Summer 8-14-2020

Impact of Immunotherapy on the Survival of Pancreatic Adenocarcinoma Patients: An Analysis of the National Cancer Database

Saber A. Amin
University of Nebraska Medical Center

IMPACT OF IMMUNOTHERAPY ON THE SURVIVAL OF PANCREATIC ADENOCARCINOMA PATIENTS: AN ANALYSIS OF THE NATIONAL CANCER DATABASE

By

Saber Amin M.D., M.B.A

A DISSERTATION

Presented to the Faculty of

the University of Nebraska Graduate College

in Partial Fulfillment of the Requirements

for the Degree of Doctor of Philosophy

Patient-Oriented Research Graduate Program

Under the Supervision of Professor Jane Meza and Chi Lin

University of Nebraska Medical Center

Omaha, Nebraska

June 2020

Supervisory Committee:

Chi Lin, MD, Ph.D.

Jane Meza, Ph.D.

Ward Chambers, MD

Michael Baine, MD, Ph.D.

Laura Bilek, Ph.D.

Acknowledgment

I would like to extend my gratitude to people who have supported me throughout this dissertation research. I am thankful for the support I have received from my advisor Dr. Jane Meza and co-advisor Dr. Chi Lin. They have devoted their time and expertise, and without their guidance, this dissertation would not have been possible. I present my most profound appreciation to Dr. Michael Baine, who helped me and supported me in developing the topic for my dissertation. I am thankful for the help and support that I received from Dr. Ward A Chambers. Thanks to Dr. Laura Bilek, who helped me during my transfer to the MSIA program. I would like to acknowledge the help I received from my friends Morshed Alam and Adam Kusi, at the Department of Biostatistics. I would also like to acknowledge the support I received from Dr. Danstan Baganda at the Department of Anesthesiology.

Especial thanks to my wife Shagofa Amin, who dedicated her time and energy to take care of the children while I was busy with classes and research work. I am grateful to my father, Mohammad Amin. He provided me the opportunity to be the first one in the family to attend college. I am also thankful to my mother, Shagul, for her support and prayers.

Finally, I would like to dedicate this work to Dr. Ward A Chambers and his wife, Suzanne Chambers, for their enormous help and support for my family and me during the last 13 years. They had helped my family and me when we needed help, and without their help, I would not have been able to pursue my Ph. D. They have provided my family and me financial, mental, and emotional support. I wish both a long, happy, and healthy life.

Abstract

IMPACT OF IMMUNOTHERAPY ON THE SURVIVAL OF PANCREATIC CANCER PATIENTS

Saber Ali Amin, Ph.D.

University of Nebraska Medical Center, 2020

Supervisor and co-supervisor: Jane Meza, Ph.D., and Chi Lin, MD, Ph.D.

Pancreatic adenocarcinoma (PDAC) represents 7.2% of all cancer deaths, and by 2030, it will become the second leading cause of death due to cancer. The median overall survival (OS) is 17-23 months in resectable and 4-6 months in metastatic PC [8-9]. The 5-year survival of resectable PC is 22%, and unresectable PC is 8%. A majority of patients treated with standard treatments such as surgery, chemotherapy, and radiation therapy eventually succumb to the disease due to widespread micrometastases at the time of diagnosis. Due to the minimal effect of the current treatments, novel treatment strategies such as immunotherapeutics have been proposed. Immunotherapy has shown excellent efficacy in many other malignancies, but its role in the survival of PC patients is unclear.

The objectives of this dissertation were to investigate the impact of immunotherapy, including the sequence of treatments on the OS of PC patients stratified by definitive surgery of the pancreatic tumor. Data from the National Cancer Database was used to address these objectives. In this study, immunotherapy was associated with improved OS compared to no immunotherapy in both patients who received definitive surgery of the pancreatic tumor and patients who did not undergo surgery. In the surgery group, patients who received

chemotherapy plus immunotherapy or chemoradiation plus immunotherapy had better OS compared to their counterparts without immunotherapy. In the no surgery group, patients who received chemoradiation plus immunotherapy had better OS compared to patients who received chemoradiation without immunotherapy. There was no significant difference in the OS of patients who started immunotherapy 31-90 days before chemotherapy, patients who started immunotherapy 91-180 days before chemotherapy, and patients who started chemotherapy and immunotherapy within 30 days of each other. There was also no significant difference in the OS of patients who started RT> 30 days before the start of immunotherapy, patients who started immunotherapy > 30 days before RT, and patients who started RT and immunotherapy within 30 days of each other. There was no significant difference in the OS of patients who received neoadjuvant immunotherapy and patients who received adjuvant immunotherapy. The study also highlighted the need for improving access to novel treatments as patients with older age, Black race, living in the rural areas, living in the areas with low education level, and diagnosis before 2011 were less likely to receive immunotherapy compared to their counterparts. The findings of the current study warrant future clinical trials of immunotherapy in PDAC patients.

Table of contents

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

CEA	Carcinoembryonic antigen
CBI	checkpoint blockade immunotherapy
CTx	Chemotherapy
CTxRTx	Chemoradiation therapy
CI	Concordance Index
CI	Confidence interval
CTLA4	Cytotoxic T-lymphocyte-associated protein 4
GM-CSF	Granulocyte-macrophage colony-stimulating factor
HR	Hazard ratio
LAK	lymphokine-activated killer
MDSC	Myeloid-derived suppressor cells
NCDB	National Cancer Database
NSCLC	non-small cell lung cancer
OR	Odds ratio
OS	Overall survival
PDAC	Pancreatic adenocarcinoma
PS	Performance status
PD-L1	Programmed death-ligand 1
RT	Radiation therapy
RR	Response rate
SD	Stable disease
TME	Tumor microenvironment

CHAPTER 1

INTRODUCTION
IMMUNOTHERAPY IN PANCREATIC CANCER

Epidemiology and Treatment Challenges

The human pancreas is a large solitary retroperitoneal organ located behind the stomach in the upper abdomen and surrounded by small intestine, liver, and spleen.[1] The pancreas is mainly composed of two portions, the exocrine pancreas, and the endocrine pancreas.[1,2] The exocrine pancreas makes more than 95% of the pancreatic mass and is responsible for producing enzymes that are essential for food digestion.[2] The endocrine cells make hormones such as insulin and glucagon which have a pivotal role in controlling blood glucose level.[2]

The majority of pancreatic tumors arise from the exocrine portion of the pancreas and resemble like pancreatic duct and are therefore called pancreatic ductal adenocarcinoma (PDAC).[2] The tumors arising from the endocrine portion are less common and are called pancreatic neuroendocrine tumors.[2] More than 85% of pancreatic cancers are PDAC.[3] Pancreatic adenocarcinoma is the seventh leading cause of global cancer deaths[4], and the third leading cause of cancer deaths in the USA.[5] Pancreatic adenocarcinoma was ranked the 11th most common cancer globally, accounting for 458,918 new cases and 432,242 deaths worldwide.[4] Each year, more than 53,000 people in the U.S. are diagnosed with PDAC, while more than 34,000 people die from it.[6] Pancreatic cancer represents 3.2% of all cancer cases, but it is responsible for 7.2% of all cancer deaths in the United States.[6] It is predicted that by 2030, PDACC will become the second leading cause of cancer death.[7] The cause of PDAC is complex and multifactorial, but certain factors are associated with the increased risk of PDAC.[8] Smoking

cigarettes is the most important and established risk factors of PC, which increases the risk of PDACC by up to 75%.[9,10] Advanced age, male gender, Black race, family history, and obesity are some other predictors of PDAC.[11-13]

Due to the lack of early detection methods, lack of signs and symptoms, late presentation, disease heterogeneity, and treatment resistance, PDAC is challenging to treat.[14] More than 80% of patients present with locally advanced (non-resectable) or metastatic PDAC, while only 20% present with resectable PDAC.[15] The five-year survival of PDAC is 8% and 22% in non-resectable and resectable patients.[16,17] Surgery is the gold standard and only curative treatment improving overall survival (OS) by ten months compared to no surgery, but more than 80% of the operable patients who undergo curative-intent surgery experience relapse.[18,19] Chemotherapy makes up the backbone of treatment for non-resectable patients, but due to the chemotherapy-resistant characteristic of PDAC, it only improves OS modestly compared to no chemotherapy.[20-22] In addition to chemotherapy, most of the non-resectable PDAC patients also receive radiation to enhance local control.[23]

A majority of patients treated with standard treatments eventually succumb to the disease due to widespread micrometastases at the time of diagnosis.[24] Due to the lack of significant benefits of the currently available treatments, there is a desperate and urgent need to develop novel treatment strategies for pancreatic cancer treatment. Immunotherapy is one of the innovative treatment strategies which has shown great success in the last few years in the treatment of various malignancies, and it is an area of exploration for the treatment of PDAC.

Cancer and Immune System

Immune evasion is one of the emerging hallmarks of cancer. Cancer development and progression is associated with the inability of the immune system to eliminate or control the

growth of cancer cells.[25] Cancer cells can evade immune destruction by modulating their cellular characteristics and, through recruitment and training of the various immune cells and the production of different cytokines and chemokines, by creating an immunosuppressive tumor microenvironment.[26-28] Immunotherapy considered the fourth pillar of cancer treatment, can overcome the immunosuppressive and immune evading properties of cancer cells by reducing the immunomodulatory alterations to cancer cells as well as manipulating the tumor microenvironment, thus allowing for the detection and destruction of cancer cells.[29-31]

Immunotherapy in PC

Types of cancer immunotherapies include checkpoint blockade immunotherapy (CBI), therapeutic cancer vaccines, and non-specific immunotherapies such as cytokines, interleukins, and interferons.[32] Checkpoint blockade immunotherapy (CBI) has been the most widely used immunotherapy to date, the mechanisms of which primarily rely on alteration of immune cell checkpoints, which are manipulated by cancer cells to allow for immune evasion.[33,34] T-cells mediate cellular immunity, which is strictly supervised and controlled by a check and balance system performed through stimulatory and inhibitory receptors.[34] The inhibitory receptors are called immune checkpoints, and their primary role is to maintain self-tolerance and limit tissues damaged during the immune response against pathogenic invasion.[25,34]

Checkpoint receptors are expressed on the surface of cytotoxic T-cells in the form of cytotoxic T lymphocyte-associated 4 (CTLA-4) and on T cells, B cells, natural killer, and dendritic cells in the form of programmed cell death protein 1 (PD-1).[35,36] When activated, these checkpoint receptors downregulate T-cell activation and effector function.[37] Specifically, CTLA-4 binds to B7-1 and B7-2 co-stimulatory molecules on antigen-presenting cells (APCs) and transmits an inhibitory signal to T-cells, which blocks T-cell activation. PD-1 and its ligands,

programmed cell death ligand 1 (PD-L1) and PD-L2, impose inhibitory signals on T-cell activation and proliferation.[37-39] Checkpoint blockade immunotherapy can inhibit CTLA-4 and PD-1 pathways and thus functions by negatively regulating the immuno-inhibitory response, removing the brakes on the immune system.[38]

Literature Overview

CBI first made inroads in cancer in the setting of metastatic melanoma with ipilimumab (an anti-CTLA-4) in 2011 and pembrolizumab (anti-PD1) in 2014.[40,41] Ipilimumab showed improved median survival of 10 months (95% CI: 8.0-13.8) and 1-year OS of 46% (95% CI: 37%-54%) compared to 6 months (95% CI: 5.5-8.7) median survival and 25% (95% CI: 18%-33%) 1-year OS in the comparison arm of a glycol protein gp100 peptide vaccine without ipilimumab. Pembrolizumab showed improved progression-free survival (HR: 0.46, CI: 0.46 to 0.72; P <0.001) and 1-year OS of (HR: 0.69; CI, 0.52 to 0.90; P < 0.0036) compared to ipilimumab. Since this time, the role for immunotherapy has expanded to include advanced melanoma, non-small cell lung cancer (NSCLC), Hodgkin's lymphoma, head and neck cancer, microsatellite instability-high cancer, gastric cancer, advanced renal cell cancer, bladder cancer, liver cancer, and Merkel cell carcinoma.[14,42-45]

Immunotherapy has not been approved for the treatment of PC but is used in an off-label setting mostly extrapolating the utility in various other malignancies.[42,46,47] However, how immunotherapy may fit into the treatment paradigms of pancreatic cancer in PC remains unclear. Immunotherapy has been currently in several clinical trials for PC, but to date, the results have been negative.[42,46,48,49] The findings of previous studies have been summarized below.

Checkpoint Inhibitors

The initial trials of the monotherapy with checkpoint inhibitors in PC failed to show any benefit.[50-54] In a total of 4 clinical trials investigating anti-PDL1 antibodies that included PC patients, results have been mixed with 3 showing no objective response, one showing stable disease, and one showing a 7% objective response rate with 21% of patient realizing disease stability. Importantly, however, PC comprised a small cohort of the included patients with the largest single-trial PC sample size being only having 29 patients. The details are in Table 1.

Synthetic Vaccines

Synthetic vaccines are made of a whole protein or peptide that matches a pre-determined antigen to induce T cell response. There have been various large clinical trials targeting the different immune-related channels, but almost none of them have shown any meaningful efficacy and improvement in OS. The majority of these trials indicated that the treatments were safe and tolerated well but failed to improve OS. The trials were not designed to investigate OS because the majority of them were phase 1 and focused predominantly on the safety and tolerability of these treatments.

Carcinoembryonic antigen (CEA). The phase 1 trials of the mono vaccination therapy targeting Carcinoembryonic antigen (CEA), expressed in 90% of the pancreatic adenocarcinoma, showed that these treatments were well tolerated, and patients had longer OS compared to a historical control group.[55,56] One study of 19 patients with resected or metastatic PC reported stable disease in 5 of the 19 (37%) patients who were alive at 32 months from the initiation of the trial including three patients with metastatic PC.[55] The second study which included two phase 1 studies, investigated the safety of PANVAC-VF that contains genes for CEA, mucin-1, and three costimulatory molecules B7.1, Lymphocyte function-associated Antigen 3 (LFA-3), and

Intra-Cellular Adhesion Molecule-1 (ICAM-1).[56] Overall, 22 patients with stage III-IV PC were enrolled in these two studies. No serious adverse event related to the vaccine was reported. The median OS was 7.9 and 6.3 months, with a one-year survival of 33% and 30%. The median OS in these studies is longer compared to the anticipated median OS of three months based on historical controls for metastatic PC.[56]

Gastrin 17. The trials of mono vaccine therapy in PC that have used gastrin reported positive immune response.[57-59] A randomized, double-blind, placebo-controlled, group-sequential multicenter trial investigated the impact of G17DT (antigastrin immunogen) in PC.[57] The study included 145 patients with advanced PC who were not able to take chemotherapy. The adjusted analysis did not show any difference between the treatment and the placebo group (HR: 0.75, CI; 0.51-1.10, P = 0.138). In unadjusted analysis, the median survival time was 152 days for G17DT and 82 days in the placebo group (P <0.03). In the study participants, more than 74% developed anti G17DT, and they had prolonged survival, 176 days compared to the nonresponders 63 or the placebo 83 days (log-rank test, p < 0.003). The treatment of G17DT was well tolerated.[57]

Another phase 2 study included 30 patients with advanced PC and investigated the antibody response, safety, tolerability, and efficacy of the anti-gastrin-17 or G17DT. In the study, 67% of the participants produced an antibody response. The response was higher in the 200 micrograms 82% compared to 46% in the 100 microgram patients (p <0.01). The median survival was significantly longer in the antibody responders compared to the nonresponders (217 days vs. 121 days, p<0.002).[58] Another trial that included 394 patients with stage II-IV PC did not report any benefit of the antigastrin vaccine.[59] In this study, the antigastrin vaccine was used in combination with gemcitabine.[59]

Table 1. Studies of checkpoint inhibitors alone or in combination with other cancer treatments in PC

Trial	Country and eligibility	Intervention and objective	Sample size	Result
Brahmer *et al*. (2014)[51] NCT00729664	U.S.A. Age>18, the life expectancy of >12 weeks, performance status of <=2, measurable disease, normal hepatic and renal tests	Single-arm: Anti-PDL1 antibody Primary objective: Assess the safety and adverse-event of anti-PD-L1 in advanced cancer patients. Secondary objective: the assessment of the antitumor activity and partial and complete response rate	N=207 with advanced cancers in whom (n=14) were PC but only seven evaluated	No objective responses in PC patients to date
Royal *et al.* (2010)[50] NCT00112580	U.S.A. Age>18, locally advanced or metastatic Stage IV PC, with > 3 months life expectancy, no surgery, no concurrent chemo	Single-arm: Anti-CTLA4 Primary outcome measures: Partial and complete response. Partial response: At least a 30% reduction in the size of all measurable lesions. Complete response: Disappearance of all clinical evidence of disease	Only PC (N=27), 20 metastatic and 7 were locally advanced (unresectable)	No acceptable response rates. A significantly delayed regression of the tumor was noticed in one patient
Patnaik *et al.* (2015)[52] *KEYNOTE-001*	International trial. Age>18, performance status 1 or 0, and normal renal and other organs test. Patients previously treated with PD-1 and patients with the auto-immune disease were excluded	Anti-PD1 monoclonal antibody Evaluate the safety, pharmacokinetics, and pharmacodynamics of pembrolizumab in patients with advanced solid tumors. Also, antitumor activity and maximum tolerated dose	N=30 with solid tumors PC=1	stable disease was reported in the patient
Herbst *et al.* (2014)[53] NCT01375842	U.S.A. Age >18, P.F. of 0 or 1, histologically confirmed diagnosis and adequate or normal functions test. Patients with auto-immune diseases, CNS, HI., Hep A, B, and C were excluded	Anti-PDL1, single-arm dose efficacy study Primary outcome: dose-limiting toxicities, a maximum tolerated dose of Atezolizumab, percentage of participants with adverse events. Secondary outcome: Objective response rate, progressive disease, and progression-free survival	Different types of cancers N=277 PC=5 Only one was evaluated	No positive results for PC, as it was combined to the category of others due to sample size

Segal *et al.* (2014)[54] NCT 01693562 Current trial	U.S.A. and other countries. Adequate organ function and performance status	Anti-PDL1 dose-escalation study Primary endpoint: safety and tolerability Secondary: antitumor activity	N=408 advanced solid tumors PC=29	Disease Control Rate 21% (6/29) and objective response rate of 7% (2/29)
Aglietta *et al.* (2014)[60] NCT00556023	Canada. Age>18, performance status 0 or 1, normal tests of other organs and not having auto-immune diseases and should not have taken ant-CTLA4 before	Anti-CTLA4, dose-escalation, and tolerability study of gemcitabine plus tremelimumab. Primary objective: Evaluating the safety of tremelimumab plus gemcitabine. Secondary purposes: Monitoring for preliminary evidence of efficacy for the combination and evaluation of drug pharmacokinetics	N=34 Metastatic PC	PR: 10.5% (2/19), SD: 7 patients had S.D. at week 10. Only two completed four cycles. OS was 7.4 months (95% CI 5.8–9.4) based on historical data
Kircher *et al. (2016*)[61] NCT01473940	U.S.A. Patients with normal blood and urine tests, P.S. of 0 or 1. No previous treatments with chemo and no auto-immune diseases	Combination of gemcitabine and ipilimumab (CTLA4). Primary outcome: Dose Limiting Toxicities. Secondary outcome: overall survival, progression-free survival, the best overall response	N=13 advanced PC	Response rate=15% (2/13), SD=38% (5/13)
Wainberg *et al*. (2017)[62] NCT02309177	U.S.A. multi-center. Age >18 and no use of the treatment before	Nivolumab With Nab-Paclitaxel Plus or Minus Gemcitabine (PD1 with two chemotherapeutic agents). Objectives: Maximum dose tolerance, OS, disease control rate, progression-free survival	N=17 locally advanced or metastatic PC, Arm A=11, B:6 A: dose-limiting toxicities, Arm B: assess the tolerability, efficacy	RR=29% (5/17), SD=41% (7/17)

PS: performance status RR.: response rate SD: stable disease

Autologous Vaccines. These are cellular-based vaccines in which the patient's dendritic cells are isolated and pulsed with a specific antigen and then reinfused back to the patient. The trials of autologous vaccines of mucin-1 showed mixed results.[63-67] A retrospective study of 17 patients of refractory and metastatic PC that used dendritic cell vaccine combined with activated lymphocyte also reported improved OS.[63] The median OS was nine months, which is longer than the expected survival in these patients. The combination of immunotherapy with chemotherapy did not show any difference in survival compared to immunotherapy alone.[63]

Another phase I/II study of mucin-1 dendritic cell vaccine in resected PC also reported the safety of the vaccine.[64] The study, which included 10 PC patients reported 33% OS of five years [80].[64] Another study investigated the efficacy of mucin-dendritic cell and cytotoxic lymphocyte combination in 20 patients with recurrent and unresectable PC.[65] In this study, one patient had complete remission of lung metastasis, and five had stable disease. The mean survival time was 9.8 months. The one-, two-, and three-year survival rates were 20%, 10%, and 5%.[65]

Another study of the mucin-1 transferred dendritic cell vaccine showed an immune response in the patients, but no survival benefit was observed.[66] The study included ten patients in which more than 90% (9/10) of the patients noticed progress in their disease.[66] A study of adoptive cell transfer mucin-1 vaccine that included 28 patients (8 with unresectable and 20 with resectable PC) reported improved survival.[67] The median survival time was five months in unresectable patients. The median survival time of adjuvant immunotherapy in the resectable patients was 17.8 months. The 1-, 2- and 3-year survival rates after resection were 83%, 32%, and 19%, which is better than the surgery alone.[67] The details of the vaccine studies in PDAC are provided in Table 2.

