to evade immunosurveillance while the adoptive transfer of engineered lymphocytes expressing tumor epitopes or chimeric receptors aims to mediate anti-tumor response[16]. After mixed results with studies employing systemic immunotherapy in PDAC, direct administration with EUS-FNI has been used.

Allogeneic mixed lymphocyte culture: The first reported clinical study that used the novel delivery technique of EUS-FNI as local injectable therapy for PDAC was reported in 2000[17]. It was also the first attempt at administering biological response modifier or cellular-based immune therapy for the treatment of PDAC. The authors used allogeneic mixed lymphocyte culture prepared by coincubation of peripheral blood mononuclear cells from the patient and an allogeneic blood donor to generate a mixed lymphocyte reaction (MLR). It was based on the hypothesis that the MLR results in a high concentration of cytokines within the tumor which upregulates host anti-tumor effector mechanisms to aid in tumor regression. Patients with unresectable PDAC underwent a single session EUS-FNI procedure using 3, 6, and 9 billion cells in a dose-escalation manner. The median OS was documented to be 13.2 mo although there were only 2 partial responses and 1 minor response on either CT or EUS. Dose-limiting toxicity (DLT) was not reached and there were no procedure-related AEs were reported. Low-grade fever was the most common AE but was not associated with leukocytosis and was treated with acetaminophen. There were three grade 3 gastrointestinal toxicities and three grade 3 elevations in bilirubin which were transient and resolved after replacing the preexisting biliary stents in the patients. The encouraging results led to a multicenter RCT comparing EUS-guided injection of the allogeneic mixed lymphocyte culture to conventional IV gemcitabine therapy, but it was not completed as interim results suggested better survival and tumor response in the gemcitabine arm.

Dendritic cells as cancer vaccine: Dendritic cells (DCs) act as potent antigen-presenting cells (APCs) to generate anti-cancer immunity through stimulation of host primary T cell response. Immature or unloaded DCs (iDCs) acquire specific tumor-derived antigens, process them in situ, and migrate to lymphoid organs for presentation to CTLs[18]. Although vaccine strategies for delivering immunogenic tumor antigens themselves as synthetic peptides have been developed for different tumors, these require identification of specific antigens and evidence of their immunogenicity in the tumor microenvironment (TME). Despite autologous tumor cell lysates being used to provide specific tumor antigens in the clinical studies involving cancers with poorly defined antigens, there is evidence to support APCs as potent vaccines to more effectively cross-prime the CTLs with apoptotic tumor cells and apoptotic bodies[19,20]. Considering the difficulty in attaining sufficient quantities of tumor cells for *ex vivo* loading of DCs, it is reasonable to inject iDCs that can get exposed to tumor antigens *in vivo* after administration of apoptosis-inducing therapy like radiotherapy or chemotherapy.

The first report of the use of iDC injection for pancreatic cancer was a pilot clinical study in patients with unsuccessfully treated (gemcitabine resistant) unresectable PDAC[21]. Seven patients underwent cycles of EUS-FNI of unpulsed iDCs in the dose of 10 million cells or more at 2 to 3 sites within the pancreatic mass. iDCs were injected on days 1, 8, and 15 with cycles repeated every 28 d. Five patients received prior radiation therapy to induce apoptosis and facilitate tumor antigen cross-presentation. No procedural AEs were noted, and all DC injections were tolerated without clinical toxicity. Two patients demonstrated a mixed response and two others had stable disease for more than 6 mo. Median patient survival was 9.9 mo despite resistance to gemcitabine.

