



## Review Article

## Endoscopic ultrasound-guided immunotherapy



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## A B S T R A C T

Anti-tumoral endoscopic ultrasound-guided fine-needle injection (EUS-FNI), with its minimally invasive access for anti-tumoral agent delivery, is the most exciting field of intervention EUS. Pancreatic cancer is regarded as a systemic disease even if imaging modalities reveal no visible metastasis. From that perspective, immunological therapy is performed. To date, several reports have described immunotherapy under EUS-guidance. The first report of EUS-FNI intended for immunotherapy for advanced pancreatic cancer was published in 2000. In that study, an allogeneic mixed-lymphocyte culture was injected into tumors of eight patients with unresectable local pancreatic adenocarcinoma. The study of dendritic cells (DCs) for cancer has continued to develop in recent years. Actually, DCs are potent antigen-presenting cells for the induction of primary T-cell dependent immune response. When injected intratumorally, DCs acquire and process tumor antigens in situ, migrate to regional lymphoid organs, and initiate a strong tumor-specific immune response. To date, three reports have described EUS-FNI of DCs into pancreatic cancer: two for unresectable and one for pre-surgical operations. Every study has indicated the feasibility and safety. Furthermore, these reports showed that EUS-guided DCs injection might be an important option for treating advanced pancreatic cancer. EUS-guided immunotherapy is a very exciting field in interventional EUS for obstinate cancers.

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## Introduction

Pancreatic cancer is among the most lethal cancers because of its attendant difficulty in early diagnosis and its resistance to standard treatments such as surgical resection, radiation, and chemotherapy. Pancreatic cancer, which entails high mortality in the United States and European countries, is the fifth leading cause of cancer deaths among men and sixth among women in Japan. With 5-year survival of less than 10%, it is the major leading cause of cancer-related death in the world.<sup>1</sup> Therefore, new therapeutic methods are required as an urgent issue.

Interventional endoscopic ultrasound (EUS) is widely performed not only for tissue diagnosis but also for treatment in patients with abnormalities of various organs. Treatment using EUS-guided fine-needle injection (EUS-FNI) has expanded the clinical utility of EUS.<sup>2–7</sup> Antitumoral EUS-FNI, with its minimally invasive access for antitumoral agent delivery, is the most exciting field of interventional EUS today. Several applications of EUS-FNI for anticancer efforts have included drug delivery into the tumors, such as ablation using ethanol, chemotherapy, gene therapy, and cytoimplantation. These procedures are divisible onto three categories based on the associated therapeutic mechanisms: physicochemical therapy, molecular biological therapy, and immunological therapy.<sup>2</sup>

Since Chang et al<sup>8</sup> first reported EUS-FNI for advanced pancreatic cancer in 2000, various antitumoral agents have been injected directly into tumors.<sup>7–14</sup> Recently, several immunotherapies using EUS have been performed using the benefits of EUS guidance: minimally invasive, direct access, and high resolution. As described in this paper, we reviewed EUS-guided oncologic immunotherapy for pancreatic cancer.

## Details of EUS-guided immunotherapy

Pancreatic cancer is regarded as a systemic disease even if imaging modalities reveal no visible metastasis. From this perspective, immunological therapy is performed. To date, four reports in the relevant literature have described immunotherapy under EUS guidance. In particular, EUS-FNI using dendritic cells (DCs) is a promising procedure as immunotherapy for cancers (Table 1).

*Allogenic mixed lymphocyte culture*

Chang et al<sup>8</sup> injected cytoimplants directly into pancreatic cancer and evaluated its technical feasibility and safety in 2000. They used allogeneic mixed lymphocyte cultures (cytoimplants). Cytoimplants were generated by coincubation of host and

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**Table 1 Published Data on EUS-guided Immunotherapy using Dendritic Cells**

Author	No. of patients	Disease	Indication	Combination therapy	Success rate of EUS-FNI	Efficacy/prognosis	Complications
Irisawa et al 2007 <sup>12</sup>	7	Unresectable pancreatic cancer with metastasis	Refractory to intravenous gemcitabine	None	100%	2 mixed response, 2 stable disease >6 mo. Median survival was 9.9 mo.	No severe event
Hirooka et al 2009 <sup>20</sup>	5	Unresectable locally advanced pancreatic cancer	First line treatment	Intravenous gemcitabine	100%	1 partial remission, 2 stable disease >6 mo.	No severe event
Endo et al 2012 <sup>21</sup>	9	Unresectable pancreatic cancer	Prior to surgery	Surgery	100%	1 patient (stage IV) with distant lymph node metastasis survived >6 mo.	No severe event

EUS-FNI, endoscopic ultrasound fine-needle injection.

allogeneic donor peripheral blood mononuclear cells, causing the release of cytokines and the activation of immune effector cells. In this clinical trial, eight patients with unresectable pancreatic cancer received EUS-guided injections of cytoimplant. The first two patients received 3 billion cells, the next three patients received 6 billion cells, and the last three patients received 9 billion cells. Results showed two partial responses and one minor response, with a median survival of 13.2 months. No procedure-related complication occurred. The study demonstrated the possibilities of EUS-FNI based on immunotherapy for unresectable pancreatic cancer. Unfortunately, no active clinical protocol has existed to evaluate cytoimplants for pancreatic cancer for more than 10 years.

