



Immunotherapy in Pancreatic Cancer; the Road Less Traveled

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Abstract

Pancreatic cancer shows extremely low responses to chemo- and radiation therapies due to its inherent genetic instability, immunosuppressive microenvironment, and the complex peritumoral stroma. They make the pancreatic cancer most lethal malignancy with 5 year survival of less than 6%, so the need of new therapeutic approaches is urgently demanded. Immunotherapies have shown promises in other multiple cancers by augmenting anti-tumor immunity, so it stood out for the alternative care of pancreatic cancer. However, up to now, immunotherapeutic agents lack efficacy in pancreatic cancer. In this review, we give an overview of immune-related therapeutic strategies, clinical trials in pancreatic cancer and current obstacles that we face. We discuss the ways to overcome those current obstacles here, and by getting over them, we hope to get over the poor prognosis of pancreatic cancer.

Keywords: Immunosuppressive microenvironment; Malignancy; Peritumoral stroma; Pancreatic cancer

Introduction

Pancreatic cancer is the most lethal malignancy with a 5 year survival of less than 6% [1]. In contrast to the increment in other cancer survival, the advance in pancreatic cancer is very slow and despite surgery, locoregional therapy, chemotherapy and radiotherapy, the overall median survival is still less than 1 year from diagnosis. Surgical resection is the only curative treatment, but even after surgery, 5 year survival does not reach to 20% [2]. The reason for low objective response rate to conventional standard therapy is because of the inherent genetic instability of pancreatic cancer cells, immunosuppressive microenvironment at the tumor site, and the complex peritumoral stroma [3,4]. As probe or inhibitor of histone methyltransferase G9a is able to induce cell senescence or death in pancreatic carcinoma, more attention has been drawn to disruption the function of G9a in the treatment of pancreatic adenocarcinoma [5,6]. However, as apart from being as a histone modifier, G9a is also essential for the maintenance of global and loci specific DNA methylation, inhibition of G9a may cause even more severe genetic instability of pancreatic cancer [7]. Therefore, new strategies, such as immunotherapeutic methods, need to be studied in order to eradicate pancreatic carcinoma. Immunotherapy started since the end of the 19th century first by European physician, William Coley, with the idea of recruiting and activating the host's T cells to recognize and attack tumor-specific antigens, by themselves [8,9]. In pancreatic cancer, from animal models, it is known that pre-invasive pancreatic lesions contain high density of immune suppressor cells, but nearly absent immune effector cells, suggesting that antitumor immunity including cytotoxic and adaptive immunity may be already defective [10]. Therefore, reactivating the antitumor immunity in pancreatic cancer has been highlighted as a new therapeutic option for the last several decades. Immunotherapies in pancreatic cancer diverse into 4 main categories; checkpoint inhibitors/immune modulators, therapeutic vaccines, adoptive T cell transfer, and monoclonal antibodies.

Checkpoint Inhibitors/Immune Modulators

When the cancer cells or cancer cells expressing proteins are recognized as antigens, tumor specific T cells are activated. Those cells are controlled by inhibitory and stimulatory signals, called immune checkpoints. The blockade of inhibitory immune checkpoints would induce T cell proliferation and prolong the life of activated T cells, consequently enhances antitumor immune responses [11]. Cytotoxic T-lymphocyte associated protein-4 (CTLA-4) and programmed cell death protein-1 (PD1), CD28 inhibitors are major negative costimulator expressed on activated T cells [12], and have become the first clinical landscape in cancer immunotherapy. Despite their success in certain malignancies, in pancreatic cancer, past phase I and II clinical trials with ipilimumab (CTLA-4 inhibitor), pidilizumab (anti-PD1 mAb), CP-870893 (monoclonal antibody of CD40 receptor), and BMS-936559 (PDL1 antibody) did not prove any effectiveness [13-15]. Due to enhancement of general immunity and temporal immunosuppression, nearly 10% of patients were undergone G3-4 adverse effects, called 'immune-related adverse events (irAEs), including dermatologic, gastrointestinal, hepatic, endocrine, and other less common inflammatory events [16,17]. To overcome those irAEs, dose adjustment and temporal use of systemic corticosteroids are recommended, depends on the severity of events.