Hirooka *et al*[22] explored DC-based vaccination as first-line therapy for unresectable LAPC and combined it with gemcitabine based on its known apoptosis-inducing effects. It was postulated to release tumor antigens slowly over time for processing and presentation by DCs. The study used DCs pulsed with OK-432, penicillin killed and lyophilized preparation of a low-virulence strain of Streptococcus pyogenes, which acts as an immunopotentiating agent reported to stimulate DC maturation and T cell activation[23,24]. Conventional lymphokine-activated killer cells stimulated with anti-CD3 monoclonal antibody (CD3-LAKs) were also administrated systemically to induce additional anti-cancer activity. The results showed the combined therapy to be safe and synergistically effective with a median survival of 15.9 mo. Effective radiological tumor response was evidenced in three patients with 1 partial response and 2 long stable diseases for more than 6 mo. Interestingly, the patient with partial remission and the longest survival of 25.4 mo also exhibited significant immunological response with respect to the number of interferon gamma producing cells in peripheral blood lymphocytes (PBLs) and tumor-antigen specific CTL activity.

Based on this study, the authors reported a recent clinical study assessing the safety and efficacy of comprehensive immunotherapy combined with IV gemcitabine as first-line therapy for patients with LAPC[25]. Twelve cycles of EUS-FNI were performed using zoledronate-pulsed DCs rather than the previously used OK-432 pulsed DCs along with systemic administration of adoptive activated T lymphocytes (αβT) and gemcitabine every 14 d. DLT was reported. Grade 3 toxicity was recorded in

four patients, including the two patients that were attributed to gemcitabine. Seven of the 15 patients showed a stable disease tumor response with most showing long-term clinical responses. Patients receiving this therapy were noted to have a higher quality of life assessments as well as the immuno-logical response which was evaluated by the ratio of the number of CD8^{+} T cells to that of regulatory T cells (CD8^{+}/Treg ratio) was found to be significantly higher in patients with stable disease. The median OS and progression-free-survival (PFS) of 15 patients were 12.0 mo and 5.5 mo, respectively. Patients with pre-treatment neutrophil/lymphocyte ratio (NLR) lower than 5.0 demonstrated significantly longer survival. In an analysis limited to patients with an NLR lower than 5.0, the patients whose CD8^{+} /Treg ratio increased more than twofold survived longer. This suggests that using precise biomarkers such as NLR and CD8^{+}/Treg ratio can make comprehensive immunotherapy more beneficial for subgroups of patients with PDAC.

Another study compared 9 patients who received EUS-FNI of iDCs and OK-432 prior to the pancre-atectomy surgery to 15 patients who did not receive this therapy[24]. The intervention group patients also received intra-operative radiotherapy to the retroperitoneal space. There were no severe toxicities following the pre-operative iDC injection except for one transient grade 3 fever. The incidence of postoperative complications was similar in both DC and non-DC groups. Although there was no statist-ically significant difference in OS times of both groups, the authors reported that 2 patients from the DC group, one of which was stage IV with distant lymph node metastasis, survived more than 5 years without requiring adjuvant therapy. Immunohistochemical examination of the surgically dissected lymph nodes revealed significantly higher CD83^{+} cells in the regional lymph nodes and higher accumulated Foxp3^{+} cells in both regional and distant lymph nodes in the DC group. This study not only demonstrated the safety and feasibility of preoperative EUS-FNI but also illustrated the potential of inducing an effective immune response against PDAC.

Chemotherapy

Although systemic chemotherapy forms the basis of standard of care (SOC) treatment for unresectable PDAC primarily LAPC, only one clinical study has so far reported the use of a chemotherapeutic agent being injected locally *via* the EUS-FNI technique.