Table 2. Studies of vaccines alone in PC

Trial	Country and eligibility	Intervention and objective	Sample size	Result
Geynisman *et al.* (2013)[55] NCT00203892	USA: performance status of 0 or 1, enough organ functions, no previous treatment with CEA, no prior systemic therapy	Phase 1 randomized pilot trial primary endpoint: determine the dose of modified CEA peptide (CAP1-6D)/ Montanide/GM-CSF-vaccine to induce an optimal CD8+ T cell response. Secondary point: dose-limiting toxicities, progression-free survival, and median OS	N=23 resectable and advanced PC, 19 received at least one dose	SD: 5/19 (37%) and were alive at 32 months after the randomization
Schuetz (2005)[56] abstract	US A PC patients with localized and metastatic cancer	Primary objective: the safety of PANVAC-VF, which contains the genes for CEA, MUC-1, and three costimulatory molecules (B7.1, LFA-3, and ICAM-1) in 2 viral vectors	N=22, stage IV (N=22) stage III (N=2). There were two studies	Median OS in the two studies was 7.9 and 6.3 months. One-year survival was 33% and 30%
Gilliam *et al.* (2012)[57] *KEYNOTE-001*	A randomized, double-blind, placebo-controlled, international multicenter trial: PC patients with Karnofsky performance score of 60 or higher, the life expectancy of > 2 months	Investigated G17DT in patients unsuitable for or unwilling to take chemotherapy. The primary objective of this study was to determine the effect of G17DT versus placebo on the survival of patients	N=154 with advanced PC, 79 received G17DT and 75 placebo	No difference in adjusted analysis (HR: 0.75, CI; 0.51-1.10, P = 0.138), but unadjusted analysis, the median OS was 152 days for G17DT and 82 days in the placebo group (P <0.03). Those who developed anti-G17DT had 176 days OS compared to the nonresponders 63 or the placebo 83 days (l P = 0.003)

Brett *et al.* (2002)[58] NCT01375842	UK single-center study	Phase II Study of Anti–Gastrin-17 Antibodies. Patients were recruited to receive three doses of 100 _g of G17DT on weeks 0, 2, and 6	N=30 patients with advanced PC	Antibody response was 82% in the 200microgram vs. 46% in the 100 microgram patients (P=0.01). Median OS was 217 days vs. 121 days, P<0.002
Shapiro *et al.* (2016)[59] Abstract	A randomized, double-blind trial. Patients with intact organ function, KPS≥70, and evaluable or measurable disease	Patients were randomized 1:1 to G17DT+Gem or identical matching placebo+Gem in two strata (disease stage II+III vs. IV). G17DT/placebo was administered im at wk 0, 4, 8, and 24; Gemcitabine (1 g/m^2 administered to subjects in both arms	N=394 patients with stage II, III, and IV PC	No positive results.
Nakamura *et al.* (2009)[63]	Japan. A retrospective single hospital-based study	A retrospective study of comparing dendritic cell (DC) vaccine plus an injection of lymphokine-activated killer lymphocytes (LAK) vs. (LAK) alone	N=17 patients with refractory and metastatic PC	OS was nine months in the entire cohort, nine months in the DC group, and d 6 months in LAK. chemo plus immunotherapy vs. immunotherapy alone had the same OS
Lepisto *et al. (2008)*[64]	USA. Patients > 18 years old) with surgically resected pancreatic or biliary tree cancer within 3-24 months of study entry	A phase I/II study of a MUC1 peptide-pulsed autologous dendritic cell vaccine as adjuvant therapy in patients with resected pancreatic and biliary tumors. Exploratory safety study	N=10 resectable PC	The median survival was 26 months (range 13-69 months) for all patients. 33% were alive after five-years

Kondo *et al.* (2008)[65]	Japan. Single hospital-based study	Objective: Evaluate the efficacy of combination mucin-dendritic cell and cytotoxic lymphocyte	N=20 with resectable or recurrence PC	One patient had a complete response. 5 had SD. The mean OS was 9.8 months. The one-, two-, and three-year survival rates were 20%, 10%, and 5%
Pecher *et al.* (2002)[66]	Germany. Single institute-based trial	A phase I/II clinical trial using human autologous DC transfected with cDNA of the human tumor antigen mucin (MUC1). Objective: Evaluate the safety of the vaccine, the induced cellular immune response, and the clinical response	N=10 patients 2 of them were PC	The immunological response was reported, but no benefit in OS was noticed
Kawaoka *et al*. (2008)[67]	Japan. Single institute-based study	Objective: analyze CTLs stimulated by YPK-1 cells and to evaluate the clinical efficacy of AIT with this type of CTL for unresectable and resectable PC	N=28, in which 8 had unresectable PC and 20 had resectable PC	The median OS was five months in unresectable patients. The 1-, 2- and 3-year survival rates after resection were 83%, 32%, and 19%, better than surgery alone. The median OS time of adjuvant immunotherapy in resectable patients was 17.8 months

CEA: carcinoembryonic antigen OS: overall survival PC=pancreatic cancer

Vaccines and Chemotherapy Combination

Mucin-1 vaccines

Studies of the combined use of peptide vaccines of mucin-1 with the SmithKline Beecham adjuvant system (SB-AS) or Incomplete Freund's reported that these treatments were well tolerated and are safe.[68,69] However, these trials did not show benefits in PC patients. The study of the peptide vaccine of mucin-1 with Incomplete Freund's included nine patients in which one patient had stable disease, while 7 developed the progressive disease after the treatment.[68] The study that used peptide vaccine of mucin-1 with SB-AS adjuvant enrolled 16 patients with resected or locally advanced PC without prior chemotherapy or radiotherapy.[69] The study reported an increase in the peripheral T cells post-vaccination. Out of 15 resectable PC patients, 13 died, and only two were alive at follow-up of 32 and 61 months. The median survival time was 12 months, which is comparable with a historic control.[69]

A study used autologous dendritic cells containing mucin-1 with gemcitabine and S1 in the 49 recruited metastatic PC.[70] The participants either received DC vaccine alone or DC vaccine plus lymphokine-activated killer (LAK) in combination with gemcitabine and or S1. Out of 49 patients, two had a complete response, five had partial remission, and 10 had stable disease. Median survival was 360 days, significantly longer than historical control. Patients who received DC vaccine+LAK in combination with chemotherapy had more prolonged survival than those who did not receive LAK (396 days vs. 229 days).[70]

Kras peptide vaccines

A phase I/II study of 38 advanced PC and ten resectable PC, used KRAS peptide vaccines in combination with Granulocyte-Macrophage Colony-Stimulating Factor.[71] Ninety percent of

the resectable and 32% of the unresectable achieved stable disease. Mean OS was 25.6 months in the resectable compared to 16.7 months in the historical control. Mean survival time in the immune responders was significantly longer than the nonresponders (148 days vs. 61 days, $p<0.002$).[71] Another study of 24 patients with resected PC, used the same combination and reported a median recurrence-free survival of 8.6 months (CI: 3.0 –19.2), and a median OS of 20.3 months (CI, 11.6–45.3). The median OS was not different in those who received adjuvant KRAS vaccine and in those who did not.[72]

A study of 39 KRAS mutated patients with resected PC investigated the efficacy of GI-4000, heat-killed recombinant S. cerevisiae plus gemcitabine vs. gemcitabine alone and reported longer median OS (526 days vs. 444 days), more prolonged recurrence-free survival (278 days vs. 255 days), and higher 1-year survival rate (72% vs. 56%) for the combined arm compared to gemcitabine alone.[73] Another study of 23 patients with resected PC reported a median OS of 27.5 months for the entire cohort and a 5-year survival rate of 29% and 22% in those who showed an immune response and those who did not.[74]

Telomerase peptide vaccines

The studies of the telomerase peptide vaccines failed to show any benefit in PC.[75-78] One of the most extensive trials of telomerase vaccine investigated the safety and efficacy of the vaccine in combination with gemcitabine in locally advanced or metastatic PC in the UK.[75] The trials assigned 1062 patients to chemotherapy, sequential chemoimmunotherapy, and concurrent chemoimmunotherapy. The median OS was 7.9 months in the chemotherapy group, 6.9 months in the sequential chemoimmunotherapy, and 8.4 months in the concurrent chemoimmunotherapy group, which was not significantly different from each other. The addition of the telomerase vaccine to chemotherapy did not improve OS.[75]

Two studies that investigated the efficacy of GV1001 in combination with gemcitabine in metastatic PC and were terminated prematurely as the preliminary analyses did not show any survival benefit.[76,77] The preliminary analysis of one study was based on 174 patients and another on 178 patients. Only one study of the telomerase peptide vaccine reported survival benefit.[78] Forty-eight patients with unresected PC were enrolled and received telomerase peptide GV1001 in three dose levels in combination with GM-CSF. The treatment was well tolerated, with no significant grade three adverse events. Vaccine-related immune response was noted in 63% of the patients. The intermediate dose group had significantly longer OS compared to the high and low dose group (8.6 vs. 4.0 vs. 5.1 months). Median survival was significantly longer in the immune responders compared to the nonresponders (7.2 vs. 2.9 months, $p < 0.001$).[78]

GM-CSF vaccines

In phase I trial of GM-CSF enrolled 14 patients with stage I, II, and III of PC[79], three patients had a disease-free survival of 25 months. In another phase II trial of 60 patients with resectable PC, patients received GM-CSF with chemotherapy or chemoradiation and a median disease-free survival of 17.3 months (CI, 14.6–22.8), and a median survival of 24.8 months (95% CI, 21.2–31.6).[80] An open-label study with 50 participants of metastatic PC, patients received GM-CSF alone or in sequence with Cyclophosphamide. The treatments were well tolerated, and a higher rate of Mesothelin-specific T cell responses was reported in the cohort who received chemotherapy prior to immunotherapy, but OS was not improved.[81] The details of the studies of vaccines combined with chemotherapy in PC are provided in Table 3.

Dendritic cells with chemotherapy or radiation therapy

A meta-analysis that included 14 clinical trials, all of which were conducted in China, investigated the efficacy and safety of dendritic cells–cytokine-induced killer (DC–CIK) cells immunotherapy in PC.[82] The study included 1,088 PC patients and the combination of immunotherapy and chemotherapy showed higher partial response rate (OR =1.49, 95% CI =1.06–2.10, *P*=0.02), higher overall response rate (OR =1.69, 95% CI =1.20–2.38, p <0.003), higher disease control rate (OR =2.33, 95% CI =1.63–3.33, *P*,0.001), and low disease progression rate (OR =0.43, 95% CI =0.30–0.61, p<,0.001) compared to chemotherapy alone.[82] The combined therapy also had higher OS compared to chemotherapy alone with the odds ratio of 1-year OS (OR =3.61, CI =2.41–5.40, *P*,0.001) and 3-year OS (OR =2.65, CI =1.56–4.50, p<0.003).

Another meta-analysis that included 11 trials with a total of 413 PC patients investigated the efficacy of dendritic cells (DCs), cytokine-induced killer cells (CIKs), natural killer cells (NKs), lymphokine-activated killer cells (LAKs), and GM-CSF.[83] The 1-year OS was 65% in the immunotherapy arm, which was combined with either radiation therapy or chemotherapy and 45% in the non-immunotherapy arm, which was either radiation therapy or chemotherapy alone. The 1-year OS was significantly improved in the immunotherapy arm ((OR: 2.95; 95% CI:1.64–5.31; p< 0.003) compared to the non-immunotherapy arm.[83] The 3-year OS rate was 38 for the PC patients receiving immunotherapy, while it was 16% for the controls. The 3-year survival was significantly improved in patients who received immunotherapy compared to the control arm (OR: 3.25; CI: 1.37–7.70; p<0.007).[83]

Table 3. Studies of vaccines with chemotherapy in PC

Trial	Country and eligibility	Intervention and objective	sample size	Result
Yamamoto *et al.* (2005)[68]	Japan. PS 0-2, and not treated for four weeks before entering to trial	A phase 1 trial of peptide vaccine of mucin-1 with Incomplete Freund's	N=9 overall, 6 were PC	Seven had progressive disease and 1 SD. There was a tendency for increased circulating anti-MUC1 IgG antibody after vaccination.
Ramanathan *et al.* (2005)[69]	U.S.A, PC patients with resectable or localized tumors. No prior chemotherapy or RT was permitted. Performance status 0-2	a phase I study with a primary clinical objective: Evaluate the toxicity and safety of the MUC1 vaccine with SB-AS adjuvant. Secondary objective: Evaluate the disease-free and overall survival of patients	N=16 patients with resected or locally advanced PC	The median OS was 12 months, and 13 patients died.
Kimura *et al.* (2012)[70]	Finland. A retrospective single hospital-based study	DC-based immunotherapy (DC vaccine alone or DC vaccine plus lymphokine-activated killer [LAK] cell therapy) in combination with the standard chemotherapeutic agents	N=49 metastatic PC	Two patients a CR, 5 had partial remission, and 10 had SD. Median OS was 360 days, longer than historical control. OS was longer in DC vaccine+LAK plus chemotherapy vs. those who did not receive LAK (396 days vs. 229 days)
Gjersten *et al.* (2001)[71]	Norway. Resectable and metastatic PC. Life expectancy > 2 months. No prior chemo or RT within four weeks	Phase I/ II study of KRAS peptide vaccines combined with Granulocyte-Macrophage Colony-Stimulating Factor	N=48, in which ten were resectable, 38 were metastatic PC	90% resectable and 32% of the unresectable achieved SD. Mean OS was 25.6 months in the resectable vs. 16.7 months in the historical control.
Abou-Alfa *et al.* (2011)[72]	South Korea. Age > 18, resectable PC with K-	A pilot study with a primary objective of assessing the safety of	N=24 patients with resectable PC	recurrence-free survival of 8.6 months (CI: 2.96 –19.2),

	RAS mutation. Single institute-based study	immunizing patients against their tumor-specific mutated K-*ras*		and a median OS of 20.3 months (CI, 11.6–45.3).However, the median OS was not different in those who received adjuvant KRAS vaccine and in those who did not
Muscarella *et al.* (2012)[73]	Multi-center phase II	A randomized, placebo-controlled, double-blind, adjuvant trial of the efficacy, immunogenicity, and safety of GI-4000 plus gem versus gem alone in patients with resected pancreas cancer with activating RAS mutations/survival and immunology analysis of the R1 subgroup	N=39 resectable PC, (GI-4000 n=19, Placebo n=20)	The GI-4000 group had an 11.4-week advantage in median overall survival (524 Days vs. 444 Days), a 16% advantage in 1-year survival (72% vs. 56%), and a 4.6-week advantage in median RFS (287 Days vs. 255 days)
Weden *et al. (2011)*[84]	Norway. A retrospective study of two previous trials	K-ras vaccination in resectable PC patients. The objective was to determine long-term survival in these patients	N=23 patients with resected PC	Median OS was 27.5 months for the entire cohort and a 5-year survival rate of 29% and 22% in those who showed an immune response and those who did not
Middleton *et al.* (2014)[75]	U.K. Multi-center phase III trial. Age >18, performance status 0-2, localized or advanced PC	Patients were randomly assigned to receive either chemotherapy alone, chemotherapy with sequential GV1001 (sequential chemoimmunotherapy), or chemotherapy with concurrent GV1001 (concurrent chemoimmunotherapy)	N=1,062 patients with localized or metastatic PC	Median OS was not different in the three groups. 7.9 months, 6.9 months, and 8.4 months in the chemotherapy group, sequential chemoimmunotherapy, and concurrent chemoimmunotherapy
Pharmexa (2008)[76]	International multi-center phase III trial	Objective: Determine the best way to use GV1001 in combination with	N=360 with resectable PC	No survival differences in

		chemotherapy in patients with non-resectable pancreatic cancer. The primary endpoint of the trial is survival, and secondary endpoints include time to progression and safety.		in the GV1001 plus gemcitabine vs. chemotherapy alone
Buanes *et al.* (2008)[77]	Phase III trial. Patients with advanced PC, performance status 0-2	This phase III trial was conducted to determine the impact on overall survival of G monotherapy vs. GV1001 in sequential combination with G in unresectable and metastatic PC. The primary endpoint was OS	N=365 patients with advanced PC	The study ended prematurely after 174 deaths occurred. Median OS was 7.3 / 5.9 months (HR 0.8; 95% CI 0.6–1.0). Median progression-free survival (PFS) was 3.7 / 1.9 months (HR 0.5; 95%CI 0.4–0.7)
Bernhardt *et al.* (2006)[78]	Norway. Phase I/II study. Age > 18, with unresectable PC and adequate liver, renal, and heart functions.	Patients were divided into three groups, given either a low dose (n=11), an intermediate dose (n=17), or a high dose of the vaccine (n=20). Objectives: investigate the safety and tolerability of GV100	N=48 unresectable PC	The median OS was (8.6 vs. 4.0 vs. 5.1 months) for intermediate, high, and low dose. Median survival was significantly longer in the immune responders compared to the nonresponders (7.2 vs. 2.9 months, P <0.001)
Jaffe *et al.* (2001)[79]	U.S.A. Single hospital-based phase I trial. Resectable PC, performance score 0-1, age >18, no metastases, stage 1, 2, and 3	A phase I trial of allogeneic GM-CSF–transduced cancer vaccine composed of these two allogeneic GM-CSF–secreting pancreatic tumor lines. Fourteen patients with stage 1, 2, or 3 with resectable PC to assess the safety and the induction of systemic antitumor immune responses	N=14 PC patients with stage 1, 2, and 3 who received surgery	Three patients had a disease-free survival of 25 months

