

EUS-guided investigational therapy for pancreatic cancer

Mary Lee Krinsky, DO, Kenneth F. Binmoeller, MD

San Diego, California

Pancreatic adenocarcinoma remains a dismal disease because of delays in diagnosis, its aggressive biologic character, and ineffective therapies. Consequently, this disease has become a target for novel therapies such as immunotherapy and gene therapy. EUS has emerged as an attractive imaging modality to guide the delivery of these new therapies. Currently underway are phase I and phase II trials exploring the feasibility and safety of EUS-guided immunotherapy and viral vectors. In addition, ablative techniques such as radiofrequency ablation therapy have been studied in animal models and provide hope for the future. These minimally invasive approaches may lead to a safe alternative for palliation and perhaps cure of unresectable pancreatic malignancies.

PANCREATIC CANCER

Pancreatic cancer is the fourth leading cause of cancer-related deaths in the United States, and its incidence appears to be increasing. It is estimated that 28,300 new cases will be diagnosed in the year 2000.¹ The disease is associated with a median survival of approximately 4 months in untreated patients. Data from the National Cancer Database show the 5-year survival rate after pancreaticoduodenectomy to be 3%.² However, if a pancreaticoduodenectomy for pancreatic head neoplasms achieves clear margins and negative lymph nodes, the 5-year survival rate approaches 25%.³ Aggressive surgery with curative intent is an option in patients with stage I disease. However, the vast majority of patients with pancreatic disease develop clinical symptoms at an advanced stage of disease (stage II or III), when surgical cure is no longer possible. The goal of treatment in these patients is primarily palliative in nature. Chemotherapy, radiation therapy, and a combination of the two may improve overall survival and quality of life. The drawback of both chemotherapy and radiation therapy, however, is their adverse side effects.

From the Division of Gastroenterology, Department of Internal Medicine, University of California, San Diego School of Medicine, and the Department of Medicine, Gastroenterology Section, VA San Diego Healthcare System, San Diego, California.

Reprint requests: Kenneth F. Binmoeller, MD, 200 West Arbor Dr., San Diego, CA 92103-8413.

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Advances in understanding the molecular basis of pancreatic cancer have allowed identification of numerous genetic alterations in oncogenes and tumor suppressor genes. Such targets include p53, K-ras, p16, and DPC-4, BRCA2. These molecular targets are amenable to novel interventional therapy using EUS.

EUS

EUS was developed in the early 1980s to overcome limitations to transabdominal US imaging of the pancreas caused by intervening gas, bone, and fat. The ability to position the transducer in direct proximity to the pancreas via the stomach and duodenum, combined with the use of high-frequency transducers, produces detailed high-resolution images of the pancreas that are far superior to those of CT and transabdominal US.

A logical progression of diagnostic endosonography was fine-needle aspiration (FNA). FNA entails the insertion of a hollow fine needle into the target organ and application of aspiration to procure a tissue sample for cytologic examination. The echoendoscope itself serves as the vehicle to deliver the needle to the target site of puncture. The echoendoscope is positioned in the stomach or duodenum at the optimal site for FNA, and a needle is passed through the instrumentation channel of the echoendoscope under EUS guidance.

The same equipment and accessories that are used for diagnostic FNA can be applied for therapeutic purposes. The injection of agents into the celiac trunk for the palliation of pancreatic pain was described in the previous article. The puncture of a pseudocyst and the placement of a guidewire for stent drainage is another example. An exciting and promising application of EUS technology is the in vivo tissue-targeted delivery of a treatment agent or device (Table 1).

IMMUNOLOGIC THERAPY

An immunologic approach to therapy of tumors uses the activation of host immune effector cells such as cytotoxic T lymphocytes. One way to activate host immune effector cells is by cytokine production. The instillation of cytokines directly within a tumor has been shown to activate immune effector cells and to induce tumor regression. Cytokine production, in turn, can be stimulated by a mixed lympho-

Table 1. Therapeutic agents used in humans in vivo or in vitro for pancreatic neoplasm