Gemcitabine

Gemcitabine has been standard therapy for surgically unresectable PDAC since 1997 with an established record of use, safety, and relative benefit when administered intravenously[26]. Given its safety profile, it was selected to be administered in a prospective study conducted at our institution[27]. Patients with locally advanced (n = 20) and metastatic (n = 13) PDAC in whom surgical resection was not performed were included to undergo a single session of EUS-FNI using gemcitabine in the concentration of 38 mg/mL injected *via* a 22-gauge needle. The needle tip was placed 0.5-1.0 cm from the distal tumor edge with injection as the needle retracted proximally to inject approximately 50% of the dose uniformly along the perimeter of the tumor at sites of local infiltration (*e.g.*, blood vessels) and 50% within the remainder of the tumor. Multiple needle passes were performed (median 3, range 1-4) until the injectate was not limited within the tumor but instead began to infiltrate along needle tract or peritumoral sites, leading to varied injection volumes. The median volume of injectate per patient was 2.5 mL (range, 0.7-7.0 mL) corresponding to an intratumoral gemcitabine dose of 95 mg (range, 27-266 mg). Patients underwent subsequent conventional multimodality therapy: Chemoradiotherapy (n = 22), chemotherapy alone (n = 10), no therapy (n = 1), or indeterminate therapy (n = 3). There were no AEs attributable to the EUS-FNI procedure. OS at 6 mo, 12 mo, and 5 years were 78%, 44%, and 3%, respectively. The median OS was 10.4 mo [95% confidence interval (CI): 2.7-68.0]. From the 20 patients with stage III unresectable disease, 4 (20.0%) were downstaged and underwent an R0 resection. Patients who had a more complete therapy based on a visual score showed the greatest increase in median survival (P < 0.0001) with a consistent trend of increasing survival as completeness increased. Although completeness of therapy corresponded to prolonged survival, the significance of this finding is potentially limited by the subjective nature of its assessment.

Oncolytic viral therapy

Oncolytic viruses (OVs) are increasingly being explored as a therapeutic option because of their ability to be engineered for tumor selectivity and express genes of interest within the tumor cells to cause cytotoxic effects and cell death[28,29]. Unlike gene therapy, OVs are replication-competent and propagate within tumor cells, generating infectious progeny that further spreads to surrounding cells after tumor cell lysis. Therefore, in theory, OVs have the potential for efficient oncolysis in solid tumor masses[30].

Onyx-015: ONYX-015 is the first replication selective virus used in clinical trials. It is a chimeric human group C adenovirus with a deletion in the E1B-55kD gene inhibiting p53 function which is already lost in most cancer cells making them susceptible to this agent[31]. In a clinical study of 21 patients with unresectable PDAC, eight sessions of EUS-FNI with ONYX-015 were administered over 8 wk along with systemic gemcitabine therapy. The viral agent was administered in the dosage of 2×10^{10} particles/session (n = 3) and 2×10^{11} particles/session (n = 3) in phase I, and 2×10^{11} particles/session (n = 15) in

phase II of the same study which was the MTD (maximum tolerated dose). EUS-FNI was performed using a transgastric or transduodenal approach with a 22-gauge needle in a fanning pattern during the withdrawal of the needle. Two cases of duodenal perforation that were observed were attributed to the stiff tip of the echoendoscope and thus the protocol was modified only allowing for transgastric FNI. Subsequently, no luminal perforations were noted. Additionally, two cases of sepsis were noted, which may have been related to the injection technique. Thus, the protocol was again modified such that the needles were not fully retracted into the lumen during repositioning and repassage of the needle. Furthermore, the study authors instituted prophylactic administration of oral ciprofloxacin. ONYX-015 itself was well tolerated. Asymptomatic grade 3 and 4 increases in amylase and lipase were detected in 10% of patients, but no clinical pancreatitis was observed. After the combination therapy, objective partial regression of > 50% was seen in 2 out of 21 patients (10%) treated. Two patients demonstrated minor radiographic response to treatment, 6 had stable disease, and the remaining 11 had progressive disease or had to go off study because of treatment toxicity. This study established that EUS guided transgastric injection of ONYX-015 adenovirus into PDAC was both feasible and safe and that such an approach may be extended to other novel biological agents.