Lutz *et al.* (2011)[80]	U.S.A. A single hospital-based study of granulocyte-macrophage colony-stimulating factor vaccine	Patients received the vaccine at specified intervals integrated with adjuvant chemoradiotherapy and Chemo. Primary and secondary endpoints: disease-free survival, OS, toxicity, induction of mesothelin-specific T cell responses	N=60 resectable PC.	The median disease-free survival was 17.3 months (95% CI, 14.6–22.8) with a median OS of 24.8 months (95% CI, 21.2–31.6)
Laheru *et al*. (2008)[85]	U.S.A. An open-label pilot study. Patients with histological confirmed PC, and normal liver, renal, and hematological functions.	Two GM-CSF secreting pancreas cancer cell lines (CG8020/CG2505) were administered. Patients received GM-CSF alone or in sequence with Cyclophosphamide. Primary and secondary objective: safety and duration of immunity, time to disease progression (TTP) and median OS	N=50 PC with metastatic disease	OS was not improved. higher rate of Mesothelin-specific T cell responses was reported in the cohort who received chemotherapy prior to immunotherapy

CR: complete response GM-CSF: granulocyte-macrophage colony-stimulating factor

Research Gaps

Overall, the majority of these trials indicated that the various types of vaccines used alone or in combination with chemotherapy were safe and well-tolerated. The data derived from these vaccine trials are promising as multiple have shown survival better than expected from historical controls. The reason that improved OS has been reported in the vaccination trials but not with checkpoint inhibitors may be due to the lack of enough patient numbers, time of the initiation of the drugs, rationale combinations, and selection of patient cohort in the checkpoint inhibitors studies. The lack of response of PC to mono immunotherapy in the initial trials is also partly attributed to the unique immunosuppressive tumor microenvironment of PC, which consists of a dense fibrotic stroma and a scarcity of T cell infiltration.[22,43] Despite the lack of data indicating the survival benefit of immunotherapy in PC[50-53], many patients are prescribed immunotherapy, and many current clinical trials are looking into the efficacy of immunotherapy in PC.[43,49] As of now, no survival data is available to guide clinicians.

However, there is a strong counter-argument that combining immunotherapy with other standard treatments has the potential to amplify the efficacy of immunotherapy in PC. Preclinical and clinical studies have indicated that chemotherapy and radiation therapy induce immunogenic cell death, increase tumor-specific T cell infiltration, decrease Treg cells and suppress Myeloid-derived suppressor cells (MDSC), which immunotherapy can utilize to improve immune response.[22,86-88] In preclinical studies of PC, immunotherapy has elicited tumor regression and improved survival when used in combination with chemotherapy.[88-90] Radiation therapy can also augment the effect of immunotherapy through the abscopal effect. After RT, injury to the tumor cells causes the release of tumor-associated antigens (TAAs), cellular danger-associated molecular patterns (DAMPs), and cytokines, thus stimulating a tumor-specific

immune response and enhancing the traffic of immune cells leading to the elimination of the tumor cells.[91-94]

These preclinical studies led to the design of some of the current clinical trials of immunotherapy combined with chemotherapy and radiation therapy, few of which have reported encouraging preliminary findings.[43,49,60-62,95] A study that enrolled 34 patients with metastatic PC investigated the combination of anti-CTLA4 with gemcitabine and reported a median OS of 7.4 months (CI: 5.8–9.4) longer than the historical data for gemcitabine alone.[60] Another trial of 16 patients with advanced PC, combined gemcitabine with ant-CTLA4 and reported a median progression-free survival (PFS) of 2.5 months (CI 0.8-4.8), and a median OS of 8.5 months (CI 2.2-10.3).[61]

An early-phase trial of anti-PD-1 with 50 patients, investigated the safety of nivolumab in combination with *nab*-paclitaxel (*nab*-P) ± gemcitabine in advanced PC.[62] The median PFS was 5.5 months, and the median OS was 9.9 months. A dose-escalation phase 1 trial which included 22 patients with advanced PC used CD40 agonist combined with gemcitabine reported a median PFS of 5.6 months, and a median OS of 7.4 months (CI: 5.5-12.8), longer than the median PFS of 2.3 and median OS of 5.7 months in Gemcitabine alone.[62] A study of a human chemokine receptor 2 (CCR2) in combination with chemotherapy that included 49 patients reported 49% overall response for CCR2 plus chemotherapy arm with 97% stable disease rate compared to no overall response and 80% of stable disease rate in the chemotherapy alone arm.[95,96]

Immunotherapy can also have a pivotal role in the early stage or resectable PC. Up to date, the utilization of immunotherapy has been in unresectable PC because nothing else has worked and immunotherapy is used a last attempt of treatment.[91,97-100] However, the newer trials are suggesting that patients with localized disease who have a high risk of

micrometastases may also benefit from immunotherapy.[101-105] More than, 60-90% of PC patients develop locoregional or distant recurrence after resection due to occult micrometastases.[106-109] Early-stage PC patients also have low tumor burden and immunotherapy may be a plausible treatment option in these patients.[101,103]

The objective of this research was to use the NCDB database, which captures 70% or more of newly diagnosed cancer cases nationwide, and performs an analysis with more robust patient numbers and investigate if immunotherapy is truly clinically beneficial and use that to help design future clinical trials.

Overall Goal and Specific Aims

The overall goal of this current research is to understand the potential role of immunotherapy in PC survival and determine how to incorporate immunotherapy into the current standard-of-care PC treatment paradigms. We hypothesize that immunotherapy will improve the survival of resectable and unresectable PC and that combining immunotherapy with other treatments may differentially alter the effect of immunotherapy on patient outcomes. The next four chapters will be used to answer research questions related to the use of immunotherapy and its impact on the OS of patients diagnosed with PDAC. The national cancer database (NCDB) was used for answering the research questions. The NCDB, a joint program of the Commission on Cancer of the American College of Surgeons and the American Cancer Society, is one of the largest cancer databases in the world which captures more than 70% of the newly diagnosed cancer cases annually in the United States of America. It is innovative because the majority of the previous studies had low power and included a small number of patients.

This dissertation identifies factors associated with receiving immunotherapy and investigates the impact of immunotherapy, including the treatment sequence on the OS of unresectable and resectable PDAC patients. The specific aims, along with their related research hypotheses and the associated manuscripts are listed below:

Manuscript 1: The impact of immunotherapy on the survival of pancreatic adenocarcinoma patients who do not receive definitive surgery of the tumor

Specific aim 1a: Identify patient and disease characteristics associated with the use of immunotherapy in unresectable PDAC

Hypothesis: Certain demographic and tumor-related factors are associated with the use of immunotherapy

Specific aim 1b: Evaluate the impact of immunotherapy in combination with other standard-of-care treatments on the survival of unresectable PDAC patients

Hypothesis: Combining immunotherapy with RT, chemotherapy, and chemoradiation has a superior impact on OS than these treatments without immunotherapy in resectable PC

Manuscript 2: The impact of immunotherapy on the survival of pancreatic adenocarcinoma patients who received definitive surgery of the pancreatic tumor

Specific Aim 2a: Identify the predictors of receiving immunotherapy in resectable PDAC patients

Hypothesis: Certain demographic and tumor-related factors are associated with the use of immunotherapy

Specific aim 2b: Examine the impact of immunotherapy with other standard-of-care treatments on the survival of resectable PDAC

Hypothesis: Combining immunotherapy with RT, chemotherapy, and chemoradiation has a superior impact on OS than these treatments without immunotherapy in resectable PC

Manuscript 3: The impact of the sequence of immunotherapy on the survival of pancreatic adenocarcinoma patients: a retrospective analysis of the national cancer database

Specific Aim 3a: Identify the treatment sequence of immunotherapy with other standard-of-care treatments on the survival of unresectable PDAC patients

Hypothesis: The OS of patients who start immunotherapy within 30 days of RT or chemotherapy is superior to those who receive treatments more than 30 days of each other in unresectable PDAC

Manuscript 4: The impact of neoadjuvant and adjuvant immunotherapy on the survival of pancreatic cancer patients: a retrospective analysis

Specific Aim 3b: Identify the treatment sequence of immunotherapy with other standard-of-care treatments on the survival of resectable PDAC patients

Hypothesis: The OS of resectable PDAC patients who receive neoadjuvant immunotherapy is improved compared to patients who receive adjuvant immunotherapy

CHAPTER 2

IMMUNOTHERAPY AND THE SURVIVAL OF UNRESECTABLE PANCREATIC CANCER PATIENTS

Abstract

Background and purpose: Immunotherapy has shown excellent efficacy in many cancers, but its role in pancreatic ductal adenocarcinoma (PDAC) remains unclear. The objective of this study is to investigate the impact of immunotherapy on the overall survival of PDAC patients who did not receive surgery of the pancreas using the National Cancer Database (NCDB). **Materials and methods:** Patients with pancreatic adenocarcinoma who did not undergo surgery were identified from NCDB. Cox proportional hazard models were employed to assess the impact of immunotherapy on survival after adjusting for age of diagnosis, race, sex, place of living, income, education, treatment facility type, insurance status, year of diagnosis, and treatment types such as chemotherapy and radiation therapy. **Results:** Of 263,886 patients who were analyzed, 911 (0.35%) received immunotherapy. Among patients who received chemotherapy (101,546), and chemoradiation (30,226) therapy, 555/101,546 (0.55%) received chemotherapy plus immunotherapy, and 299/3,0226 (0.99%) received chemoradiation plus immunotherapy. In a multivariable analysis adjusted for the factors mentioned above, immunotherapy was associated with significantly improved OS (HR: 0.87, CI: 0.80-0.94; P<0.001) compared to no immunotherapy. Chemotherapy plus immunotherapy was significantly associated with improved OS (HR: 0.85, CI: 0.77-0.94; P <0.001) compared to chemotherapy without immunotherapy.Further, chemoradiation plus immunotherapy was associated with significantly improved OS (HR: 0.80, CI: 0.71-0.94; P <0.001) compared to chemoradiation alone. **Conclusion:** In this study, the addition of immunotherapy to chemotherapy and chemoradiation therapy was

associated with significantly improved OS in PDAC patients without definitive surgery. The study warrants further future clinical trials of immunotherapy in PDAC.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) represents 3.2% of all cancer cases, but it is responsible for 7.2% of all cancer deaths in the United States.[6] Each year, more than 53,000 people in the U.S. are diagnosed with PDAC, while more than 34,000 people die from it.[6] It is predicted that by 2030, PDAC will become the second leading cause of cancer death.[7] Due to the lack of early detection methods, lack of early signs and symptoms, late presentation, disease heterogeneity, and treatment resistance, PDAC is challenging to treat.[14] More than 80% of the patients present with locally advanced (non-resectable) or metastatic disease, while only 20% present with resectable cancer.[15] The five-year survival is 8.1% and 22% in non-resectable and resectable PDAC patients.[16,48] Surgery is the only curative treatment of pancreatic cancer (PC) that improves overall survival (OS) by only ten months.[110] Chemotherapy makes up the backbone of treatment for non-resectable patients, but due to the chemotherapy-resistant characteristic of PDAC, it only improves OS from 6 to 11 months.[21,22,111] In addition to chemotherapy, most of the non-resectable patients also receive radiation therapy (RT) to improve local control or prevent future symptoms.[112]

Due to the minimal effect of the current treatments, novel treatment strategies such as immunotherapeutics have been proposed and occasionally used in an off label setting in PDAC, mostly extrapolating the utility in various other malignancies. Many current clinical trials are looking into the efficacy of immunotherapy in PDAC[43,49,113], but no survival data is available to guide clinicians. Despite the lack of data indicating the survival benefit of immunotherapy in PDAC[50-53], many patients are prescribed immunotherapy. The lack of response of PDAC to mono immunotherapy in the initial trials is partly attributed to the unique immunosuppressive tumor microenvironment, which consists of a dense fibrotic stroma and a scarcity of T cell infiltration.[22,113] It is also possible that the negative results were due to the small sample size and

inclusion of heavily pretreated advanced PDAC patients. There is a strong counterargument that combining immunotherapy with other standard treatments has the potential to amplify the efficacy of immunotherapy in PDAC.

Pre-clinical and clinical studies have indicated that chemotherapy and RT induce immunogenic cell death, increase tumor-specific T cell infiltration, decrease Treg cells and suppress Myeloid-derived suppressor cells (MDSC), which immunotherapy can utilize to improve immune response.[22,87,88] In pre-clinical studies of PDAC, immunotherapy has elicited tumor regression and improved survival when used in combination with chemotherapy.[89,90,114] Pre-clinical studies have also found that the combination of RT and targeted Programmed cell death receptor 1, and programmed cell death receptor ligand 1 therapy activates cytotoxic T-cells, reduces MDSC, and induces an abscopal response.[38,114,115] A pre-clinical study demonstrated that RT is synergistic with anti-cytotoxic T-lymphocyte antigen 4 (anti-CTLA-4) antibody and induces systemic anti-tumor responses in a poorly immunogenic carcinoma compared to anti-CTLA-4 monotherapy.[116]

The results of these pre-clinical studies in various cancers have led to the design of some of the current clinical trials of immunotherapy combined with chemotherapy and RT[43,49,113]. Early phase trials of combining immunotherapy, especially checkpoint inhibitors with chemotherapy in pancreatic cancer, have reported some encouraging findings[60-62,95,96]. These trials have reported improved median OS for patients who received checkpoint inhibitors with chemotherapy compared to historical data[60-62,95,96].

The objective of this study is to investigate the impact of immunotherapy on the overall survival of PDAC patients who did not receive definitive surgery of the pancreas using the National Cancer Database (NCDB). This manuscript only includes patients who did not receive

definitive because patients who do or do not receive definitive surgery are two different populations of patients. Patients who receive surgery do significantly better than those who do not undergo surgery. The median survival is 17-23 months in resectable and 4-6 months in nonresectable PDAC[117,118].

Methods

Data Source

The data were extracted from the National Cancer Database (NCDB), which is a joint program of the Commission on Cancer of the American College of Surgeons and the American Cancer Society. It captures 70% or more of newly diagnosed malignancies in the United States annually. Since all patient information in the NCDB database is de-identified, this study was exempt from institutional review board evaluation

Study Population

Patients age 18 or older, diagnosed with PDAC between 2004 and 2016, were included in the study. Patients who received definitive surgery of the tumor, and those who had missing information on RT, chemotherapy, and immunotherapy were excluded. Patients with unknown or missing information about other covariates were not included in the adjusted multivariable analysis. The surgical site-specific code was used to identify patients with definitive surgery of the tumor and exclude them. There was not enough sample size for immunotherapy plus RT vs. RT alone, and therefore the analysis for this group was not performed. The ICD-O-3 histology codes of 8000, 8010, 8020-8022, 8140, 8141, 8211, 8230, 8500, 8521, 8050, 8260, 8441, 8450, 8453, 8470-8473, 8480, 8481, 8503,8250,8440, 8560 were used for defining PDAC.

End Points

The primary outcome was overall survival (OS) calculated from the date of diagnosis to the date of death from any cause. Those alive or lost to follow up were censored at the date of the last contact.

Predictors or explanatory variables

The main predictors of this study were immunotherapy, immunotherapy combined with chemotherapy, and immunotherapy combined with chemoradiation. Age of diagnosis, gender, race, urban and rural living status, income, education, treatment facility type, comorbidity score, insurance status, year of diagnosis, and receipt of chemotherapy, radiation therapy, and immunotherapy were other explanatory variables included in the study.

Statistical Analyses

Descriptive statistics for categorical and continuous variables were reported. Multivariable logistic analysis was performed to identify predictors of receiving immunotherapy, and the odds ratio was reported as the measure of association with the probability of using immunotherapy. Kaplan-Meier curves and log-rank tests were utilized to report the difference in median OS between groups. Multivariable analysis of OS was conducted using Cox proportional hazards regression analysis and estimated hazard ratios with associated 95% confidence intervals (CI). A P-value of 0.05 was used for a significant level, which was based on two-sided tests. Separate multivariable Cox proportional hazard regression models were developed for the hazard ratio of immunotherapy combined with chemotherapy and chemoradiation as these combinations are mutually explosive variables. The SAS 9.4 software was used for the analysis.

To assess the quality and robustness of the final model and prevent overfitting of the logistic and Cox regression models, the final model was validated by splitting the data to testing and validation sets using the bootstrap sampling method. Bootstrap sampling is a statistical

technique for random sampling with replacement and is determined to be an excellent method for model performance. We performed 1,000 bootstrapping samples and compared the concordance index (C-Index) of the bootstrap model with the C-Index of the original model. The C-Index of the original model for multivariable logistic regression analysis was 0.81, and the C-Index after 1,000 bootstraps was 0.81. The C-Index bias for the logistic model was 0.00. The C-Index for the original survival model of immunotherapy was 0.685, and for the survival model after 1,000 bootstraps was 0.685. The C-Index bias for the survival model was 0.00. The details of the original and bootstrap models, including the hazard ratio and C-Index, are provided in supplemental tables 1-5. SAS 9.4 and R 6.2 were used for analysis and bootstrap sampling.

Results

In total, 263,886 patients diagnosed with PDAC between 2004 and 2016 who did not receive definitive surgery met the inclusion criteria and were included for the analysis. Of the 263,886 patients, 911 (0.35%) received immunotherapy. Among patients who received chemotherapy (101,546), RT (5,111), and chemoradiation (30,226) therapy, 555/101,546 (0.55%) received chemotherapy plus immunotherapy, 9/5,111 (0.18%) received RT plus immunotherapy, and 299/30,226 (0.99%) received chemoradiation plus immunotherapy. The median age was 71.00, with a range of (18.0-90.0) years. The majority of patients were White, insured, living in the urban areas, had Charlson/Deyo Score of zero, had a high school degree, had income >=$35,000, and received chemotherapy. In the multivariable logistic analysis, older age, black race, no insurance, Charlson/Deyo Score of 1 and 2, community hospital, being less educated, diagnosed before 2011, not receiving chemotherapy, and not receiving RT were all less likely to receive immunotherapy compared to their counterparts (Table 4).