Therapeutic agent	Proposed method of action	In vitro/in vivo/humans/+EUS guidance
Immunotherapy (target manipulation) —cytotoxic T lymphocytes	Once activated or cytotoxic T-lymphocyte mucin-specific clones are developed, they induce tumor regression.	Y/Y/Y/Y (phase I clinical study) ⁴⁻⁶
Viral vectors—attenuated adenovirus	Adenovirus lacking E1B protein is replication incompetent and not infectious, but preferentially transduces p53-deficient cells. Provokes tumor destruction by replication and stimulation of an immune response.	Y/Y/Y/Y (phase I clinical study) ^{7,8,16,23}
Gene therapy	Viral or nonviral (liposomes, naked DNA) vectors transfer genetic constructs, which when expressed in tumor may alter their biology.	Y/Y/N/N ^{7,9-14}
Ablative therapy—radiofrequency ablation —other: microwave radiation, laser (laserthermia), and photodynamic therapy	Tissue-induced coagulation induces cell death. Hyperthermia-induced cell death.	N/Y/N/Y ^{18,21,23} Y/Y/Y/N ²⁰⁻²²

cyte reaction generated by the coincubation of host and allogeneic donor peripheral blood mononuclear cells. In a rat model of liver cancer, a derived cytoimplant of mixed lymphocytes was injected directly into the tumor. This was found to confer a survival advantage over the control group (mean survival of 38 days compared with 68 days [$p < 0.02$]).⁴

A further immunologic mechanism to treat patients with pancreatic cancer involves the instillation of mucin-specific cytotoxic T-lymphocyte clones. Cytotoxic T-lymphocyte clones have been developed to mucin, which is expressed on the surface of tumor cells. Once these clones are introduced, they immediately recognize and lyse mucin-bearing tumor cells.⁵

Clinical investigation of immunotherapy by EUS guidance

Chang et al.⁶ reported the first application of EUS to deliver immunologic therapy of pancreatic cancer. A derived cytoimplant of mixed lymphocytes was injected into unresectable pancreatic cancer (stage II, III, IV) in eight patients. A single injection of 3, 6, or 9 billion cytoimplant cells was administered. The injection was performed through a 22-gauge 10 cm FNA needle in conjunction with a curved linear array echoscope. Clinical variable studies included toxicities, serious adverse events, tumor response, Karnofsky performance scale, and survival. Overall, no dose-limiting toxicity was reached, and all toxicities were reversible or self-limited. These included low-grade fevers, nausea, vomiting, transaminase elevations, and hyperbilirubinemia that reversed with biliary stent exchange. Tumor regression occurred in 3 of 8 patients, no change in 3 of 8 patients, and increased growth in 2 of 8 patients. There was no correlation of tumor response with dose or survival. The medi-

an survival was 13.2 months, ranging from 4.2 to more than 36 months.

GENE THERAPY AND ATTENUATED VIRAL VECTORS

Gene therapy involves the transfer of genetic constructs, which alter the neoplastic potential of the cancer cell. Vectors used in gene transfer may be viral and nonviral. Viral vectors are used for gene transfer due to their high transduction efficiency.⁷ Once genetic transfer has taken place, expression of the gene product may alter the biologic behavior of the tumor. This alteration can occur due to blocking transformation of known oncogenes, restoration of tumor suppressor function, or augmentation of the immunologic attack against cancer cells. In addition, viral constructs can be altered to eliminate proteins to create attenuated viruses that are not considered “gene therapy.” However, they replicate specifically in and destroy cancer cells but are not infectious. The viral replication is permitted due to tumor suppressor gene deficiencies, and this replication combined with a provoked immune response may mediate tumor destruction.⁸

Presently, gene therapy of pancreatic cancer is limited to preclinical studies using in vitro and in vivo models. The results from these preclinical studies have been encouraging. One new approach uses gene transfer to enhance the sensitivity of pancreatic tumor cells to recombinant human tumor necrosis factor (TNF). By transfection of a gene for the TNF receptor in combination with high-affinity mutant TNF, both pancreatic cancer cell lines and tumor models demonstrated increased susceptibility to TNF administration.⁹

Furthermore, gene therapy producing cytokines (viral transgene) combined with a viral vector specific for p53-deficient pancreatic cells has been

shown to reduce the volume of tumor in severe combined immunodeficient mice.¹⁰

Preclinical gene therapy studies

A spectrum of gene mutations in pancreatic cancer have been the target of gene therapy. K-ras and p53 have been attractive targets due to their frequent mutation in pancreatic cancer. The retinoblastoma tumor suppressor gene has been shown to be mutated in pancreatic cancer, although with a lower incidence compared with p53 and K-ras.¹¹ When transferred to *in vitro* human pancreatic cancer cells by an adenoviral vector, the retinoblastoma gene was found to inhibit cancer cell growth.¹² Other investigators have used the adenoviral vector to overexpress the p21 tumor suppressor gene to inhibit the proliferation of pancreatic cancer *in vitro*.¹³

There are several reports on the transfer of drug susceptibility genes in experimental models of pancreatic cancer. These genes, once expressed by the cancer cell, render the tumor susceptible to therapeutic agents by a process called directed enzyme pro-drug therapy. The two genes most commonly used are the herpes simplex virus thymidine kinase gene, which increases sensitivity to ganciclovir, and the bacterial enzyme cytosine deaminase, which converts 5-fluorocytosine to the active form 5-fluorouracil.