#### *Immature DCs for unresectable pancreatic cancer*

Immunotherapy is a novel approach that has been investigated using different types of tumors including pancreatic cancer. During the past 2 decades, adoptive immunotherapy based on tumor-infiltrating lymphocytes or lymphokine-activated killer (LAK) cells has been used in clinical trials.<sup>15,16</sup> These early results provided the first evidence that the manipulation of the immune system represents a promising tool in cancer immunotherapy. Furthermore, Steinman et al<sup>17</sup> discovered the crucial role of DCs for the induction of primary T cell-dependent immune responses. DCs are now regarded as the best adjuvant for antitumor immunity.

The first results for an animal model of pancreatic cancer were reported in 2002 by Akiyama et al.<sup>18</sup> They demonstrated that tumor growth was significantly inhibited by 82% in hamsters treated (subcutaneous injection) using DCs pulsed with tumor lysate and N[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium methylsulfate, and suggested that DC-based immunotherapy might be a useful approach for the treatment of pancreatic cancers. Techniques of interventional EUS permit direct intratumoral injection of DCs. Intratumorally injected DCs would acquire and process tumor antigens *in situ*, migrate to regional lymphoid organs via lymphoid vessels, and initiate a significant tumor specific immune response. In addition, DCs have antigen-capturing abilities and processing abilities as well as trafficking abilities only during their immature phase.<sup>19</sup>

Based on this background, the author performed EUS-FNI of immature DCs into unresectable pancreatic cancer in seven patients with prior chemotherapy (e.g., gemcitabine) failure.<sup>12</sup> Immature DCs were generated by the patients' peripheral blood mononuclear cells after stimulation with granulocyte-macrophage colony-stimulating factor and interleukin 4. The patients received intratumoral injections of 10 billion or more immature DCs at two to three sites using EUS-FNI. Five of the seven patients underwent irradiation prior to the initial DC injection to facilitate tumor-associated antigens for DC cross-presentation. DC was administered on Day 1, Day 8, and Day 15. The cycles were repeated every 28 days to the greatest extent possible. All DC injections were tolerated, with no clinical toxicity. No complication associated with

the procedure was noted. The median patient survival was 9.9 months in spite of being refractory to gemcitabine. Results show that intratumoral injection of unpulsed DCs into the pancreatic cancer using EUS-FNI is safe, and that it can induce some clinical responses in patients with advanced diseases. Later, Hirooka et al<sup>20</sup> reported the results of a combination therapy of gemcitabine with immunotherapy for patients with inoperable, locally advanced pancreatic cancer. Five patients received the treatment course, which consisted of an intravenous gemcitabine administration at 1000 mg/m (Day 1) and the EUS-FNI of OK432-pulsed DCs into a tumor, with subsequent intravenous infusion of LAK cells stimulated with anti-CD3 monoclonal antibody (CD3-LAKs; Day 4), at 2-week intervals. Results showed no severe treatment-related adverse events. Moreover, three of the five patients demonstrated effective responses to this clinical trial: one patient had partial remission; and two patients had long stable disease lasting longer than 6 months. In the patient with partial remission, results demonstrated that DC-based vaccination combined with gemcitabine administration induces tumor antigen-specific cytotoxic T lymphocytes. Consequently, they concluded that the combined therapy is to be regarded as synergistically effective and that it might have a role in the therapy of pancreatic cancer for inducing tumor antigen-specific cytotoxic T lymphocytes.

#### *Immature DCs for resectable pancreatic cancer as neoadjuvant therapy*

Recently, Endo et al<sup>21</sup> reported the feasibility and safety of EUS-FNI using immature DCs with OK-432 into resectable pancreatic cancer prior to the surgical operation as neoadjuvant therapy. Nine patients enrolled in this trial (DC group) were compared with 15 patients who had undergone the operation without immature DC injection (non-DC group). The authors evaluated not only the clinical utility but also the histological changes within the tumor and lymph nodes by immunohistochemical examination of infiltrating inflammatory cells (CD4+, CD8+, Foxp3+, and CD83+). The authors reported that two patients, one of whom was stage IV with distant lymph node metastasis, had remarkably survived more than 5 years without requiring adjuvant therapy. Additionally, they showed that CD83+ cells had accumulated significantly in the regional lymph nodes of the DCs injection group as well as Foxp3+ cells in the regional and distant lymph nodes in comparison with the no DCs injection group. EUS-FNI of immature DCs as neoadjuvant therapy might be useful for improved prognosis in patients with resectable pancreatic cancer.

#### **Conclusion**

EUS-guided intervention has opened new and exciting clinical applications for the management of malignancies. In particular, EUS-guided immunotherapy is an exciting field in interventional EUS for obstinate cancers. For greater development in this field, it is

anticipated that endosonographers, basic scientists, and engineers will collaborate fruitfully with much greater mutual effort. Once an effective therapy becomes available, it is likely to become important for cancer treatment. This effort remains a work in progress.

### Conflicts of interest

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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