Therapeutic Vaccines

Autologous pancreatic cancer cells express several specific antigens, including Wilms' tumor gene 1 (WT1) (75% of patients), mucin 1 (MUC1) (over 85%), human telomerase reverse transcriptase (hTERT) (88%), mutated K-RAS (73%), and carcinoembryonic antigen (CEA) (over 90%) [18-24]. With the idea of administering these tumor antigens to stimulate the patients' own immune system to recognize them, tumor vaccinations have been tested. There are two major categories of therapeutic vaccines; whole-cell vaccines and antigen-specific vaccines. Whole-cell vaccines are made by transfecting human tumor cell lines with or without granulocyte-macrophage colony-stimulating factors (GM-CSF); GVAX (GM-CSF) and Algenpantucel-L

(α -1,3 galactosyl transferase) [25-28]. Despite their promises in post-surgical patients, for metastatic pancreatic cancer patients, they did not show any benefits. Antigen-specific vaccines, peptide vaccines, try to target antigens and other factors expressed by tumor cells; GV1001 (hTERT), Tacemotide (MUC-1), Kras (K-RAS), WT1 (WT1), and KIF-20A (HLA-A24) [29-34]. This approach has not delivered any improvement in overall survival or disease free survival. Nonspecific vaccines like IMM-101 (contains a wide variety of pathogen-associated molecular patterns (PAMPs)), dendritic cells (DC) also tried, but no single agent of vaccines proved its efficacy [35,36].

Adoptive T Cell Therapy

T cells are removed from a patient and genetically engineered to recognize tumor specific proteins, and then re-introduced to the patient aiming to enhance antitumor responses. Chimeric antigen receptors (CARs) are transmembrane proteins comprising an antibody-derived single-chain variable fragment specific for a tumor antigen fused to a hinge region, a spacer, a membrane spanning element and signaling domain [37]. CAR therapy has been an

innovative strategy to redirect T cells against tumors and shows promises in leukemia targeting CD 19 antigen. Up to now, due to less identified potential antigens and its expensive and time-consuming process, the progress in pancreatic cancer is very slow [38]. However, if those problems would be overcome, engineered T cells in CAR therapy would seem to prove its clear ability to kill tumor cells significantly.

Monoclonal Antibodies

Monoclonal antibodies (mAbs) bind to targeted antigens, same epitopes, expressed on tumor cells and play an inhibitory role by preventing formation of antigen-ligand complex [39]. MUC-1, overexpressed in 90% of pancreatic cancer, has been the first target of mAb in pancreatic cancer; PAM4, clivatuzumab. Starting from MUC-1 mAb, endothelial growth factor receptor (EGFR), EGFR2 (HER2), vascular endothelial growth factor (VEGFR), CD40, MUC5a, etc. have been targeted of mAbs, and started clinical trials; no significant improvement in phase III clinical trials was shown, yet [40-46] (Table 1).