Based on results from the Kaplan Meier curves, patients who received immunotherapy had significantly improved median overall survival compared to patients who did not receive

immunotherapy (Figure 1a) with an absolute median OS benefit of 6.33 [10.60 vs. 4.27; p <0.0001] months. Subset analysis revealed that patients who received chemotherapy plus immunotherapy had significantly improved median OS compared to those who receive chemotherapy alone (Figure 1b) with an absolute median OS benefit of 2.33 [9.30 VS. 6.97; p <0.0001] months. Similarly, patients who received chemoradiation plus immunotherapy had significantly improved median OS compared to patients who received only chemoradiation (Figure 1c) with an absolute median OS benefit of 3.38 [14.42 vs. 11.04; p <0.0001] months.

In univariate Cox Proportional analysis (Table 5), immunotherapy was associated with significantly improved OS with a hazard ratio (HR) of 0.59 (CI: 0.55-0.64.1; P <0.0001). Significantly improved OS was also noticed in Immunotherapy plus chemotherapy vs. chemotherapy alone (HR: 0.82, CI: 0.75-0.90; P <0.0001), and immunotherapy plus chemoradiation vs. chemoradiation alone (HR: 0.74, CI: 0.65-0.83; P <0.0001). In the univariate Cox analysis, older age, low education, low income, treatment at a community hospital, Charlson/Deyo Score of 1 and 2, diagnosis before 2011, not receiving RT, and not receiving chemotherapy were all associated with significantly decreased OS, while Black race and non-white non-black race were associated with significantly improved OS.

In the multivariable Cox proportional hazard analysis (Table 5), receipt of immunotherapy, female sex, and the non-white non-black race was associated with significantly improved OS, while older age, low income, treatment at a community hospital, Charlson/Deyo of one and two, diagnosis before 2011, not receiving chemotherapy, and not receiving RT were associated with significantly decreased OS. In the multivariable analysis adjusted for all the above factors, immunotherapy was associated with significantly improved OS (HR: 0.88, CI: 0.81-0.95; P<0.0001) compared to no immunotherapy. The results stayed the same when patients with no treatments were excluded from the analysis. Treatment with chemotherapy plus

immunotherapy was significantly associated with improved OS (HR: 0.86, CI: 0.78-0.98; P <0.001) compared to chemotherapy without immunotherapy. Further, chemoradiation plus immunotherapy was associated with significantly improved OS (HR: 0.80, CI: 0.70-0.92; P <0.004) compared to chemoradiation alone. Both models were adjusted for the same factors mentioned previously. The one- and two-year survival rate was 60% (CI: 54%-66%) and 23% (CI: 18%-28%) for chemoradiation plus immunotherapy, 37% (CI: 33%-42%) and 11% (CI: 8%-13%) for chemotherapy plus immunotherapy, 45% (CI: 45%-46%) and 14% (CI: 13%-14%) for chemoradiation alone, and 28% (CI: 27%-28%) and 9% (CI: 8%-9%) for chemotherapy alone. Table 6 has the results of the univariate and multivariable analysis.

Table 4. Multivariable logistic analysis of the factors associated with the receipt of immunotherapy in PDAC patients with no surgery

Variable		Immunotherapy 911 (0.35%)	No Immunotherapy 262,975 (99.65%)	Total 263,886	Odds Ratio	95% CI	P
Age at diagnosis, Median (range)		64.00 (21-90)	71.00 (18-90)	263,886	0.97	0.97-0.98	0.0001
Sex	Male	497 (54.56)	131,965 (51.18)	132,462 (50.20)	1	Reference	
	Female	414 (45.44)	131,010 (49.82)	131,424 (49.80)		NS	0.331
Race	White	784 (87.21)	217,747 (83.77)	218,531 (83.78)	1	Reference	
	Black	75 (8.34)	33,124 (12.74)	33,199 (12.73)	0.66	0.52-0.85	0.002
	Other	40 (4.45)	9,067 (3.49)	9,107 (3.49)	1.08	0.76-1.54	0.68
	Unknown	12	3,037	3,049			
Education	>=13% HG	317 (35.11)	114,060 (43.55)	114377 (43.52)	0.77	0.66-0.90	0.001
	<13%	586 (64.89)	147,832 (56.45)	148,418 (56.48)	1	Reference	
	Unknown	8	1,083	1,091			
Income	>=$35,000	593 (65.74)	152,161 (58.13)	152,754 (58.16)	1	Reference	
	<35,000	309 (34.26)	109,590 (41.87)	109,899 (41.84)		NS	0.52
	Unknown	9	1,224	1,233			
Place of Living	Urban	862 (97.95)	251,360 (98.11)	252,222 (98.11)	1	Reference	
	Rural	18 (2.05)	4,843 (1.89)	4,861 (1.89)		NS	0.49
	Unknown	31	5,768	6,803			
	Academic	589 (65.59)	100,414 (38.43)	101,003 (38.52)	1	Reference	

Hospital Type	Community	309 (34.41)	160,897 (61.57)	161,206 (61.48)	0.38	0.33-0.45	0.0001
	Unknown	13	1,664	1,677			
Insurance Status	Insured	847 (98.26)	249,219 (96.94)	250,066 (96.95)	1	Reference	
	Not insured	15 (1.74)	7,856 (3.06)	7,871 (3.05)	0.44	0.27-0.78	0.010
	Unknown	49	59,00	5,949			
Charlson/Deyo Score	0	716 (78.59)	171,219 (65.11)	171,935 (65.16)	1	Reference	
	1	154 (16.90)	63.980 (24.33)	64,134 (24.30	0.78	0.65-0.93	0.007
	>=2	41 (4.50)	27,776 (10.56)	27,817 (10.54)	0.61	0.44-0.84	0.003
M stage	M0	449 (51.14)	116,598 (45.95)	117047 (45.97)	1	Reference	
	M1	429 (48.86)	137,142 (54.05)	137,571 (54.03)		NS	0.79
Chemotherapy	Yes	854 (93.74)	130,918 (49.78)	131,772 (49.94)	1	Reference	
	No	57 (6.26)	132057(50.22)	132,114 (50.06)	0.12	0.08-0.14	0.0001
Radiation Therapy	Yes	308 (33.81)	35,029 (13.32)	35,337 (13.39)	1	Reference	
	No	603 (66.19)	227,946 (86.68)	228,549 (86.61)	0.61	0.52-0.71	0.0001
Year of Diagnosis	2004-2010	451(49.51)	126180 (47.98)	126,631 (47.99)		NS	0.65
	2011-2016	460 (50.49)	136,795 (52.02)	137,255 (52.01)	1	Reference	

Figure 1a: Overall survival of unresectable PDAC patients with (red) or without (blue) immunotherapy

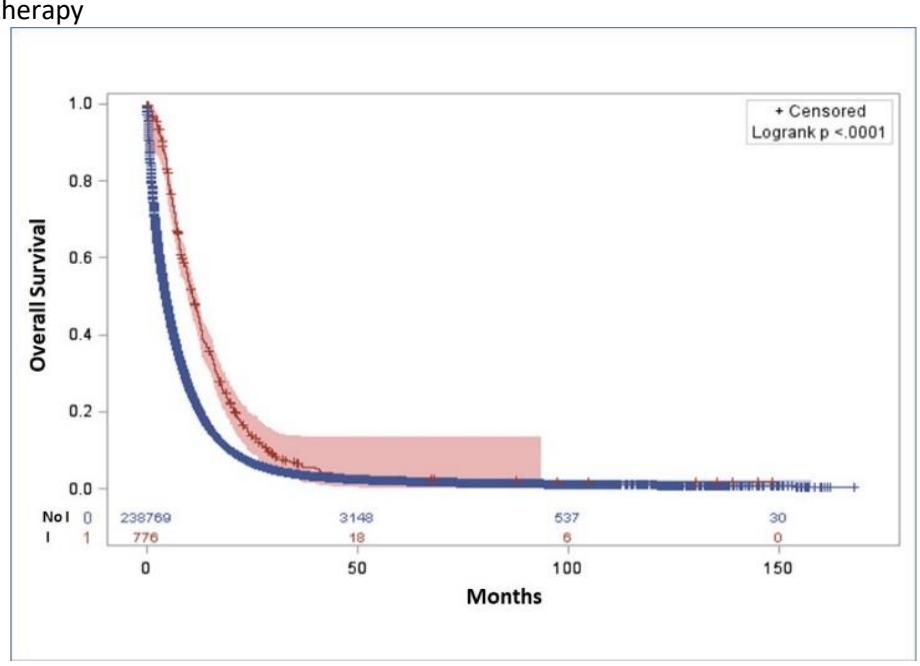


Figure 1b: Overall survival of unresectable PDAC patients who received chemotherapy with (Red) or without (blue) immunotherapy

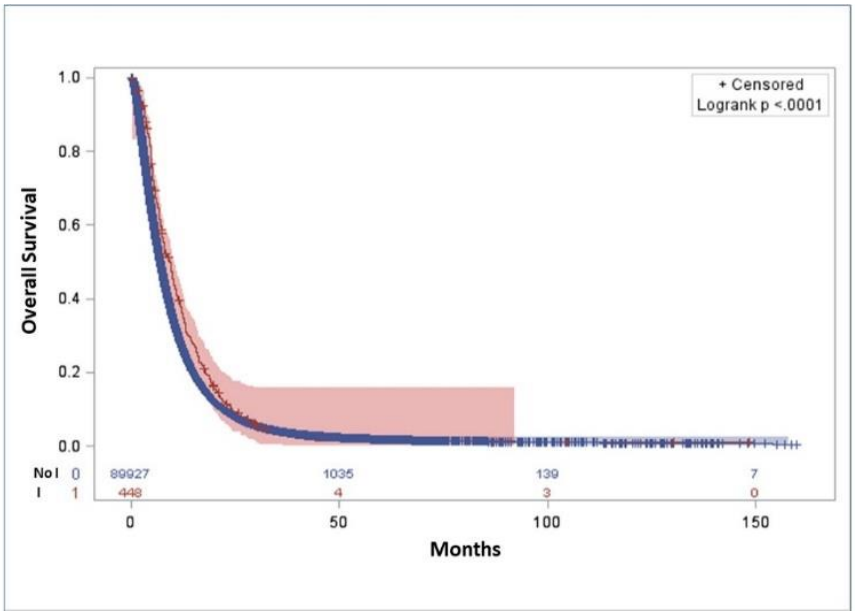


Figure 1c: Overall survival of unresectable PDAC patients who received chemoradiation with (red) or without (blue) immunotherapy

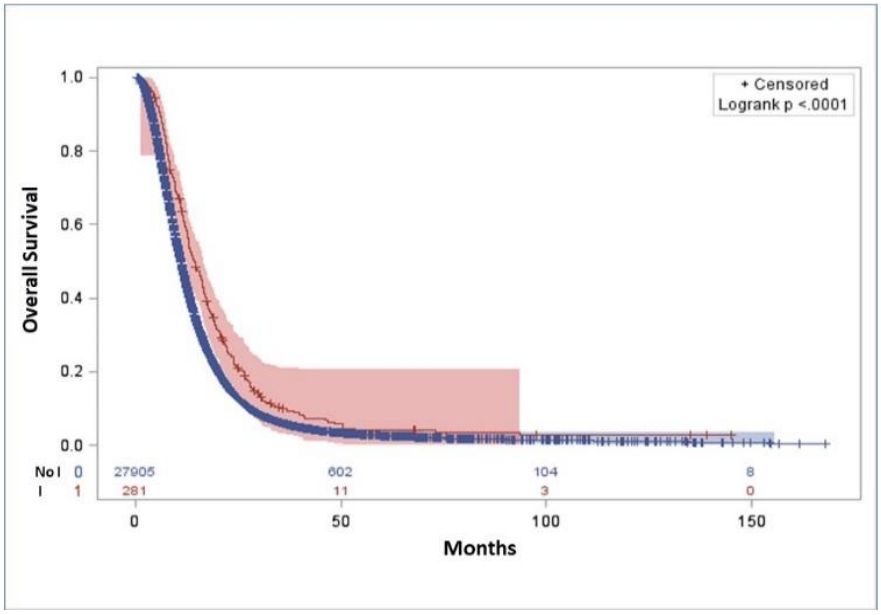


Table 5. Univariable and multivariable Cox analysis and the OS of PC patients who did not receive definitive surgery

Variable		Univariable analysis		Multivariable analysis	
		Hazard Ratio (95% CI)	P	Hazard Ratio (95% CI)	P
Age at diagnosis (continuous)		1.02 (1.02-1.02)	<0.0001	1.01 (1.01-1.01)	<0.0001
Sex	Male	Reference		Reference	
	Female	0.99 (0.99-1.00)	<0.18	0.94 (0.94-0.95)	<0.0001
Race	White	Reference		Reference	
	Black	0.97 (0.96-0.98)	<0.0001	0.99 (0.98-1.01)	<0.21
	non-white non-black	0.87 (0.85-0.89)	<0.0001	0.89 (0.86-0.91)	<0.0001
Education	>=13% HG	1.05 (1.04-1.06)	<0.0001	0.99 (0.98-0.99)	0.021
	<13% HG	Reference		Reference	
Income	>=$35,000	Reference		Reference	
	<$35,000	1.09 (1.08-1.100)	<0.0001	1.07 (1.06-1.08)	<0.0001
Place of Living	Urban	Reference		Reference	
	Rural	1.08 (1.05-1.11)	<0.0001	1.05 (1.01-1.08)	0.008
Hospital Type	Academic	Reference		Reference	
	Community	1.28 (1.27-1.29)	<0.0001	1.18 (1.17-1.19)	<0.0001
Insurance Status	Insured	Reference		Reference	
	Not insured	0.98 (0.95-1.00)	0.066	1.07 (1.04-1.09)	<0.0001
	0	Reference		Reference	

Charlson/Deyo Score	1	1.17 (1.16-1.18)	<0.0001	1.11 (1.11-1.13)	<0.0001
	>=2	1.52 (1.50-1.54)	<0.0001	1.35 (1.33-1.37)	<0.0001
Year of Diagnosis	2004-2010	1.18 (1.17-1.19)	0.0001	1.18 (1.17-1.19)	0.0001
	2011-2016	Reference		Reference	
M stage	M0	0.66 (0.65-0.66)	0.0001	0.56 (0.56-0.57)	0.0001
	M1	Reference			
Chemotherapy	Yes	Reference		Reference	
	No	2.15 (2.13-2.17)	<0.0001	2.10 (2.08-2.12)	<0.0001
Radiation Therapy	Yes	Reference		Reference	
	No	1.76 (1.73-1.78)	<0.0001	1.11 (1.09-1.12)	<0.0001
Immunotherapy	Yes	0.59 (0.55-0.64)		0.87 (0.80-0.94)	
	No	reference	<0.0001	reference	<0.0004

Table 6. Univariate and multivariate analysis of Combining Immunotherapy with Chemotherapy and Radiation therapy

Variable		N (%)	Univariable analysis		Multivariable analysis	
			Hazard Ratio (95% CI)	P	Hazard Ratio (95% CI)	P
Chemo and immunotherapy combination	Chemotherapy Only	100,991 (99.45%)	Reference		Reference	
	Chemo plus Immunotherapy	555 (0.55%)	0.82 (0.75-0.90)	<0.0001	0.85 (0.77-0.94)	0.001
Chemoradiation plus immunotherapy combination	Chemoradiation Only	29,927 (99.01%)	Reference		Reference	
	Chemoradiation plus Immunotherapy	299 (0.99%)	0.74 (0.65-0.83)	<0.0001	0.81 (0.71-0.94)	0.004

Two different models were developed for the multivariable analysis of table 3 because the treatment combination variables were mutually exclusive.

Discussion

The current study compared the survival outcomes of PDAC patients without surgery who received chemotherapy with and without immunotherapy and those who received chemoradiation with and without immunotherapy. Our analysis demonstrated that adding immunotherapy to either chemotherapy or chemoradiation therapy led to a significant OS benefit in both univariate and multivariable Cox regression analysis. What is unique about our study is that chemoradiation plus immunotherapy was associated with a significantly improved OS, which to our knowledge, has not been investigated yet.

The resistance of PDAC to the standard-of-care treatments is multifactorial[24]. Local therapies such as surgery and RT failed to show significant success because PDAC metastasizes microscopically early in the disease course, which limits the effectiveness of these treatments [109,119]. The presence of a strong desmoplastic stroma and the ability of the PDAC cells to go through a profound oncogenic alteration contributes to the failure of systemic therapies in PDAC[24,120,121]. The tumor microenvironment (TME) of PDAC evades immune response by up-regulating programmed-death ligand 1, downregulating CTLA4, recruitment of MDSC, and tumor-associated macrophages[122-127]. Based on these characteristics of the tumor, a multidisciplinary treatment approach of combining various systemic therapies such as immunotherapy and chemotherapy with each other or with local therapies such as RT may deliver better results. Immunotherapy may produce synergetic interaction with chemotherapy and radiation therapy as they increase tumor-specific T cell infiltration, decrease Treg cells, and suppress MDSC[86,88,128,129]. Various combination treatment strategies have been proposed to overcome the resistance of PDAC to immunotherapy. The combination of immunotherapies with chemotherapy and chemoradiation in PDAC represents a promising strategy to stimulate

immunogenicity, improve antigen recognition, increase the presentation of neoantigen, utilize abscopal effect, inhibit tumor-mediated immunosuppression, and improve survival[130-132].

The OS of patients who only received immunotherapy was not significantly different from the OS of patients who only received chemotherapy or chemoradiation, indicating that using immunotherapy alone in PDAC is not more effective compared to other treatments alone (data not shown). The results of the study remained the same when the analysis was restricted to patients who received immunotherapy within six months of chemotherapy or chemoradiation. There was no difference in the OS of patients who received immunotherapy concurrently with chemotherapy or chemoradiation compared to patients who received immunotherapy before chemotherapy or chemoradiation, and patients who received immunotherapy after chemoradiation. No difference may be due to the small sample size of the non-concurrent groups (the data is not shown). The sequence was investigated separately for immunotherapy plus chemotherapy and immunotherapy plus chemoradiation.