TNFerade: TNFerade™ Biologic (also called "AdGVEGR.TNF.11D" or "TNFerade"), is a replication-deficient adenoviral vector for selective delivery of tumor necrosis factor-α (TNF-α) into tumor cells. It consists of Egr-1 promoter gene located upstream to the cDNA which is radiation inducible, hence providing spatial and temporal control of the cytotoxicity by TNF-α[9]. TNF-α may also act as a radiosensitizer and enhance the effect of subsequent radiation therapy[32]. In a multicenter study investigating the safety and feasibility of intratumoral gene therapy with TNFerade Biologic along with standard chemoradiotherapy as first-line treatment for LAPC, subjects were administered either EUS-FNI or percutaneous injection under ultrasound or CT guidance[9]. Twenty-seven patients underwent EUS-FNI and 23 received a percutaneous injection of TNFerade once a week for 5 wk in a dose-escalation manner. A total volume of 2 mL was administered per session of EUS-FNI and injected as four 0.5 mL injections into different areas of the tumor. The maximum tolerated dose was calculated as 4×10^{11} PU after the appearance of DLT of pancreatitis and cholangitis in 3 patients at the highest dose. Overall grade 3 and 4 toxicities included GI bleeding, deep vein thrombosis (DVT), pulmonary emboli, pancreatitis, and cholangitis which had an unclear attributability to the treatment which was therefore concluded to be well tolerated. There occurred 1 complete response (2%), 3 partial responses (6%), 4 minor response (8%) and 12 had stable disease (24%). Seven patients subsequently had an operative resection, 6 of which had clear margins, and 3 had a survival of more than 24 mo. Furthermore, the overall outcome was not influenced by the mode of delivery of TNFerade Biologic (either by EUS or percutaneous injection).

Based on these results, a multicenter RCT was conducted in patients with LAPC who were assigned to receive either TNFerade along with SOC chemoradiotherapy or SOC alone[33]. TNFerade intrat-umoral injection was delivered in the dose of 4×10^{11} PU using a CT or ultrasound-guided percutaneous transabdominal approach (PTA) or EUS-guided transgastric or transduodenal approach before the first fraction of radiotherapy each week for 5 wk. The mode of delivery was based on the discretion of the individual study sites. SOC consisted of continuous infusion 5-fluorouracil and radiotherapy, followed by gemcitabine or gemcitabine plus erlotinib maintenance therapy. The trial was discontinued based on futility after planned interim analysis. Median OS of TNFerade plus SOC *vs* SOC alone (10.0 mo *vs* 10.0 mo; HR: 0.90; 95%CI: 0.66-1.22; *P* = 0.26) and median PFS (6.8 mo *vs* 7.0 mo, respectively; HR: 0.96; 95%CI: 0.69-1.32; *P* = 0.51) were similar in both groups. Multivariate analyses showed that the EUS-FNI approach rather than the percutaneous transabdominal approach was a risk factor for lower PFS (HR: 2.08; 95%CI: 1.06-4.06; *P* = 0.032). Higher rates of definite or probable grade 1 and 2 fever and chills were observed in TNFerade plus SOC *vs* SOC arm alone. Significantly more grade 2 to 4 toxicities were present in the TNFerade plus SOC arm, but this was not dose-limiting suggesting that conditional expression of TNF-α through Egr-1 promoter limits systemic toxicity. TNFerade administration in this study did not prove effective in prolonging survival in patients with LAPC.

HF-10: HF-10 is a spontaneously mutated oncolytic herpes simplex virus-1 reported to have high tumor selectivity and reduced neuro invasiveness[34]. A phase 1 trial published in 2018 evaluated the safety and anti-tumor effectiveness of a triple combination therapy consisting of EUS-guided intratumoral injection of HF-10 along with systemic gemcitabine and erlotinib therapy for unresectable LAPC[35]. Patients underwent twice-weekly HF10 injections to a total of four injections unless DLT appeared. Three cohorts were designed in a dose escalation of 1×10^6, 3×10^6, and 1×10^7 pfu/d. Five patients developed grade III myelosuppression due to chemotherapy and two had serious AEs (perforation of duodenum and grade IV hepatic dysfunction) which were concluded to be unrelated to HF-10. Out of the nine subjects who completed the treatment, the tumor response was three partial responses and four stable diseases. Although the median PFS was relatively short as 6.3 mo, the median OS was 15.5 mo and two patients achieved long-term survival over 3 years. Infiltration of CD4+ or CD8+ cells was well documented in surgical specimens of two patients who ultimately downstaged and underwent surgery, highlighting the idea that oncolytic viruses might not only aid in tumor destruction but may also trigger host anti-tumor response.