Therapeutic category	Investigator	Phase	Target	Agent	Patients/N	Outcomes
Checkpoint inhibitors	Royal et al. [13]	II	CTLA-4	Ipilimumab	Advanced/27	PR: 3.7%
	Anjali et al. [14]	Ib	CTLA-4	Ipilimumab	Advanced/ 13	PR: 15% SD: 38%
	Brahmer et al. [15]	I	PDL-1	Nivolumab	Advanced/14	No objective responses
Therapeutic Vaccines						
Whole cell vaccines	Jaffee et al. [47]	I	GM-CSF vaccine	GVAX with chemotherapy	Resected/ 14	DFS greater than 10 years
	Laheru et al. [48]	II	GM-CSF vaccine	GVAX with cyclophosphamide (Cy)	Advanced/ 50	OS benefit with Cy: 2.4 months
	Lutz et al. [28]	II	GM-CSF vaccine	GVAX with chemoradiotherapy (5-FU)	Resected/ 60	DFS: 17.3 months OS: 24.8 months
	Hardacre et al. [25]	II	Algenpantucel-L	Algenpantucel-L with chemotherapy	Resected/ 70	DFS: 62% OS: 86%
	Lutz et al. [49]	Pilot	GM-CSF vaccine	GVAX with cyclophosphamide (Cy)	Resected/ 54	Vaccine-induced intratumoral tertiary lymphoid aggregation was observed. Upregulation of PD-1 and PD-L1 in the tumor was observed after vaccination.
Antigen-specific vaccines	Abou-Alfa et al. [29]	I	K-ras	Kras	Resected/ 24	DFS: 8.6 months OS: 20.3 months
	Bernhardt et al. [30]	I/II	hTERT	GV1001	Advanced/ 48	OS: 8.6 months
	Middleton et al. [31]	III	hTERT	GV1001 with sequential chemotherapy	Advanced/ 1052	PFS: 4.5%, OS: 6.9%

				GV1001 with concurrent chemotherapy		PFS: 6.6%, OS: 8.4%
				Chemotherapy only		PFS: 6.4%, OS 7.9%
	Ramanathan et al. [50]	I	MUC-1	MUC-1	Advanced/ 16	OS: 12.0 months
	Mayanagi et al. [32]	I	WT1	DC	Advanced/ 32	PFS: 4.2 months OS: 8.1 months
	Asahara et al. [34]	I/II	HLA-A24	KIF20A	Advanced/ 31	PFS: 2.0 months OS: 4.7 months
Adoptive T cell therapy	Beatty et al. [38]	I	Mesothelin	CARTmeso	Advanced/ 2	Acceptable safety in both IV and intratumoral injection
Monoclonal antibodies	Gulec et al. [40]	I	MUC-1	PAM4	Advanced/ 20	PR: 15%, SD: 20%
	Ocean et al. [41]	I	MUC-1	PAM4 with gemcitabine	Advanced/ 38	PR: 15%, SD: 27%
	Philip et al. [42]	III	EGFR	Cetuximab with gemcitabine	Advanced/ 745	PR: 8%, SD: 37%
				Gemcitabine		PR: 7%, SD: 30%
	Assenat et al. [43]	I/II	EGFR/HER2	Cetuximab/Trastuzumab	Advanced, Previously treated/ 38	PFS: 1.8 months OS: 4.6 months
	Kindler et al. [44]	III	VEGFR	Bevacizumab with gemcitabine	Advanced/ 535	CR/PR: 13%, SD: 42%, PFS: 3.8 months, OS: 5.8 months
				Gemcitabine		CR/PR: 10%, SD: 34%, PFS: 2.9 months, OS: 5.9 months
Beatty et al. [45]	I	CD40	CP 807,893 with gemcitabine	Advanced/ 21	PR: 19%, SD: 52% PFS: 5.6 months, OS: 7.4 months	
PR; partial response, SD; stable disease, CR; complete response, PFS; progression free survival, DFS; disease free survival, OS; overall survival						

Table 1: Summary of main clinical trials of immunotherapy in pancreatic cancers.

Challenges in Immunotherapy

Up to now, any single agent of immunotherapies in pancreatic cancer did not prove its efficacy significantly. There are some reasons for that, but mainly it seems to be thought that pancreatic cancer is such a heterogeneous disease, requiring polyvalent approaches [51]. It might be not sufficient to remove the inhibitory immune regulators or to active innate and cytotoxic immunity, alone. In order to bypass the immunosuppressive environments and boost immune responses in pancreatic cancer, an orchestrated approach would be required. Many preclinical studies support evidences for combination of vaccines with immune checkpoint inhibitors, and clinical trials begin to show promises in the treatment of pancreatic cancer; especially clinical trials for the combination of PD-1 inhibitor and GVAX do [52,53]. To make further steps possible, we still face two simple questions; 1) whom to treat, and 2) how to deliver drugs through microenvironment.