The improved OS with the addition of immunotherapy to standard treatments reported in our study may be synergistic. Chemotherapy can recruit and activate dendritic cells, trigger the release of tumor-specific antigens, and reduce Treg cells[130]. Chemotherapy, especially gemcitabine, has been associated with an increase in tumor-specific T cell infiltration, a decrease in Treg cells, and the suppression of MDSC in pre-clinical and clinical studies [86,129,133]. Chemotherapy causes immunogenic death, which promotes antigen presentation and leads to the priming of the tumor-specific T cells[86,129]. Radiation therapy promotes the translocation of calreticulin, which will enable T cells to clear tumor cells[133]. More importantly, through the abscopal effect, RT causes the release of tumor-associated antigens[134], which stimulates a tumor-specific immune response, allowing the immune cells (T-cells) to recognize and attack both the primary tumor and metastatic disease in a sort of auto-vaccination[92,103,135-138]. The

irradiated tumor cells may also release cellular danger-associated molecular patterns and cytokines that enhance the traffic of immune cells leading to the elimination of the tumor cells[92,136]. Chemotherapy and RT also cause the release of neoantigens and upregulation of inflammatory cytokines, which promote the presentation of the neoantigens in the TME and thereby increase the immunogenicity of the tumor cells, making them better targets for immunotherapy[94,137-141].

Our results are consistent with the preliminary findings of the ongoing phase 1 trials of immunotherapy and chemotherapy[60-62,95,96]. The median OS reported in these trials is similar to the median OS reported in our study. In phase I trial of 34 patients with metastatic PC, patients who received anti-CTLA4 with gemcitabine had a median OS of 7.4 months, much longer than the historical data from chemotherapy alone[60]. Another trial which included 16 patients with advanced PC and investigated the combination of gemcitabine with anti-CTLA4 reported a median OS of 8.4 months[61]. An early-phase trial with 50 patients investigated anti-PD-1, nivolumab in conjunction with *nab*-paclitaxel (*nab*-P) ± gemcitabine in advanced PDAC, reported a median OS of 9.9 months with a 6-months OS rate of 73%[62]. A dose-escalation phase 1 trial of CD40 agonist combined with gemcitabine of advanced PDAC which include 22 patients reported a median OS of 7.4 months for patients who received CD40 with gemcitabine compared to 5.7 months for gemcitabine alone[95]. A study of PF-04136309, a human chemokine receptor 2 (CCR2) in combination with chemotherapy in patients with borderline resectable or advanced PDAC that included 49 patients reported 49% overall response rate and 97% stable disease in the combined arm, while in the chemotherapy alone arm, there was no overall response reported, but 80% achieved stable disease[96].

In this study, chemotherapy plus immunotherapy was associated with significantly improved OS with a hazard ratio of ((HR: 0.857, CI: 0.776-0.984; P <0.001) compared to

chemotherapy without immunotherapy. chemoradiation plus immunotherapy was associated with significantly improved OS (HR: 0.804, CI: 0.702-0.921; P <0.001) compared to chemoradiation without immunotherapy

To our knowledge, the current study is the first to use an extensive database such as NCDB and investigate the impact of immunotherapy on the OS of PAD patients who did not get definitive surgery. The findings of our study, together with early results of some clinical trials, warrant future large phase III clinical trials of immunotherapy combined with chemotherapy or chemoradiation in PAD patients.

The strength of the current study is the large sample size. A large sample size allowed us to adjust for the important patient and tumor characteristics in the multivariable analysis. More importantly, we were able to stratify patients by definitive surgery. However, our research is not without limitations, and those limitations are inherent to NCDB which include incomplete data and ascertainment bias, lack of data about the cause of death, lack of detailed information on the use of multi-agent chemotherapy regimens, and lack of information on the type of immunotherapy and if a single or combined immunotherapy was used. Also, the NCDB does not provide data on the microsatellite-instability status for PDAC patients who are more likely to respond to immunotherapy. Due to the small sample size, the analysis of comparing the impact of RT plus immunotherapy vs. RT alone was not performed.

Nevertheless, NCDB provided sufficient patient numbers to assess the impact of immunotherapy on the OS of PDAC patients, which is difficult to quantify from small early-phase clinical trials, most of which are single arm. To our knowledge, this study is the most extensive retrospective study of the use of immunotherapy and its impact on the OS of unresectable PDAC patients. This research included the majority of patients treated in the United States and is the

best available resource outside multicenter, randomized trials to investigate the impact of novel treatments such as immunotherapy on the OS of unresectable PDAC patients.

Conclusion

To our knowledge, the current study is the first study with a robust investigation of the impact of immunotherapy in combination with chemotherapy and chemoradiation on the OS of PDAC patients using the NCDB. This research study found significantly improved OS in patients receiving standard therapies such as chemotherapy and chemoradiation when combined with immunotherapy. These findings warrant further clinical trials looking into the impact of immunotherapy combined with chemotherapy and chemoradiation in PDAC patients.

CHAPTER 3

IMMUNOTHERAPY AND THE SURVIVAL OF RESECTABLE PANCREATIC CANCER PATIENTS

Abstract

Purpose: Immunotherapy has paved the way for new therapeutic opportunities in cancer but has failed to show any efficacy in Pancreatic Adenocarcinoma (PDAC), and its therapeutic role remains unclear. The objective of this study is to examine the impact of immunotherapy in combination with chemotherapy, RT, and chemoradiation on the overall survival (OS) of PDAC patients who received definitive surgery of the tumor using the National Cancer Database (NCDB). **Methods and Materials:** Patients with PDAC who received definitive surgery of cancer and were diagnosed between 2004 and 2016 from the NCDB were identified. Cox proportional hazard analysis was used to assess the survival difference between patients who received chemotherapy plus immunotherapy and chemoradiation therapy plus immunotherapy and their counterparts who only receive these treatments without immunotherapy. The multivariable analysis was adjusted for age of diagnosis, race, sex, place of living, income, education, treatment facility type, insurance status, year of diagnosis, and treatment types such as chemotherapy and radiation therapy. **Results:** In total, 63,154 PDAC patients who received definitive surgery of the tumor were included in the analysis. Among the 63,154 patients, 636 (1.01%) received immunotherapy. Among patients who received chemotherapy (21,355), and chemoradiation (21,875), 157/21,355 (0.74%) received chemotherapy plus immunotherapy, and 451/21,875 (2.06%) received chemoradiation plus immunotherapy. In the multivariable analysis, patients who received immunotherapy had significantly improved OS compared to patients who did not receive immunotherapy (HR: 0.90; CI: 0.81-0.99; P <0.039). Patients who received chemoradiation plus immunotherapy had significantly improved OS compared to their

counterparts who only received chemoradiation without immunotherapy (HR: 0.85 CI: 0.75-0.95; P <0.008). **Conclusions:** In this study, the addition of immunotherapy to chemoradiation therapy but not chemotherapy alone was associated with significantly improved OS in PDAC patients who received definitive surgery. The study warrants further future clinical trials of immunotherapy in PDAC.

Introduction

Pancreatic adenocarcinoma (PDAC) is the 7th leading cause of global cancer deaths and the third leading cause of cancer deaths in the United States[4]. In 2019, there were an estimated 56,000 new cases of PDAC and 450,00 deaths[142]. It is predicted that PDAC will become the second leading cause of cancer deaths by 2030, after lung cancer[7]. There are no early detection tests, and most patients with localized disease have no recognizable symptoms or signs. Therefore, most PDAC patients are diagnosed after their cancer has metastasized to other organs[143]. The five-year survival rate for all stages remains at 5% and has not changed in the last 30 years[142].

Surgery is the only curative treatment, but unfortunately, only 15-20 % of patients present with cancer that is amenable to resection[144]. Despite significant improvement in surgical techniques, the five-year survival rate after resection remains at 10-20% with a median survival of 24 months[144,145]. A Locoregional and distant recurrence rate of up to 80% after surgery is reported, which is likely secondary to the presence of occult micrometastatic disease at the time of resection[146,147]. The majority of locoregional or distant recurrence occurs within two years after resection[146,147]. A rapid autopsy series of patients with known PDAC found that only 30 % of the patients died with a locally destructive disease with no evidence of distant metastasis. In comparison, 70 % died with widespread metastatic disease[148]. The potential of PDAC for early metastases have convinced scientists to hypothesize that PDAC is a systemic disease at the time of diagnosis, even when there is no radiographic evidence of distant metastases[145].

Chemotherapy and/or chemoradiation have been combined with surgery to improve disease control and survival. Unfortunately, the outcomes of combined treatment are still not very promising. Therefore, there is a desperate need for more effective systemic therapy that could be combined with the current standard treatment to improve the overall survival (OS) of the resectable PDAC patients. Strategies of combining novel treatments such as immunotherapy with surgery have been proposed and could provide a potential successful curative option for PDAC patients. After making first inroads in cancer in the setting of metastatic melanoma in 2011, immunotherapy has now been approved for advanced melanoma, non-small cell lung cancer (NSCLC), Hodgkin's lymphoma, head and neck cancer, microsatellite instability-high cancer, gastric cancer, advanced renal cell cancer, bladder cancer, liver cancer, and Merkel cell carcinoma[42,149].

Immunotherapy is not approved for PDAC but has been occasionally used in an off-the-label clinical setting for metastatic PDAC extrapolating the utility in various other malignancies. Despite the inconclusive results of the initial trials of mono immunotherapy in metastatic PDAC, to date, the utilization of immunotherapy has been primarily in the metastatic setting as a last-ditch effort following the failure of currently FDA approved therapies[50,150-152]. However, new evidence indicates that immunotherapy could be effective and useful in patients with localized disease who have a high risk of micrometastases a critical hallmark of PDAC[103-105]. Occult metastases and the fact that early-stage cancer presents with the more intact immune system and lower tumor burden underline the rationale for the use of immunotherapy in resectable PDAC[102,103].

Immunotherapy may be useful in PDAC patients who receive definitive surgery if it is combined with other treatments such as chemotherapy and radiation therapy (RT). Preclinical and clinical evidence demonstrates that immunotherapy can have synergistic interaction with

chemotherapy and RT as they increase tumor-specific T cell infiltration, decrease Treg cells, and suppress Myeloid-derived suppressor cells (MDSCs)[87,88]. In preclinical studies of PDAC, immunotherapy has elicited tumor regression and improved survival when used in combination with other treatments of cancer, especially chemotherapy[89,90]. The objective of the current study is to investigate the impact of immunotherapy combined with chemotherapy and chemoradiation on the overall survival of PDAC patients who received definitive surgery of PDAC using the National Cancer Database (NCDB).

Methods

Data Source

The data for this study was extracted from the National Cancer Database (NCDB), which is a joint program of the Commission on Cancer of the American College of Surgeons and the American Cancer Society. It captures 70% or more of newly diagnosed malignancies in the United States annually. This study was exempt from the Institutional Review Board (IRB) because the de-identified file of the NCDB data was used.

Study Population

The study included patients age 18 or older who were diagnosed with PADC between 2004 and 2016 and received definitive surgery of the tumor. Only patients who were diagnosed with PDAC were included using the ICD-O-3 histology codes of 8000, 8010, 8020-8022, 8140, 8141, 8211, 8230, 8500, 8521, 8050, 8260, 8441, 8450, 8453, 8470-8473, 8480, 8481, 8503,8250,8440, 8560. The surgical site-specific code was used to identify patients with definitive surgery of the tumor. Patients who were missing information about RT, chemotherapy, and immunotherapy were excluded. We also excluded patients with the M1

stage and those with unknown or missing information about other covariates in the adjusted multivariable analysis.

End Points

The primary outcome of the current study was the OS of the patients, which was calculated from the date of diagnosis to the date of death. Patients who were alive or lost to follow up were censored.

Explanatory variables

The main predictors of OS in this study were immunotherapy, immunotherapy combined with chemotherapy, and immunotherapy combined with chemoradiation. The age of diagnosis, gender, race, urban and rural living status, income, education, treatment facility type, comorbidity score, insurance status, year of diagnosis, and receipt of chemotherapy, RT, and immunotherapy were other explanatory variables used in the analysis.

Statistical Analyses

Descriptive statistics were reported for categorical and continuous variables. Multivariable logistic analysis was used to identify the predictors of receiving immunotherapy and reported the odds ratio as a measure of association with the probability of receiving immunotherapy. The p-value of 0.10 was used as a cut-off point for a variable to stay in the final model. OS rates were determined using the Kaplan–Meier method and were compared between groups using log-rank statistics. Survival time was measured in months from the date of diagnosis to the date of death. Cox proportional hazards model was used to determine the significant predictors of OS and estimate the hazard ratio of death as well as its 95% confidence interval (CI). The potential variables to be adjusted in the multivariable Cox models were the age

of diagnosis, gender, race, urban and rural living status, income, education, treatment facility type, comorbidity score, insurance status, year of diagnosis, and receipt of chemotherapy and RT. Variables with a p-value of 0.2 in the univariate analysis were selected for the multivariable analysis. A p-value of 0.10 was used as a cut-off point for a variable to stay in the final model. The p-value of 0.05 was considered significant. Separate multivariable Cox proportional hazard regression models were developed for the hazard ratio of immunotherapy combined with chemotherapy and chemoradiation as these combinations are mutually explosive variables. The C-Index of the original model for multivariable logistic regression analysis was 0.80, and after 1,000 bootstraps was 0.80 with C-index bais of 0.00. The C-Index for the original survival model of immunotherapy was 0.59, and the survival model after 1,000 bootstraps was 0.59, with a C-Index bias of 0.00. The details of the original and bootstrap models, including the hazard ratio and C-Index, are provided in supplemental tables 6-10. SAS 9.4 and R 6.2 were used for analysis and bootstrap sampling.

Results

In total, 63,154 patients diagnosed with PDAC between 2004 and 2016 who received definitive surgery of the tumor were included in the analysis. Among the 63,154 patients, 636 (1.01%) received immunotherapy. Among patients who received chemotherapy (21,355), and chemoradiation (21,875), 157/21,355 (0.74%) received chemotherapy plus immunotherapy, and 451/21,875 (2.06%) received chemoradiation plus immunotherapy. The majority of the patients were White, from urban areas, with high school degrees, Charlson/Deyo Score of zero, the income of >=$3,5000, received chemotherapy, and treated in academic hospitals. In the multivariable logistic analysis, older age, female sex, Black race, Charlson/Deyo Score of 1 and 2, treatment at a community hospital, being less educated, diagnosed before 2011, not receiving chemotherapy, and not receiving RT were significantly less likely to receive immunotherapy.

Insurance status, income level, place of living, and non-white non-black race were not significantly associated with the receipt of immunotherapy. The odds ratio of these factors is provided in Table 7.

PDAC patients who received immunotherapy had significantly improved median overall survival OS with an absolute median OS benefit of 7.1 [28.45 vs. 21.36; p <0.0001] (Figure 2a) months compared to their counterparts without immunotherapy. Patients who received chemoradiation plus immunotherapy had significantly improved median OS compared to patients who only received chemoradiation with an absolute median OS benefit of 5.7 [29.31 vs. 23.66; p <0.0001] months (Figure 2c). There was no significant difference in the median OS of patients who received chemotherapy plus immunotherapy and those who only received chemotherapy [26.28 vs. 22.70; p <0.051] months (Figure 2b).

Table 7. Multivariable logistic analysis of the predictor of immunotherapy in patients who received definitive surgery of the pancreatic tumor

Variable		Immunotherapy 636 (1.01%)	No Immunotherapy 62,518 (98.99%)	Total 63,154	Odds Ratio	95% CI	P
Age at diagnosis, Median (range)		62.00 (29-90)	67.00 (18-90)	63,154	0.97	0.97-0.98	<0.0001
Sex	Male	352 (55.35)	31,719 (50.74)	32,071 (50.78)	1	Reference	
	Female	284 (44.65)	30,799 (49.26)	31,083 (49.22)	0.84	0.72-0.99	0.046
Race	White	574 (92.13)	53,761 (86.84)	54,335 (86.89)	1	Reference	
	Black	28 (4.49)	5982 (9.66)	6,010 (9.61)	0.48	0.32-0.71	0.0003
	Other	21 (3.37)	21,68 (3.50)	2,189 (3.50)	0.79	0.48-1.28	0.338
	Unknown	13	607	620			
Education	>=13% HG	167 (26.47)	24,941 (40.05)	25,108 (39.91)	0.65	0.54-0.78	0.0001
	<13%	464 (73.53)	37,336 (59.95)	37,800 (60.09)	1	Reference	
	Unknown	5	241	246			
Income	>=$35,000	459 (72.74)	38,308 (61.54)	38,767 (61.65)	1	Reference	
	<35,000	172 (27.26)	23,944 (38.46)	24,116 (38.35)		NS	0.160
	Unknown	5	266	271			
Place of Living	Urban	604 (99.02)	59,667 (98.11)	60,271 (98.12)	1	Reference	
	Rural	6 (0.98)	1,150 (1.89)	1,156 (1.88)	0.41	0.15-1.11	0.081
	Unknown	26	1701	1,727			
Hospital Type	Academic	505 (80.41)	34,074 (55.04)	34,579 (55.30)	1	Reference	
	Community	123 (19.59)	27831 (44.96)	27,954 (44.70)	0.261	0.21-0.32	0.0001

	Unknown	8	613	621			
Insurance Status	Insured	623 (98.89)	60,145 (97.73)	60,768 (97.74)	1	Reference	
	Not insured	7 (1.11)	1,399 (2.27)	1,406 (2.26)	0.50	0.24-1.07	0.074
	Unknown	6	974	980			
Charlson/Deyo Score	0	486 (76.42)	40,852 (65.34)	41,338 (65.46)	1	Reference	
	1	125 (19.65)	16,270 (26.02)	16,395 (25.96)	0.73	0.59-0.90	0.003
	>=2	25 (3.93)	5,396 (8.63)	5,421 (8.58)	0.52	0.34-0.79	0.002
Chemotherapy	Yes	608 (95.60)	42,622 (68.18)	43,230 (68.65)	1	Reference	
	No	28 (4.40)	19896 (31.82)	19,924 (31.55)	0.21	0.14-0.32	0.0001
Radiation Therapy	Yes	459 (72.17)	22,068 (35.30)	22,527 (35.67)	1	Reference	
	No	177 (27.83)	40,450 (64.70)	40,627 (64.33)	0.35	0.29-0.43	<0.0001
Year of Diagnosis	2004-2010	330 (51.89)	27,978 (44.75)	28,308 (44.82)	1.27	1.07-1.50	<0.005
	2011-2016	306 (48.11.)	34,540 (55.25)	34,846 (55.18)	1	Reference	

When we excluded insurance status and place of living the results were the same; therefore, we included them in the multivariable analysis

In the univariate Cox Proportional analysis (Table 8), patients who received immunotherapy had significantly improved OS compared to their counterparts without immunotherapy (HR: 0.77, CI: 0.70-0.85; P <0.0001). Patients receiving chemoradiation plus immunotherapy had significantly improved OS compared to chemoradiation alone (HR: 0.80, CI: 0.71-0.89; P <0.008). In the univariate Cox Proportional analysis, patients who received chemotherapy plus immunotherapy did not notice significantly improved OS compared to their counterparts (HR: 0.81, CI: 0.66-1.00; P <0.064). Female sex and non-white non-black race were associated with significantly improved OS, while older age, living in the rural area, treatment at a community hospital, low income, low education, not receiving chemotherapy or RT, and diagnosis before 2011 were all associated with significantly decreased OS.