Ad5-DS: Ad5-DS is a second-generation, replication-competent oncolytic adenovirus containing double suicide genes that convert prodrugs, 5-fluorocytosine, and valganciclovir, to active cytotoxic metabolites. In a recent Phase I study, nine patients with newly diagnosed LAPC received EUS-guided injection of Ad5-DS with concomitant oral 5- fluorocytosine and valganciclovir along with standard-dose intravenous gemcitabine[36]. The dose cohorts were 1×10^{11}, 3×10^{11}, and 1×10^{12} viral particles (viral particles)/mL. The therapy was reported to be well tolerated no DLT occurred. Tumor response from nine patients who underwent this therapy showed that one patient had a partial response while the other eight had stable disease at 12 wk. The overall response rate was 11%, and the disease control rate was 100%. Disease progression was noted in two patients at 6.5 mo (median PFS of 11.4 mo). Adenoviral DNA was detected in the peripheral blood of 4 patients at 8 wk. Although the trends in tumor size and carbohydrate antigen 19-9 levels seemed more favorable in patients who received higher doses of Ad5-DS, no dose-response relationship was established statistically[37].

Other gene transfer therapies

STNM01: Carbohydrate sulfotransferase 15 (CHST15) is a specific enzyme that has been shown to initiate pancreatic cell mobilization and invasion through its product chondroitin sulphate-E which cleaves CD44 and releases the sCD44 variant into the extracellular space. STNM01 is a synthetic double-stranded RNA oligonucleotide that selectively represses CHST15 expression[38,39]. Six patients with unresectable pancreatic cancer were administered STNM01 *via* a single EUS-FNI procedure with 16 mL (250 nM) injectate using a conventional 22-gauge needle in an open-labeled trial[40]. The agent was injected into 16 different sites within the tumor (1 mL each). Additional STNM01 injections were delivered after 4 wk of observation for AEs and were continued until disease progression occurred. All patients tolerated the procedure well and no AEs were observed. Median tumor size changed from 31 mm to 29 mm along with a significant decrease in median serum soluble CD44 variant, 6 which may reflect CHST15 inhibition and decreased cleavage of CD44, although this finding is limited by the lack of a reference range for sCD44v6 in healthy individuals. Histological evidence of high baseline expression of CHST15 positive cancer cells was noted which showed a large reduction in 2 patients after 4 wk of treatment. Interestingly, these patients also demonstrated tumor necrosis and longest OS (15.5 mo and 18 mo, respectively) indicating that STNM01 acts on CHST15 positive cells to reverse invasion and induce local tumor necrosis although this needs further confirmatory data in future studies.

BC-819: BC-819 is a double-stranded DNA plasmid that carries the cytotoxic gene for diphtheria toxin. Its expression is controlled by the presence of the H19 promoter sequence, which is overexpressed in some tumors like PDAC, leading to selective tumor cell destruction[41]. In a clinical study involving nine patients with unresectable locally advanced PDAC (positive for H19 expression), 2 wk of twice-weekly intratumoral injection of BC-819 under either CT (*n* = 3) or EUS (*n* = 6) guidance was administered[42]. The mode of delivery was determined by the principal investigator depending on tumor size, location, and ease of injection. Injection volumes of 1 mL (4 mg of BC-819) and 2 mL (8 mg of BC-819) were delivered in a dose-escalation manner in the two cohorts using a 21- to a 22-gauge needle in a clockwise alternating injection site scheme for maximum distribution. The treatment was safe and well-tolerated. Asymptomatic elevation of lipase in one patient was considered as DLT but MTD was not reached. Partial response was observed in 3 of the 6 patients treated with the higher dose (8 mg) at three-month follow-up. Resectability assessment showed that two individuals who received chemotherapy or chemoradiation therapy after experimental treatment were down staged to resectable PDAC at three months with one patient subsequently undergoing surgery with negative margins. This indicates that BC-819 may provide additional therapeutic benefits for advanced PDAC along with systemic chemotherapy.