Whom to treat

Recent data support that overall response rate to immunotherapy is around 20% [54-60]. That emphasizes the importance to find the right person for immunotherapy who would show good response. There could be two possible approaches; select patients with biomarkers or with cancer status. Eventually, immunotherapy should extend the indications to all stages of pancreatic cancer, but it would be more promising to start with patients of possible minimal residual disease after resection to prevent recurrence with minimal toxicities. Past studies were focused on finding serum biomarkers, but yet, no biomarkers show any benefit. For the GV1001, telomerase peptide vaccine, high eotaxin levels at baseline were once reported to correlate with a longer overall survival, but following studies did not find any correlation between eotaxin levels and overall survival. This distress in finding serum biomarkers may be due to complex peritumoral stroma of pancreatic cancer which separates tumor burden from systemic circulation. If we can stabilize tumor cells obtained by endoscopic or

percutaneous biopsy from patients, and analyze the specific antigens for each patient, we can specify the immunotherapy that compatible to each patient; 'half-individualized immunotherapy'.

How to deliver

Like other failures of chemotherapy, immunotherapy also has the same issue; how to deliver immune regulating drugs or activated T lymphocytes to the cancer. There would be three available approaches to improve; 1) combination with chemotherapy, 2) combination with radiotherapy, and 3) *in situ* immunization. It had been assumed that chemotherapy could not be used in combination with immunotherapy because of its immunosuppressive activities for a long time. However, in the aspects of reducing immune suppressive cells, increasing antigen presenting cells by cytotoxic activities, and alternative mechanisms of immunogenicity, chemotherapy is now recognized as providing additional benefit to immunotherapy. Many preclinical studies support that theme [61-68], and clinical trials begin to show positive results [69-77]. Radiotherapy also was known to have potential to be a good combination therapy with immunotherapy. As we can imagine easily from its 'abscopal effect', radiation can convert the irradiated tumor into an 'immunogenic hub' [78]. Once, tumor have damaged with radiation exposure, cancer cells dye of apoptosis and necrosis, and those dead cancer cells promote uptake by dendritic cells, presentation of tumor-derived antigens to T cells, activation of cytotoxic T cells, exposure of specific proteins on tumor cell surface, and release of ATP and high mobility group protein B1 (HMGB1) [79]. Thus, radiation seems to play an important role in modifying systemic immune system into more suitable conditions for immunotherapy. Concepts of '*in situ* immunization' were started from the idea of how to invade cancer cells directly. This use of immunotherapy show significant antitumor effect in bladder cancer, melanoma, and head and neck cancer [80-82]. For the pancreatic cancer, it is not that difficult to inject drugs into cancer cells with techniques of endoscopic ultrasonography. *In situ* immunization can provide promises to immunotherapy in pancreatic cancer.

Conclusion

In spite of advances in anticancer treatment, pancreatic cancer remains a lethal malignancy. Surgery, locoregional therapies, chemotherapy and radiotherapy could not prove the survival more than 1 year. Regarding to its immune surveillance characteristics, immunotherapy has potentials to treat pancreatic cancer more efficiently. Since 19th century, many advances and clinical trials have done in the field of immunotherapy, but in pancreatic cancer, it is still evolving and unexplored landscape. So far, no significant efficacy was proven in clinical trials of pancreatic cancer and people begin to face obstacles; whom to treat and how to deliver. If we can get the tumor tissue from patients efficiently, it might be possible to choose individualized appropriate target antigens. And combination with chemotherapy or radiotherapy, or *in situ* immunization can improve the efficacy of immunotherapies. There are far ways to go, and many obstacles to overcome, but as we overcome one obstacle, one patient can live one month longer.

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