In the multivariable Cox Proportional analysis, immunotherapy, female gender, and non-white non-black race were associated with significantly improved OS, while older age, Black race, treatment at a community hospital, low income, low education, not receiving chemotherapy or RT, not having insurance, Charlson/Deyo of one and two, and diagnosis before 2011 were associated with significantly decreased OS (Table 8). The multivariable analysis was adjusted for age of diagnosis, race, sex, place of living, income, education, hospital type, insurance status, year of diagnosis, and Charlson/Deyo score. Patients who received immunotherapy had significantly improved OS compared to patients who did not receive immunotherapy (HR: 0.90; CI: 0.81-0.99; P <0.039). Patients who received chemoradiation plus immunotherapy had significantly improved OS compared to their counterparts who only received chemoradiation without immunotherapy (HR: 0.85 CI: 0.75-0.95; P <0.008) (Table 9). The 1-year and 2-year survival rates were 88% and 60% for chemoradiation plus immunotherapy patients compared to 81% and 49% in patients who only received chemoradiation (data not shown). Chemotherapy plus immunotherapy was not associated with significantly improved OS.

Figure 2a: Overall survival of resectable PDAC patients with (red) or without (blue) immunotherapy

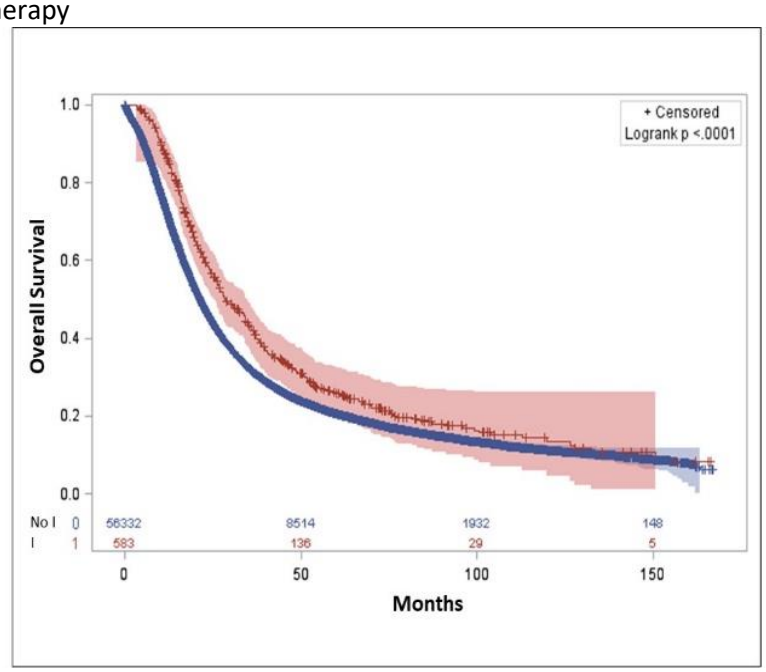


Figure 2b: Overall survival of resectable PDAC patients who received chemotherapy with (red) or without (blue) immunotherapy

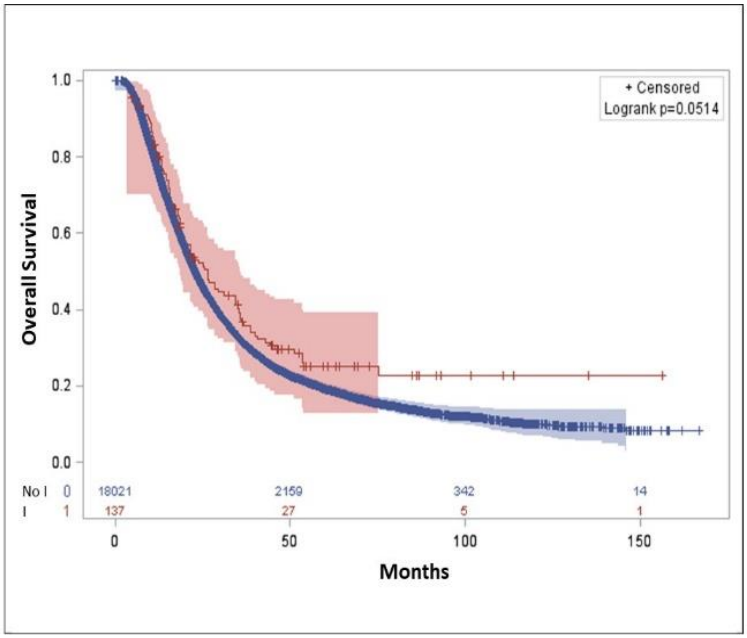


Figure 2c: Overall survival of unresectable PDAC patients who received chemoradiation with (red) or without (blue) immunotherapy

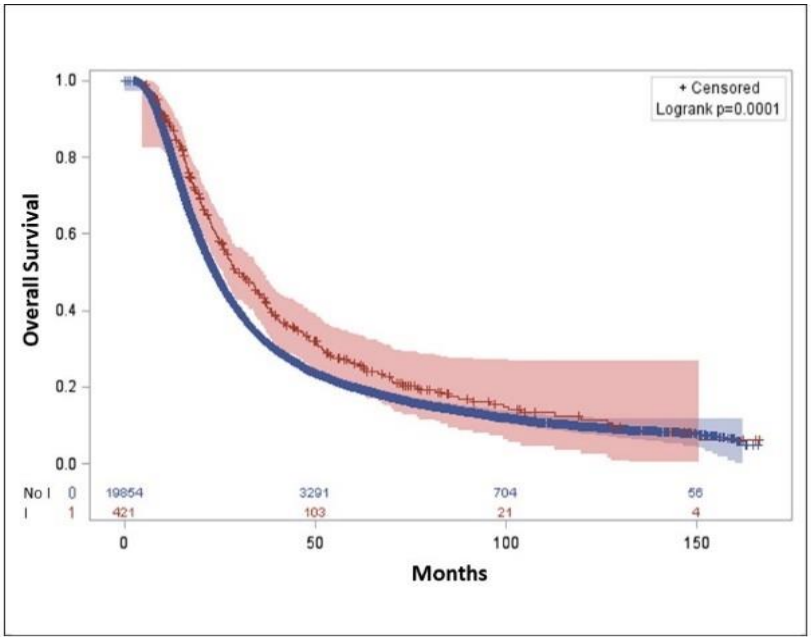


Table 8. Univariable and multivariable Cox analysis of PDAC patients who received definitive surgery of the pancreatic tumor

Variable		Univariable analysis		Multivariable analysis	
		Hazard Ratio (95% CI)	P	Hazard Ratio (95% CI)	P
Age at diagnosis (continuous)		1.01 (1.01-1.02)	<0.0001	1.01 (1.01-1.01)	<0.0001
Sex	Male	Reference		Reference	
	Female	0.94 (0.92-0.96)	<0.0001	0.93 (0.91-0.94)	<0.0001
Race	White	Reference		Reference	
	Black	1.02 (0.99-1.05)	<0.23	1.03 (0.99-1.06)	<0.10
	non-white non-black	0.82 (0.77-0.87)	<0.0001	0.86 (0.81-0.91)	<0.0001
Education	>=13% HG	1.12 (1.10-1.14)	<0.0001	1.07 (1.05-1.10)	<0.0001
	<13% HG	Reference		Reference	
Income	>=$35,000	Reference		Reference	
	<$35,000	1.15 (1.12-1.17)	<0.0001	1.09 (1.07-1.12)	<0.0001
Place of Living	Urban	Reference		Reference	
	Rural	1.140 (1.06-1.22)	<0.0002	NS	0.150
Hospital Type	Academic	Reference		Reference	
	Community	1.20 (1.18-1.22)	<0.0001	1.20(1.17-1.22)	<0.0001
Insurance Status	Insured	Reference		Reference	
	Not insured	0.96 (0.90-1.03)	0.20	1.08 (1.01-1.16)	<0.024
	0	Reference		Reference	

Charlson/Deyo Score	1	1.10 (1.08-1.12)	<0.0001	1.06 (1.04-1.09)	<0.0001
	>=2	1.30 (1.26-1.35)	<0.0001	1.23 (1.19-1.28)	<0.0001
Year of Diagnosis	2004-2010	1.16 (1.13-1.18)	0.0001	1.16 (1.13-1.18)	0.0001
	2011-2016	Reference		Reference	
Chemotherapy	Yes	Reference		Reference	
	No	1.22 (1.19-1.24)	<0.0001	1.14 (1.11-1.17)	<0.0001
Radiation Therapy	Yes	Reference		Reference	
	No	1.12 (1.10-1.14)	<0.0001	1.03 (1.01-1.06)	<0.008
Immunotherapy	Yes	0.77 (0.70-0.85)		0.90 (0.81-0.99)	
	No	reference	<0.0001	reference	<0.039

Table 9. Univariate and multivariate Cox analysis of Combining Immunotherapy with other treatments in patients who received definitive surgery of the pancreatic tumor

Variable		N (%)	Univariable analysis		Multivariable analysis	
			Hazard Ratio (95% CI)	P	Hazard Ratio (95% CI)	P
Chemo and immunotherapy combination	Chemotherapy Only	21,198 (99.26%)	Reference		Reference	
	Chemo + Immunotherapy	157 (0.74%)	0.82 (0.67-1.00)	<0.052	NS	0.44
Chemoradiation and immunotherapy combination	Chemoradiation Only	21,424 (97.94%)	Reference		Reference	
	Chemoradiation + Immunotherapy	452 (2.06%)	0.80 (0.72-0.90)	<0.0001	0.85 (0.76-0.96)	0.008

Two different models were developed for the multivariable analysis of Table 6 because these variables were mutually exclusive

Discussion

Using the NCDB, this study examined the impact of immunotherapy in combination with chemotherapy and chemoradiation on the OS of PDAC patients who received definitive surgery of the tumor. Chemoradiation but not chemotherapy alone plus immunotherapy was associated with significantly improved OS in the univariate and multivariable Cox Proportional analysis adjusted for age of diagnosis, gender, race, income, education treatment facility type, Charlson/Deyo score, place of living, year of diagnosis, and insurance status.

The tumor microenvironment of PDAC is non-immunogenic and immunosuppressive[87]. Pancreatic cancer itself induces local and systemic immune dysfunction or immunosuppression to avoid being recognized and attacked by effector immune cells[153,154]. The tumor cells use mechanisms such as the up-regulation of immune checkpoint signaling program (PD-L1, CTLA-4), the blockage of co-stimulation to activate T cells, and the recruitment of MDSCs, and tumor-associated macrophages to achieve immune suppression[122,123,155]. The tumor microenvironment reflects a lack of tumor-infiltrating lymphocytes and dendritic cells and plenty of suppressor T cells[127,156]. The immunosuppressive tumor microenvironment of PDAC is one of the reasons for mono immunotherapy not to show the response and success in PDAC that has been reported in many other malignancies[42,149]. However, various rational combination treatment strategies have been proposed to overcome the resistance of PDAC to immunotherapy. The combination of immunotherapies with chemotherapy and chemoradiation in PDAC represents a promising strategy that could stimulate immunogenicity, improve antigen recognition, and inhibit tumor-mediated immunosuppression[131,132].

Chemoradiation can work synergistically with immunotherapy and improve OS compared to chemoradiation alone. Chemotherapy and RT cause the release of neoantigens and upregulation of inflammatory cytokines, which promote the presentation of the neoantigens in

the tumor microenvironment and thereby increase the immunogenicity of the tumor cells making them better targets for immunotherapy[137,140,157].

Checkpoint blockade immunotherapy has resulted in impressive responses in the metastatic setting of various tumors and, more recently, has been tested in the adjuvant setting after surgery[127,156]. FDA has approved a couple of checkpoint inhibitors for adjuvant use in advanced melanoma, cervical cancer, bladder cancer, and renal cancer[156,158]. Various types of immunotherapies, including checkpoint inhibitors and vaccines therapies in combination with chemotherapy and chemoradiation, have been studied in early-stage and metastatic PDAC but have not led to the FDA approval of immunotherapy for pancreatic cancer[86]. The use of immunotherapy in neoadjuvant or adjuvant setting combined with chemoradiation in PDAC has been limited. Some clinical trials studying the efficacy of immunotherapy in resectable PDAC combined with chemoradiation therapy have shown positive response and measurable activity[80,159-161]. More extensive studies are needed to confirm these findings.

Our results are consistent with the findings of a few other clinical trials and retrospectives studies. A phase II trial involving 60 patients with resected PDAC, investigated the impact of granulocyte-macrophage colony-stimulating factor (GM-CSF) with chemoradiation reported a median survival of 24.8 months (95% CI, 21.2–31.6)[80]. A dose-escalating study with 24 patients evaluated Gene-mediated cytotoxic immunotherapy (GMCI™) in combination with chemoradiation therapy for resected PDAC in adjuvant setting reported a median OS of 12 months and a 1-year OS of 50%[160]. A multi-institutional open-label phase II study evaluated algenpantucel-L in combination with chemoradiation therapy in 70 patients with resectable PDAC and reported the 12-months OS rate of 86%[161]. In the current study, we found a median OS of 26.2 months, a 12-months OS rate of 88%, and a 24-months OS rate of 60% comparable to these studies.

To our knowledge, the current study is the first to use an extensive database such as NCDB and investigate the impact of immunotherapy on the OS of PAD patients who receive definitive surgery. In this study combining immunotherapy with chemoradiation was associated with significantly improved OS. The results stayed the same when patients who received immunotherapy more than six months before or after chemoradiation were excluded. The findings of our study, together with early findings of some clinical trials, warrant future clinical trials of immunotherapy combined with chemoradiation in PAD patients. Chemotherapy and immunotherapy both induce a systemic immune response, and the addition of RT to chemotherapy and immunotherapy may be required to overcome the local and systemic immune suppression. The negative results of chemotherapy plus immunotherapy compared to chemotherapy indicates that both systemic and local immune response is necessary to overcome the immune evasion of pancreatic cancer cells. It is also possible that the number and quality tumor-infiltrating T cells and neoantigens produced by chemotherapy are not enough for immunotherapy to induce complete immune response as opposed to chemoradiation. The immunostimulatory effect of chemotherapy, especially in the adjuvant setting is through the inhibition of T regulatory cell and MDSCs rather than the stimulation and increase of T cells[162-164]. The significant improved OS associated with chemoradiation and immunotherapy is biologically justified. Evidence indicates that chemoradiation, especially after surgery, can significantly increase the number and function of dendritic cells by reducing immunosuppressive cytokines[165]. Dendritic cells are an essential part of the immune system and play a critical role in tumor cell recognition and T cells stimulation[166]. Chemoradiation is also capable of producing humoral or cellular immune responses, and its combination with immunotherapy has shown to mount long-term T cell reactivity[74,85,167].

The major strength of our study is the large sample size of the patients. The large sample size enabled us to adjust for some important confounding factors. Our study has several limitations. The NCDB, like many other large cancer databases, is prone to selection bias affecting the receipt of immunotherapy. The database does not provide information about the cause of death and the type of immunotherapy, such as checkpoint inhibitors and vaccine therapy. The NCDB also does not collect information about the type of chemotherapy, and the use of multi-agent chemotherapy regimens. Nonetheless, the NCDB is the largest cancer database in the world which capture the majority of the newly diagnosed cancer cases in the United States and serves as an excellent source outside of multicenter clinical trials for examining the impact of novel treatments such immunotherapy on the OS of PDAC patients who received definitive surgery of the tumor.

Conclusion

This study is the first large study with a robust analysis using the NCDB that has investigated the impact of immunotherapy in combination with chemotherapy, RT, and chemoradiation on the OS of PDAC patients who received definitive surgery of the tumor. In this study, combining chemoradiation therapy with immunotherapy was associated with significantly improved OS of the patients. The findings of the current study, together with the results of other previous studies of the use of immunotherapy with other standard-of-care cancer treatments in PDAC patients who receive surgery, warrant the need for future clinical trials of investigating the impact of immunotherapy in this group of patients.

CHAPTER 4

THE IMPACT OF THE SEQUENCE OF IMMUNOTHERAPY ON THE SURVIVAL OF PANCREATIC ADENOCARCINOMA PATIENTS: A RETROSPECTIVE ANALYSIS OF THE NATIONAL CANCER DATABASE

Abstract

Background: Immunotherapy has shown great success in various malignancies. However, its efficacy in pancreatic ductal adenocarcinoma (PDAC) remains a challenge, and the lack of understanding about the appropriate timing of immunotherapy with other standard-of-care cancer treatments may be one of the causes. The objective of the current study is to investigate the impact of the timing of immunotherapy with chemotherapy and RT on the overall survival (OS) of PDAC patients who did not receive surgical resection of the pancreatic tumor. **Materials and methods:** Patients with pancreatic adenocarcinoma who did not undergo surgical resection of the pancreatic tumor were identified from the National Cancer Database (NCDB). Cox proportional hazard models were employed to compare the OS between patients who received immunotherapy with chemotherapy or RT with a different sequence of treatment. The multivariable analysis was adjusted for age of diagnosis, race, sex, place of living, income, education, treatment facility type, insurance status, and year of diagnosis. **Results:** In total, 705 patients received chemotherapy and immunotherapy, while 226 received radiation therapy and immunotherapy. In the multivariable analysis, there was no significant difference in the OS of patients who started immunotherapy 31-90 days before the start of chemotherapy (HR:1.06, CI: 0.72-1.56; $p < 0.78$) and patients who started immunotherapy 91-180 days before the start of chemotherapy (HR: 0.90, CI: 0.58-1.39; $p < 0.64$) compared to patients who started chemotherapy and immunotherapy within 30 days of each other. There was also no significant difference in the OS of patients who started RT> 30 days before the start of immunotherapy

(HR: 0.64, CI: 0.35-1.17; p <0.15) and patients who started immunotherapy > 30 days before the start of RT (HR: 0.66, CI: 0.33-1.33; p <0.25) compared to patients who started RT and immunotherapy within 30 days of each other. **Conclusion:** The sequence of immunotherapy with chemotherapy or RT was not associated with improved OS. Future studies with a larger subgroup sample size investigating the impact of the timing of immunotherapy with chemotherapy and RT on the OS of PDAC patients who did not receive surgical resection of the pancreatic tumor are needed.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) represents 3.2% of all cancer cases, but it is responsible for 7.2% of all cancer deaths in the United States[6]. It is predicted that PDAC will become the second leading cause of cancer deaths by 2030, after lung cancer[168]. The median 5-year survival rate is 28-30% for localized diseases and only 8% for all stages. Due to the lack of sensitive biomarkers for early detections, more than 80% of the patients present with a locally advanced (non-resectable) disease[169,170].

Surgery is the only curative treatment, but unfortunately, only 15-20 % of patients present with cancer that is amenable to resection[171]. Resectable patients undergo curative surgery followed by a combination of fractionated radiation therapy (RT) and chemotherapy as adjuvant therapies, while unresectable patients receive chemotherapy or chemo-RT[170]. A median OS of up to 54.4 months has been reported for patients with resectable PDAC who receive modern adjuvant chemotherapy[21]. Nevertheless, a majority of the patients treated with standard treatments eventually succumb to the disease and, due to the minimal effect of the available treatments, new effective therapies for PC are urgently needed.