Clinical investigation of attenuated viral vector administration via EUS guidance

Replacement of mutated p53 with wild-type p53 by retroviral and adenoviral gene therapy has been used in phase 1 clinical trials to treat patients with non-small cell lung cancer.¹⁴ Clinical trials in patients with pancreatic cancer have not been carried out.

Onyx-015 is an attenuated adenovirus that will replicate primarily in nonfunctioning p53 tumor cells. Mutations in the p53 gene have been identified in nearly 70% of pancreatic cancer samples.¹⁶ The University of California Los Angeles and the MD Anderson Cancer Center have combined the molecular alterations of pancreatic adenocarcinoma with the Onyx-15 viral vector technology in a phase I/II study.¹⁷ Twenty-one patients with unresectable pancreatic adenocarcinoma underwent eight sessions of EUS-guided injection of this viral system. This was performed over a 2-month period, and four cycles of Gemcitabine were administered concomitantly with the last four viral injections. Each endoscopic session consisted of numerous injections into the tumor that were dictated by the geometry of the lesion. The injections were performed using a linear array echoscope with a 22-gauge FNA needle. Clinical end

Table 2. Overall response rates in pancreatic adenocarcinoma treated with EUS-guided Onyx-015¹⁶

Response	No.	Percentage
Partial	2	10
Minor	2	10
No change	6	29
Progressive disease	11*	52

*Nine patients with progressive disease, 2 toxicity failures.

points were toxicities, tumor response, and survival. Overall, no dose-limiting toxicity was reached, and hence after the first cohort of three patients, doses were increased tenfold. Bacterial sepsis was noted in two patients, and subsequently prophylactic antibiotics were given to the remaining patients. Transduodenal injections led to two duodenal perforations that were successfully oversewn and resulted in a protocol change to transgastric fine-needle injection only. No clinical pancreatitis was noted; however, numerous patients had elevations in their pancreatic enzymes. All other toxicities were reversible or hematologic and were associated with the chemotherapy. Tumor regression occurred in 4 of the 21 patients, progressive disease in 11, and stable size in 6 (Table 2). Six-month survival occurred in 67% of the patients.

RADIOFREQUENCY ABLATION

Percutaneous ablative techniques have been adapted for the treatment of focal neoplasms, particularly hepatic tumors. These imaging-guided approaches include cryotherapy, microwaves, photodynamic therapy, lasers, alcohol injection, and radiofrequency ablation.¹⁷⁻²¹ EUS-guided radiofrequency ablation has been studied in the normal porcine pancreas.²¹ Complications have included thermal injury to adjacent organs, mild pancreatic enzyme elevations, and a peripancreatic cyst in 1 of the 13 pigs. Surprisingly, pancreatitis was not found to be a significant complication.

DISCUSSION AND FUTURE DIRECTIVES

In pilot studies EUS-guided injection therapy for pancreatic cancer has been well tolerated. The next step is to explore the true efficacy and the optimal dosing. The endosonographic assessment of tumor response is fraught with confounding factors of inflammation in the tumor bed, fibrotic changes, and necrosis. Therefore, a more instructive end point may be quality of life in addition to survival, which is emphasized in contemporary cancer management. This was partially addressed in the cytoimplant study. Furthermore, the Onyx-015 study demonstrated technical difficulties, with two

perforations and two significant infections. Hopefully the implemented protocol changes will lead to safer techniques and fewer complications.

Therapeutic approaches have been evaluated in cell cultures and an animal model, but experience in humans is lacking. Innovative animal models are being developed that use real-time fluorescence of human pancreatic cell growth in nude mice that reflects pancreatic metastasis.²³

EUS-guided therapies are a welcome addition to the endosonographer's interventional armamentarium for pancreatic cancers. These injected agents and devices exploit the rapid advances in molecular and engineering technology. Furthermore, these unique approaches to gene therapy, attenuated viral therapy, and immunotherapy will pave the way for the development of newer treatments, randomized controlled studies, and multimodality regimens for unresectable aggressive, minimally responsive tumors.

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