DISCUSSION

Pancreatic cancer remains a highly lethal malignancy. One of the challenges hypothesized with systemic administration of therapeutic agents is their lack of penetration into the pancreatic tumor bed owing to surrounding desmoplasia. Direct injection therapies are an attractive option as they can lead to greater intratumoral concentration of the drug or biologic agent while minimizing systemic side effects. EUS-FNI has emerged as an attractive delivery option as this modality can visualize the tumor and surrounding structures in real-time. This allows for precise intratumoral delivery of biological agents while minimizing the risk for AEs such as avoiding vascular or surrounding structures. Multiple candidate agents for local therapy have been identified.

Successful local delivery of chemotherapeutics is a logical option given their proven safety profile with systemic therapy. Some of these agents have been delivered into normal pancreatic tissue in animal studies using EUS-FNI with no significant AEs[43,44] Results of the clinical study using EUS-guided gemcitabine injections are encouraging although additional data is required to confirm these findings with regulated delivery of standard multimodality therapy and controlled trials assessing the effect of multiple sessions and escalating doses towards significant clinical advantage. In theory, immuno-

therapy is an attractive option, but various challenges with PDAC include the immune-suppressive TME including stromal cellular and molecular components, and other multiple immunological barriers making PDAC a "cold" tumor. Direct delivery of these iDCs into the tumor mass makes tumor antigen loading theoretically more effective for inciting T-cell response mechanisms and has provided a renaissance in the exploration of immunotherapy for PDAC. Although the mentioned limited clinical reports have established safety, feasibility, and some immunological response in their studies, there appears to be a need for increased understanding of the complex immunotherapeutic pathways in PDAC for determining the most efficacious DC activating agent and most suited combination therapy for improving outcomes. Further studies are required to confirm survival benefits, explore synergism with immune checkpoint inhibitors, and select the most appropriate patient population to benefit from these immunotherapies based on precise biomarkers like neutrophil to lymphocyte ratio (NLR) and CD8^{+}/Treg ratio[25] Novel molecular markers may also help in identifying patients with predominantly locoregional complications from PDAC that would benefit from localized therapies.

Oncolytic viral therapy is among the most promising agents for local delivery in pancreatic tumors. Although different strains of adenovirus, herpes virus, measles virus, and other viruses have shown positive results in cancer cell lines and preclinical models, limited clinical studies have performed intratumoral injection of these agents using EUS-FNI[45,46]. As PDAC mass consists of islands of neoplastic cells interspersed with dense stroma which can hinder the spread of injectable agents, OVs can overcome this problem to some extent through their replicative potential and hence increased dissemination within the tumor. Other future areas of research can include viruses targeted towards extracellular matrix disruption and combination with anti-stromal agents to allow better penetration of viral therapy.

EUS-FNI technique can further enhance tumor penetration regardless of tumor cell distribution and composition of the surrounding stroma. Injection into multiple sites in the pancreatic mass using EUS-FNI may assist with even distribution of the agent throughout the tumor. Furthermore, EUS can be used to assess response, and allow for subsequent FNI therapy. EUS, however, is associated with the need for sedation, which may add additional cost and risks associated with sedation. Further studies are needed to compare and firmly establish the most effective EUS-FNI delivery technique including the use of multiple injection sites within the tumor, multiple passes, or use of newer designed needle devices for enhanced dispersion of the agents within the desmoplastic pancreatic stroma[47].