In recent years, immunotherapy has shown great success in various malignancies, but is not approved by the FDA for the treatment of PDAC and is used in the clinic as a last attempt after the failure of the current standard treatments[172-178]. Due to the negative results of the mono immunotherapy trials in PDAC, most recent trials have focused on combining immunotherapy with chemotherapy and RT[43,49,60-62,95,96,113]. Chemotherapy and RT cause the release of neoantigens and upregulation of inflammatory cytokines, which are critical

for the optimal function of immune cells stimulated by immunotherapy[137,139-141,157]. The preliminary findings of these trials have reported improved median OS for patients who received immunotherapy with chemotherapy compared to historical data[61,62,95,96]. The sequence of immunotherapy with chemotherapy and RT need to be balanced with the transient immunosuppressive impact of chemotherapy and RT to achieve the optimal effect of the combination. Chemotherapy and RT both cause a temporary increase in immunosuppressive myeloid cells, circulating tumor-macrophages, depletion of T cells, and an increase in Treg cell, which can suppress the immune system[131,179,180].

In a previous study that is currently submitted for publication, we found that immunotherapy combined with chemotherapy is associated with improved OS compared to chemotherapy alone in PDAC patients who did not receive definitive surgery of the pancreatic tumor. Improved OS was also noticed in patients who received chemoradiation plus immunotherapy compared to chemoradiation without immunotherapy. There is no consensus about the sequence of immunotherapy with RT, chemotherapy, and chemoradiation, and there is no study that has investigated the sequence of immunotherapy with other cancer treatments in PDAC as most of the trials of immunotherapy in PDA are in their early phases. The objective of this study is to investigate the impact of the sequence of immunotherapy with chemotherapy, and chemoradiation on the OS of PDAC patients using the National Cancer Database (NCDB) in an attempt to determine the appropriate treatment sequence that could be used to mitigate the immunosuppressive effects of the current treatments and maximize the impact of immunotherapeutic.

Methods

Data Source

The data for this study was obtained from the National Cancer Database (NCDB), which is a joint program of the Commission on Cancer of the American College of Surgeons and the American Cancer Society. The National Cancer Database is the largest in the world, and it captures 70% or more of newly diagnosed malignancies in the United States annually. The institutional review board evaluation was not obtained because the database provides de-identified data.

Study Population

Patients age 18 or older, diagnosed with PDAC between 2004 and 2016, were included in the study. Patients who received definitive surgery of the primary pancreatic cancer and those who had missing information on RT, chemotherapy, and immunotherapy were excluded. Patients with unknown or missing information about other covariates were not included in the adjusted multivariable analysis. The chemotherapy plus immunotherapy treatment sequence was divided into chemotherapy and immunotherapy within 30 days of each other, immunotherapy 31-90 days before chemotherapy, and immunotherapy 91-180 days before chemotherapy. There was not enough sample for chemotherapy >30 days before immunotherapy. The RT and immunotherapy treatment sequences were divided into RT and immunotherapy within 30 days of each other, 30 <RT <=180 days before immunotherapy, and 30<immunotherapy <=180 days before RT. Patients who started immunotherapy > 6 months before chemotherapy were excluded. Patients who started RT >6 months before immunotherapy or immunotherapy >6 months before RT were also excluded

End Points

The primary outcome of the current study was overall survival (OS), which was calculated from the date of diagnosis to the date of death from any cause. Those alive or lost to follow up were censored at the date of the last contact. We also reported the treatment patterns related to the use of immunotherapy.

Statistical Analyses

Descriptive statistics for categorical and continuous variables are reported. The association of various demographic and tumor-related factors with the type of treatment sequence was tested using the chi-square test of association. Kaplan-Meier curves and log-rank tests were utilized to report the difference in median OS between the treatment sequences. Multivariable Cox analysis was conducted to assess the OS of patients. The estimated hazard ratios with associated 95% confidence intervals (CI) were reported. A P-value of 0.05 was considered for a significant level. The SAS 9.4 software was used for the analysis.

Results

Chemotherapy and immunotherapy with or without RT

In total, 705 patients were eligible for the final analysis of this group. Among them, 621/705 (88.09%) started chemotherapy and immunotherapy within 30 days of each other, 41/705 (5.82%) started immunotherapy 31-90 days before the start of chemotherapy, and 43/705 (6.10%) started immunotherapy 91-180 days before the starting chemotherapy. Among 621 patients who started chemotherapy and immunotherapy within 30 days of each other 470/621 (75.68%) started the two treatments on the same day, 525/621 (84.54%) started within two days, and 551/621 (88.57%) started within seven days of each other. The last two proportions are cumulative.

The median age of diagnosis for the entire cohort was 64.00, with a range of 21-90 years. The median age of the diagnosis was 64.00 (21-90) years for patients who started chemotherapy and immunotherapy within 30 days of each other, 65.00 (44-83) years for the group who started immunotherapy 31-90 days before chemotherapy, and 64.00 (40-79) years for patients who started immunotherapy 91-180 days before chemotherapy. The majority of the patients were White, had high school degrees, had income >=$35,000, were insured, living in the urban areas, were treated in academic hospitals, and had a comorbidity score of zero. There was no association between the baseline characteristics of the patients and the treatment sequence except the hospital type and the year of diagnosis. Among patients who started chemotherapy and immunotherapy within 30 days of each other, 63.93% were treated at academic facilities. In patients who started immunotherapy 31-90 days before the start chemotherapy, 46.34% were treated at academic hospitals. In comparison, 72.09% of the patients who started immunotherapy 91-180 days before the start of chemotherapy were treated at academic hospitals. The proportion of patients who were diagnosed in 2011 and later were 46.22%, 63.41%, and 83.72% for those who started chemotherapy and immunotherapy within 30 days of each other, started immunotherapy 31-90 days before chemotherapy, and those who started immunotherapy 91-180 days before the start chemotherapy. The baseline characteristics are provided in Table 10.

Based on the KM curves, the OS of the treatment categories was not significantly different from each other (Figure 3). The median OS was 10.68 (CI: 9.79-11.66) months for patients who started chemotherapy and immunotherapy within 30 days of each other, 7.82 (CI: 5.85-11.93) months for patients who began immunotherapy 31-90 days before the start of chemotherapy, and 9.72 (6.67-14.62) months for patients who started immunotherapy 91-180 days before the start of chemotherapy Table 11.

In the multivariable Cox Proportional analysis (Table 12) adjusted for the age of diagnosis, sex, race, education, income, hospital type, comorbidity score, and year of diagnosis, there was no significant difference in the OS of patients who started immunotherapy 31-90 days before the start of chemotherapy (HR:1.06, CI: 0.72-1.56; p <0.781) compared to patients who started chemotherapy and immunotherapy within 30 days of each other. There was also no difference in the OS of patients who started immunotherapy 91-180 days before the start of chemotherapy (HR: 0.90, CI: 0.58-1.39; p <0.64) compared to patients who started chemotherapy and immunotherapy within 30 days of each other. The 1-year survival rates were 44% (CI: 40%-48%) for patients who started chemotherapy and immunotherapy within 30 days of each other, 32% (CI: 16%-48%) for those who started immunotherapy 31-90 days before starting chemotherapy, and 38% (CI: 20%-56%) for patients who started immunotherapy 91-180 days before beginning chemotherapy.

Radiation therapy and immunotherapy with or without chemotherapy

Among the 226 patients who received RT and immunotherapy, 177/226 (78.32%) started RT and immunotherapy within 30 days of each other, 34/226 (15.04%) started RT > 30 days before starting immunotherapy, and 15/226 (6.64%) started immunotherapy > 30 days before starting RT. Importantly, among those who began Rt and immunotherapy within 30 days of each other, 107/177 (60.45%) started the two treatment on the same day, 140/177 (79.66%) started the two treatments within 2 days from each other, and 153/177 (86.44%) patients started the two treatment within a week of each other indicating a pattern of care that clinicians are in favor of administrating the two treatment close to each other.

The median age of this cohort was 62.0 (33-85) years. The median age of those who started RT and immunotherapy within 30 days of each other was 61.0 (33-85), while the median

age of the patients who started RT > 30 days before starting immunotherapy was 64.0 (80-37) years, and patients who received immunotherapy > 30 days before the start of RT was 70.0 (47-80). Except for hospital type, comorbidity score, and year of diagnosis, no other variables were associated with the treatment sequence of RT and immunotherapy. Among patients who started RT and immunotherapy within 30 days of each other, 78.29%% were treated at academic hospitals, while 75.76% of the patients who started RT > 30 days before immunotherapy and 33.33% of patients who started immunotherapy > 30 days before RT were treated at academic hospitals. Among the patients who started RT and immunotherapy within 30 days of each other, 84.18% had comorbidity score of zero, while 73.53% of the patients who started RT> 30 days before the start of immunotherapy, and 46.47% of the patients who started immunotherapy > 30 days before the start of RT had comorbidity score of zero.

Among the patients who started RT and immunotherapy within 30 days of each other only 16.38% were diagnosed between 2011 and 2016, while 85.29% of the patients who started RT> 30 days before the start of immunotherapy, and 60.00% of the patients who started immunotherapy > 30 days before the start of RT were diagnosed between 2011 and 2016.

The characteristics of the patients are shown in Table 13. Based on KM, there was no significant difference in the median OS of the treatment sequence groups (Figure 4; p=0.497). The median OS was 12.39 (CI: 10.84-13.54) months for patients who started RT and immunotherapy within 30 days of each other only, 13.27 (CI: 11.20-19.19) months patients who started RT> 30 days before the start of immunotherapy, and 8.54 (CI: 5.09-15.67) months patients who started immunotherapy > 30 days before the start of RT (Table 14).

In the multivariable analysis, there was no significant difference in the OS of patients who started RT> 30 days before the start of immunotherapy (HR: 0.64, CI: 0.35-1.17; p=0.15)

compared to patients who started RT and immunotherapy within 30 days of each other. The OS was also not different between patients who started immunotherapy > 30 days before the start of RT (HR: 0.66, CI: 0.33-1.33; p=0.25) compared to patients who started RT and immunotherapy within 30 days of each other (Table 15). The 1-year survival rates were 51% (CI: 44%-59%) for patients who started RT and immunotherapy within 30 days of each other, 43% (CI: 17%-69%) for those who began immunotherapy > 30 days before beginning RT, and 62% (CI: 61%-79%) for patients who started RT > 30 days before starting RT.

Discussion

To our knowledge, the current study is the first and the most extensive research on reporting treatment patterns in the use of immunotherapy and comparing the impact of the timing of immunotherapy with chemotherapy and RT in PC patients who did not get definitive surgery of the pancreatic tumor.

This study provides information about the timing pattern of immunotherapy treatment in PDAC patients. The findings indicate that the majority of patients receive immunotherapy within 30 days of chemotherapy or RT. The results also suggest that clinicians tend to start immunotherapy close to the start of chemotherapy or RT. As noticed, the majority of the patients began immunotherapy on the same day with starting chemotherapy or RT. Current clinical guidelines favor the concurrent use of immunotherapy with chemotherapy or RT. However, starting immunotherapy on the same day with chemotherapy or RT may not deliver the optimal benefits as chemotherapy, and RT both cause transient immunosuppression. Starting immunotherapy during that window of systemic and local immunosuppression may minimize the synergetic effect of the interaction of immunotherapy with chemotherapy and RT. The majority of the patients who received immunotherapy with 30 days of chemotherapy or RT were treated at academic centers, and these centers tend to recommend the concurrent use of

immunotherapy with chemo and RT. Current ongoing clinical trials which some of these centers may be participating in are also administering the concomitant use of immunotherapy with other treatments. Data are lacking to either confirm or oppose the current treatment sequence used in these clinical trials.

In the current study, the treatment sequence of immunotherapy with chemotherapy and RT was not associated with improved OS. In our unpublished data, we found that immunotherapy is associated with improved OS when combined with chemotherapy or chemoradiation. Based on the findings of that data, we decided to investigate the timing of immunotherapy with other cancer treatments and see if the timing of immunotherapy matters. However, the results of the current study indicate that the improved OS associated with the use of immunotherapy in combination with chemotherapy or chemoradiation therapy does not depend on the sequence of the treatments.

The optimal time of immunotherapy may depend on the mechanism of the immunotherapy drug and the cancer type[181]. For example, a preclinical study of colorectal carcinoma found that the optimal timing for the anti-CTLA4 blockade is before RT, while for anti-OX40 agonists, the best time is after RT[182]. These findings have been supported by clinical studies and case series of metastatic melanoma, gastrointestinal cancers, NSCLC, lymphoma, and head and neck cancer patients in which patients received immunotherapy first and then received RT or chemotherapy[183-189]. Contrarily a few other studies which only included brain metastasis (BMs) patients from melanoma reported better results when SRS was administered either before immunotherapy or concurrently[190-193]. Patients with BMs from melanoma who received whole-brain RT plus SRS before ipilimumab had better median OS compared to ipilimumab before SRS or concurrently with [26 vs. six vs. 18] months[190].

Nonetheless, the majority of these studies were not designed to investigate the treatment sequence due to the absence of comparison group, had small sample size, only looked into SRS or RT in BMs, and only included ipilimumab. Current ongoing clinical trials are designed to deliver immunotherapy concurrently with RT or after RT, ignoring the reports that giving anti-CTLA4 before palliative RT may improve response rate[194,195].

The negative results of the study may be in part due to the small sample size of some of the treatment sequence groups, especially for immunotherapy plus RT cohort. The insignificant results of the sequence of RT with immunotherapy may be due to the use of a low dose of conventional RT fraction in most of these patients. The majority of the patients received conventional RT with 1.8-2 Gray per fraction, and past reports have suggested that higher fractional doses such as those provided with SBRT are required to improve immunotherapy when combined with RT. It is also possible that the benefit of immunotherapy with RT is drowned by using immunotherapy with chemo and vice versa. It is also possible that the sequence of immunotherapy with other treatments such as chemotherapy and RT does not matter, and immunotherapy is associated with improved OS, as found in our unpublished data. Our findings are consistent with the results of other studies in which there was no difference in the OS of BMs patients when RT was delivered before immunotherapy or after immunotherapy or if RT was administered concurrently with immunotherapy or sequentially [196-198].

The strength of this study is the relatively large sample size, which allows for adjusting for various critical patient and tumor-related factors. However, the study is not without several limitations. The limitations include selection bias, lack of information on the cause of death, lack of information about the type of immunotherapy, and if a single or combined immunotherapy was administered, and lack of detailed information on the use of multi-agent chemotherapy.

The small sample size for some treatment sequence groups in both immunotherapy plus chemotherapy and immunotherapy plus RT was another limitation of the study.

Conclusion

To our knowledge, the current study is the first and the most extensive research that has compared the timing of immunotherapy with chemotherapy and RT. There was no association between the treatment sequence of immunotherapy with chemotherapy or RT and the OS of the patients. Future studies with a large sample size for each subgroup of the treatment sequences are needed to investigate the timing of immunotherapy with chemotherapy and RT.

Figure 3. Overall survival of unresectable PDAC patients chemotherapy plus immunotherapy regardless of RT; chemotherapy and immunotherapy started within 30 days of each other (blue), immunotherapy started 31-90 days before chemotherapy (red), immunotherapy started 91-180 days before chemotherapy (green)

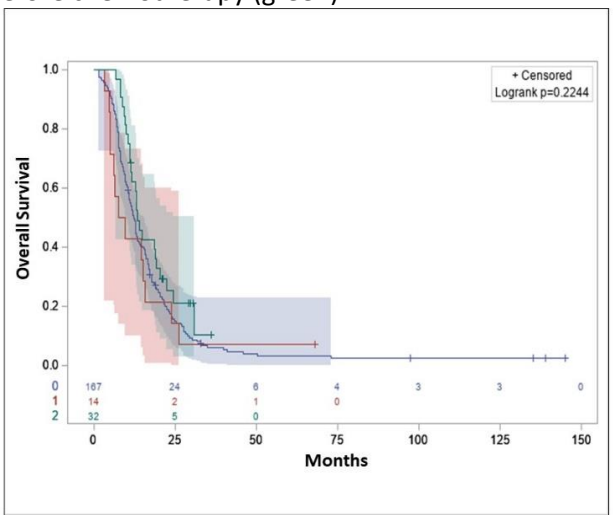


Figure 4. Overall survival of unresectable PDAC patients with RT plus immunotherapy regardless of chemotherapy: RT and immunotherapy started within 30 days of each other (blue), immunotherapy started >30 days before the start of radiation therapy (red), radiation therapy started > 30 days before the start of chemotherapy (green)

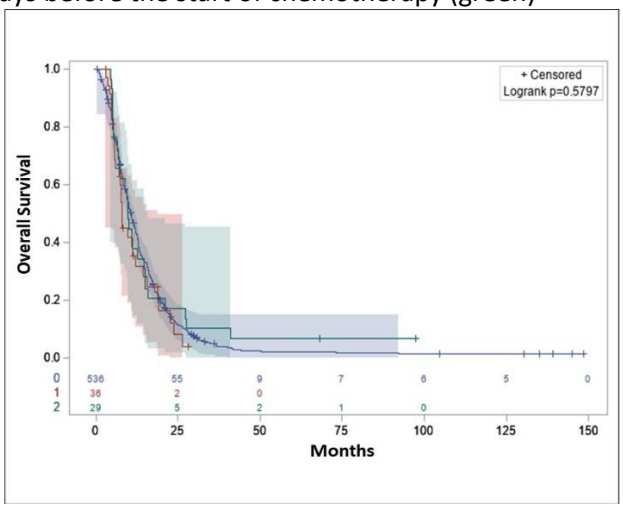


Table 10. Baseline characteristics of the sequence of immunotherapy with chemotherapy in PDAC patients with no surgery

Variable		CTx and Immx within 30 days of each other 621 (88.09%)	Immx 31-90 days bf CTx 41 (5.82%)	Immx 91-180 days bf CTx	Total 705	P
Age at diagnosis (mean)		64.0 (21-90)	65.0 (44-83)	64.0 (40-79)	64.0 (21-90)	
Sex	Male	354 (57.00)	24 (58.54)	23 (53.49)	401 (56.88)	0.88
	Female	267 (43.00)	17 (41.46)	20 (46.51)	304 (43.12)	
Race	White	538 (87.48)	37 (90.24)	40 (95.24)	615 (88.11)	0.62
	Black	56 (9.11)	3 (7.32)	1 (2.38)	60 (8.60)	
	Other	21 (3.41)	2.44	1 (2.38	23 (3.30)	
	Unknown	6	0	1	7	
Education	>=13% HG	216 (35.06)	11 (27.50)	10 (23.26)	237 (33.91)	0.19
	<13%	400 (64.94)	29 (72.50)	33 (76.74	462 (66.09)	
	Unknown	5	1	0	6	
Income	>=$35,000	402 (65.26)	28 (71.79)	29 (67.44)	459 (65.76)	0.69
	<35,000	214 (34.74)	11 (28.21)	14 (38.56)	239 (34.24)	
	Unknown	5	2	0	7	
Living place	Urban	587 (97.83)	38 (97.44)	42 (100.00)	667 (97.94)	0.62
	Rural	13 (2.17)	1 (2.56)	0 (0.00)	14 (2.06)	
	Unknown	21	2	1	24	
Hospital Type	Academic	390 (63.93)	19 (46.34)	31 (72.09)	440 (63.40)	0.037
	Community	220 (36.07)	22 (53.66)	12 (27.91)	254 (36.60)	