EUS-FNI is an emerging modality for enhanced local intratumoral drug delivery[48]. Current data demonstrate that EUS-guided injectable therapies are safe for the treatment of PDAC. Larger studies, including RCTs should consider using EUS-FNI and these data are needed to establish efficacy and survival data, identify the most suitable anti-tumor agents, including combination therapy, and determine the best patient populations that may benefit from local drug delivery. Regenerative therapies, including the use of immunotherapy, DCs, and oncolytic viruses offer new hope in the management of PDAC. These advances towards novel EUS-FNI therapies should more actively involve endoscopists as part of the multidisciplinary treatment team as we hope to improve survival of our patients with PDAC.

CONCLUSION

EUS-guided injectable therapies, including immunotherapy, chemotherapy, and viral or other biological therapies have shown minimal AEs and potential efficacy in the treatment of PDAC. Comparative studies, including controlled trials, are required to confirm improved survival and establish the most effective therapeutic options. Further research is needed to offer novel EUS-based therapies as a promising treatment for patients with PDAC in the future.

ARTICLE HIGHLIGHTS

Research background

Many new treatment options for pancreatic cancer are being explored owing to its poor prognosis. Advent of therapeutic Endoscopic ultrasound (EUS) guided therapies in recent years paved the way to explore the local delivery of injectable agents. In the last 22 years, very few studies have explored the use of EUS-guided fine-needle injection (EUS-FNI) to treat pancreatic ductal adenocarcinoma (PDAC). These are mostly phase I/II clinical studies using different agents and varied methodologies with mixed results.

Research motivation

EUS-FNI has the theoretical advantage of targeted delivery of anti-tumor agents under real-time visualization and minimal invasiveness. It can also overcome the limitations of systemic therapy mainly the low penetration of these agents into the desmoplastic tumor mass of PDAC. Limited literature and

heterogeneity in methodologies and outcomes necessitated a systematic review of the present literature to understand and guide future research in this promising field.

Research objectives

To evaluate the current status of research in the novel area of EUS-guided injectable treatment for PDAC. This has helped to understand the progress made so far and draw meaningful conclusions based on the limitations and gaps found in the literature. This has also enabled the development of focused future directives for research on this topic which can potentially advance the treatment of PDAC.

Research methods

A systematic and comprehensive review of clinical studies which used EUS-guided injectable therapy for the treatment of PDAC was done. Expert librarian assisted in the electronic search of various databases. Screening of papers for eligibility was done by two study members independently. Data were collected in a standardized manner with regard to the methodologies and outcomes of these studies. A critical appraisal of the present literature on this topic was performed.

Research results

Our study demonstrates that immunotherapy, chemotherapy, oncolytic viral, and other biological therapies have been used *via* EUS-guided injection technique in different ways to study the safety and efficacy of such treatment in PDAC patients. The review of the present literature indicates that these therapies are well tolerated and feasible overall. Mixed results are demonstrated in terms of clinical efficacy.

Research conclusions

This study concludes that EUS-FNI based treatment may be administered to patients with advanced PDAC without significant toxicity. Clinical efficacy with respect to the standard of care (SOC) is not yet established. Further research should be undertaken to find out the most effective therapeutic agent, dose, and techniques that may be employed to the appropriate population of PDAC patients who would benefit the most from these.

Research perspectives

The direction of future research should be to design controlled studies and phase III trials using the data from present literature to establish efficacy in terms of tumor response and survival with respect to the SOC. Anti-tumor agents may be administered at higher doses and multiple EUS-FNI sessions to maintain the appropriate concentration in the tumor bed. Studies using appropriate combination therapies (using chemotherapy and/or radiotherapy) and different EUS-FNI techniques, for example, multiple needle passes should be encouraged as they may help in overcoming hostile tumor microenvironment of pancreatic cancer.

FOOTNOTES

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