	Unknown	11	0	0	11	
Insurance	Yes	572 (89.45)	40 (97.56)	39 (97.50)	651 (98.34)	0.83
	No	9 (10.55)	1 (2.44)	1 (2.50)	11 (1.66)	
	Unknown	41	0	2	43	
Charlson Score	0	486 (78.26)	30 (73.17)	34 (79.07)	550 (78.01)	0.83
	1	108 (17.39)	8 (19.51)	8 (18.60)	124 (17.59)	
	>=2	27 (4.35)	3 (7.32)	1 (2.33)	31 (4.40)	
Year of Diagnosis	2004-2010	334 (53.78)	15 (36.59)	7 (16.28)	356 (50.50)	0.0001
	2011-2016	287 (46.22)	26 (63.41)	36 (83.72)	349 (49.50)	

CTx=chemotherapy Immx=immunotherapy bf=before

Table 11. Median OS of chemotherapy and immunotherapy sequence groups

Variable	Median OS (95% CI)
Chemotherapy and immunotherapy within 30 days of each other	10.68 (9.79-11.66)
Immunotherapy 31-90 days before chemotherapy	7.82 (5.85-11.93)
Immunotherapy 91-180 days before chemotherapy	9.72 (6.67-14.62)

Table 12. Univariate and multivariable Cox analysis of the sequence of chemotherapy and immunotherapy in PDAC patients with no surgery

Variables		Univariate Analysis		Multivariable Analysis	
		HR (95% CI)	P	HR (95% CI)	P
CT plus immunotherapy	CTx and Immx within 30 days	Ref		Ref	
	Immx 31-90 days before CTx	1.18 (0.82-1.71)	0.38	1.06 (0.72-1.56)	0.78
	Immx 91-180 days before CTX	0.90 (0.61-1.33)	0.60	0.90 (0.58-1.39)	0.64

Table 13. Baseline characteristics of the sequence of radiation therapy with immunotherapy in PDAC patients with no surgery

Variable		RT and Immx within 30 days of each other 177 (78.32)	Immx >30 days before RT 15 (6.64)	RT>30 days before Immx 34 (15.04)	Total 226	P
Age at diagnosis (mean)		61.0 (33-85)	70.0 (47-80)	64.0 (80-37)	62.0 (33-85)	
Sex	Male	99 (55.93)	10 (66.67)	15 (44.12)	124 (54.87)	0.29
	Female	78 (44.07	5 (33.33)	19 (55.88)	102 (45.13)	
Race	White	154 (89.53)	12 (80.00)	29 (85.29)	195 (88.24)	0.37
	Black	12 (6.98)	2 (13.33)	5 (14.71)	19 (8.60)	
	Other	6 (3.49)	1 (6.67)	0 (0.00)	7 (3.17)	
	Unknown	5	0	0	5	
	>=13% HG	58 (32.95)	7 (46.67)	11 (32.35)	76 (33.78)	0.55

Education	<13%	118 (67.05)	8 (53.33)	23 (67.65)	149 (66.22)	
	Unknown	1	0	0	1	
Income	>=$35,000	123 (69.89)	8 (53.33)	24 (70.59)	155 (68.89)	0.40
	<35,000	53 (30.11)	7 (46.67)	10 (29.41)	70 (31.11)	
	Unknown	1	0	0	1	
Place of Living	Urban	168 (97.67)	15 (100.00)	33 (100.00)	216 (98.18)	0.57
	Rural	4 (2.33)	0 (0.00)	0 (0.00)	4 (1.82)	
	Unknown	5	0	1	6	
Hospital Type	Academic	137 (78.29)	5 (33.33)	25 (75.76)	167 (74.89)	0.0006
	Community	38 (21.71)	10 (66.67)	8 (24.24)	56 (25.11)	
	Unknown	2	0	1	3	
Insurance	Yes	148 (98.67)	14 (100.00)	33 (97.06)	195 (98.48)	0.70
	No	2 (1.33)	0 (0.00)	1 (2.94)	3 (1.52)	
	Unknown	27	1	0	28	
Charlson Score	0	149 (84.18)	7 (46.67)	25 (73.53)	181 (80.09)	0.001
	1	23 (12.99)	5 (33.33)	8 (23.53)	36 (15.93)	
	>=2	5 (2.82)	3 (20.00)	1 (2.94)	9 (3.98)	
Year of Diagnosis	2004-2010	148 (83.62)	6 (40.00)	5 (14.71)	159 (70.35)	0.0001
	2011-2016	29 (16.38)	9 (60.00)	29 (85.29)	67 (29.65)	

RT=radiation therapy Immx=immunotherapy bf=before

Table 14. Median OS of RT and immunotherapy sequence groups

Variable	Median OS (95% CI)
RT and immunotherapy within 30 days of each other	12.39 (10.84-13.54)
RT > 30 days before immunotherapy	13.27 (11.20-19.19)
Immunotherapy > 30 days before RT	8.54 (5.09-15.67)

Table 15. Univariate and multivariable Cox analysis of the sequence of radiation therapy and immunotherapy in PC patients with no surgery

Variables		Univariate Analysis		Multivariable Analysis	
		HR (95% CI)	P	HR (95% CI)	P
Radiation therapy plus immunotherapy	RT and Immx within 30 days	Ref		Ref	
	RT >30 days bf Immx	0.71 (0.47-1.09)	0.11	0.63 (0.35-1.17)	0.146
	Immx >30 days bf RT	1.17 (0.66-2.06)	0.59	0.66 (0.33-1.33)	0.245

CHAPTER 5

THE IMPACT OF NEOADJUVANT AND ADJUVANT IMMUNOTHERAPY ON THE SURVIVAL OF PANCREATIC CANCER PATIENTS: A RETROSPECTIVE ANALYSIS

Abstract

Background: Immunotherapy has become an essential part of cancer treatment after showing excellent efficacy in various malignancies. However, its effectiveness in pancreatic adenocarcinoma (PDAC), especially in resectable pancreatic cancer, has not been studied. The primary objective of this study is to compare the OS impact of immunotherapy between PDAC patients who receive neoadjuvant immunotherapy and patients who receive adjuvant immunotherapy. The secondary aim is to investigate the impact of neoadjuvant and adjuvant immunotherapy in combination with chemotherapy and chemoradiation by performing subsets analyses of these two groups. **Methods:** Patients diagnosed with PDAC between 2004 and 2014 were identified from the National Cancer Database (NCDB). Multivariable Cox proportional hazard analysis was performed to examine the difference in the OS of patients who received adjuvant and neoadjuvant immunotherapy in combination with chemotherapy and chemoradiation. The multivariable analysis was adjusted for essential factors such as the age of diagnosis, sex, race, education, income, place of living insurance status, hospital type, comorbidity score, and year of diagnosis was used to assess the OS of the patients. **Results:** Overall, 526 patients received immunotherapy, among whom 408/526 (77.57%) received neoadjuvant immunotherapy, and the remaining 118/526 (22.43%) received adjuvant immunotherapy. Neoadjuvant immunotherapy was not associated with improved OS (HR: 1.10, CI: 0.79-1.41; p=0.71) compared to adjuvant immunotherapy in the multivariable analysis. In the subset analysis of neoadjuvant and adjuvant therapies patients, immunotherapy combined with chemotherapy or chemoradiation was not associated with improved OS compared to chemotherapy or chemoradiation without immunotherapy. **Conclusion:** In this study, no

difference in the OS between patients who received neoadjuvant immunotherapy and patients who received adjuvant immunotherapy was noticed. Future studies comparing neoadjuvant adjuvant immunotherapy combined with chemotherapy, radiation therapy, and chemoradiation are needed.

Introduction

The majority of pancreatic cancer (PC) patients are diagnosed with unresectable PC, while less than 20% are diagnosed with resectable cancer[19,199]. The current standard-of-care treatment for resectable PC is upfront surgery followed by adjuvant single or combined chemotherapy[200]. The median overall survival (OS) after surgery is between 15-24 months with a five-year survival rate of 20% with some recent data showing a median OS of up to 54 months[21,201-203]. Up to 80% of patients who undergo surgery experience recurrence, owing significantly to micrometastases, which occur early in the disease, or microscopic residual disease in the tumor bed[19,199]. These difficulties have brought adjuvant therapy to the forefront of PC treatment. Despite the improvement in surgical techniques, radiation therapy (RT), and chemotherapeutic options, only a modest increase in the OS has been noticed[204]. Due to the lack of current standard-of-care treatments, novel treatment strategies such as the use of immunotherapeutics are desperately needed.

Immunotherapy has worked well in many solid cancers, but its use in PC is not clear[42,149]. The use of immunotherapy to date has been mainly in the metastatic setting. However, new evidence indicates that immunotherapy could be useful in patients with localized disease who have a high risk of micrometastases[22,86,102-105]. Chemotherapy and RT increase tumor-specific T cell infiltration, decrease T regulatory cells, and suppress Myeloid-derived suppressor cells (MDSCs), and can have synergistic interaction with immunotherapy[22,88,129]. Immunotherapy was associated with tumor regression and improved OS in preclinical studies of PDAC when used in combination with other treatments[89,90]. To achieve the optimal OS effect of the use of immunotherapy with chemotherapy and chemoradiation in PC, the sequence of the treatment is

critical. The sequence of treatment even becomes more important in resectable PS due to the potential interactions of systemic therapy with surgery. Due to the higher rate of recurrence after surgery, the early implementation of systemic therapy is needed[205].

Neoadjuvant treatment (NAT) strategies have emerged and been employed as an attractive option for resectable and potentially resectable PC[206,207]. Neoadjuvant treatment can also turn those initially borderline resectable or even some unresectable disease into resectable[206,207]. This strategy provides an opportunity for an early start of systemic therapy in contrast to upfront surgery, where more than half of the patients may not receive adjuvant therapy due to postoperative complications and declining performance status[208-210]. Recent clinical trials and systematic reviews have reported the survival benefit of NAT[211-214]. However, the effectiveness of NAT in resectable PC remains unclear as there are still many questions to be addressed before NAT become a standard of care[215].

The neoadjuvant and adjuvant use of immunotherapy both could be justified. Neoadjuvant immunotherapy with chemotherapy or chemoradiation could shrink the tumor, downstage nodal disease, and increase the chance of margin negative resection as reported for neoadjuvant systemic therapy[216,217]. It may also work with chemotherapy or chemoradiation to mitigate the risk of micrometastases[218,219]. Conversely, adjuvant immunotherapy may be useful when the bulk of the tumor is removed, and there is a minimal residual disease, which T cells can target and eliminate. Also, the timing of adjuvant immunotherapy needs to be appropriately chosen as surgery is associated with transient immunosuppression[220,221]. The use of immunotherapy in neoadjuvant or adjuvant setting combined with chemoradiation in PDAC has been limited. Some clinical trials studying the efficacy of immunotherapy in resectable PDAC combined with chemoradiation therapy have shown positive response and measurable activity[80,159-161]. However, large studies of neoadjuvant and adjuvant immunotherapy in

resectable PC are lacking. The objective of this study is to investigate the impact of neoadjuvant and adjuvant immunotherapy in combination with chemotherapy and chemoradiation on the OS of resectable PDAC patients using the National Cancer Database (NCDB).

Methods

Data source

The data for this study was extracted from a de-identified file of the National Cancer Database (NCDB). The NCDB is a joint program of the Commission on Cancer of the American College of Surgeons and the American Cancer Society. It captures 70% or more of newly diagnosed malignancies in the United States annually. This study was exempt from the Institutional Review Board (IRB) because the de-identified data were used.

Study population

The study included patients age 18 or older who were diagnosed with PADC between 2004 and 2016 and received definitive surgery of the tumor. The ICD-O-3 histology codes of 8000, 8010, 8020-8022, 8140, 8141, 8211, 8230, 8500, 8521, 8050, 8260, 8441, 8450, 8453, 8470-8473, 8480, 8481, 8503,8250,8440, 8560 were used to identify PADC. The surgical site-specific code was used to identify patients with definitive surgery of the pancreas. Patients who were missing information about RT, chemotherapy, immunotherapy, and sequence of these treatments with each other and surgery were excluded. Patients with the M1 stage and those with unknown or missing information about other covariates in the adjusted multivariable analysis were also excluded. The analysis of the sequence of immunotherapy with RT alone was not performed due to the small sample size. The variable of days from diagnosis to the start of the treatment was used to identify neoadjuvant and adjuvant immunotherapy, chemotherapy, and chemoradiation. If chemotherapy, RT, and immunotherapy were delivered more than eight

months before or after surgery, those patients were excluded. If immunotherapy was received more than six months before or after chemotherapy or RT, those patients were also excluded. The primary outcome of the current study was the OS of the patients, which was calculated from the date of diagnosis to the date of death. Patients who were alive or lost to follow up were censored. The subset analysis of the neoadjuvant group only included patients who received only neoadjuvant chemotherapy, immunotherapy, RT, and chemoradiation. If any of the treatment was not neoadjuvant, they were excluded for this subset analysis. Patients with no treatment were also excluded. The subset analysis of adjuvant treatment comparison included patients who only received adjuvant chemotherapy, immunotherapy, RT, and chemoradiation. If any of the treatment was not adjuvant, those patients were excluded for adjuvant subset analysis. Patients with no treatment were also excluded from this subset analysis.

Explanatory variables

The main predictors of OS in this study were immunotherapy combined with chemotherapy, and immunotherapy combined with chemoradiation. The age of diagnosis, gender, race, urban and rural living status, income, education, treatment facility type, comorbidity score, insurance status, year of diagnosis, and receipt of chemotherapy, RT, and immunotherapy were other explanatory variables used in the multivariable analysis.

Statistical analyses

Descriptive statistics for categorical and continuous variables are reported. A Chi-square test was used to report the association of the explanatory variables with the treatment sequence of immunotherapy with chemotherapy and chemoradiation therapy. The difference in the median OS between the different treatment sequences was reported using the Kaplan-

Meier curves based on the log-rank test. The Cox proportional analysis was used to determine the OS of the patients. The estimated hazard ratio (HR) with its associated 95% confidence intervals (CI) was reported. A p-value of 0.05 was considered significant which based on the two-side t-test. The analysis was conducted using the SAS 9.4 software.

Results

Neoadjuvant immunotherapy vs. adjuvant immunotherapy. Among 526 patients who received immunotherapy, 408/526 (77.57%) received neoadjuvant immunotherapy, and the remaining 118/526 (22.43%) received adjuvant immunotherapy. The median age of diagnosis among patients who received immunotherapy was 62 with a range of (29-88) years. The median age of diagnosis of patients who received neoadjuvant immunotherapy was 62.0 (34-88) years, while it was 62.5 (29-86) years in patients who received adjuvant immunotherapy. A majority of the patients were White, living in the urban areas, had a high school degree, had income >=$35000, had insurance, were treated in academic hospitals, and had a Charlson/Deyo Score of zero. There was no association between the baseline characteristics of the patients and receiving neoadjuvant or adjuvant immunotherapy except the year of diagnosis. Among patients who received neoadjuvant immunotherapy, 41.67% were diagnosed after 2011, while among patients who received adjuvant immunotherapy, 66.10% were diagnosed after 2011. Among those diagnosed after 2011, 68.55% received neoadjuvant immunotherapy compared to 31.45% who received adjuvant immunotherapy, while among those who were diagnosed before 2011, 85.61% received neoadjuvant immunotherapy compared to 14.39% who received adjuvant immunotherapy. The baseline characteristics of the study population are shown in Table 16. We did not report the baseline characteristics of the neoadjuvant and adjuvant subsets analyses due to insignificant results of these subsets.

The KM curves did not show any significant difference in the median OS of patients who received neoadjuvant immunotherapy compared to adjuvant immunotherapy (Figure 5). The median OS of patients who received neoadjuvant immunotherapy was 26.78 months (CI: 23.92-31.24) vs. 34.37 months (CI: 24.21-42.28 months; p=703) in patients who received adjuvant immunotherapy. In the multivariable Cox analysis, neoadjuvant immunotherapy was not associated with improved OS (HR: 1.10, CI: 0.79-1.41; p=0.71) compared to adjuvant immunotherapy (Table 17).

Subset analyses

Only neoadjuvant subset analysis. This group was restricted to patients who only received neoadjuvant treatments such as chemotherapy, RT, chemoradiation, and immunotherapy. If any of the treatment was not neoadjuvant, those observations were excluded from this subset analysis. Based on KM curves, patients who received neoadjuvant immunotherapy had significantly improved OS with an absolute median OS benefit of 2.6 months compared to patients who did not receive immunotherapy (25.10 months, CI: 21.42-27.96 vs. 22.51 months, CI: 22.21-22.77) (Figure 6a). There was no difference in the median OS of patients who received neoadjuvant chemotherapy plus immunotherapy compared to neoadjuvant chemotherapy alone (Figure 6b), and patients who received neoadjuvant chemoradiation plus immunotherapy compared patients who received only neoadjuvant chemoradiation (Figure 6c). In the univariate Cox proportional analysis, neoadjuvant immunotherapy was associated with improved OS (HR: 0.88, CI: 0.78-0.98; p <0.026) compared to no immunotherapy. However, in the multivariable analysis, this association became nonsignificant (Table 18). In the multivariable analysis, there was no difference in the median OS of patients who received neoadjuvant chemotherapy plus immunotherapy compared to neoadjuvant chemotherapy alone (HR: 0.93, CI: 0.73-1.20; p=0.97) and patients who received

neoadjuvant chemoradiation plus immunotherapy compared to neoadjuvant chemoradiation alone (HR: 0.94, CI: 0.81-1.09; p=0.425) (Table 18).

Adjuvant subset. This analysis included patients who only received adjuvant chemotherapy, RT, chemoradiation, and immunotherapy. If any of the treatment was not adjuvant, those patients were not included in this subset analysis. Based on KM curves, there was no difference in the median OS of patients who received adjuvant immunotherapy compared to patients who received other adjuvant treatment but did not receive immunotherapy (Figure 7a). There was no difference in the median OS of patients who received adjuvant chemotherapy plus immunotherapy or chemoradiation plus immunotherapy compared chemotherapy or chemoradiation without immunotherapy (Figure 7b). In the multivariable analysis, there was no significant difference in the OS of patients who received adjuvant immunotherapy compared to no immunotherapy (HR:1.00, CI: 0.76-1.32; p=0.99). A significant difference in the OS was also not observed between patients who received adjuvant chemotherapy plus immunotherapy or chemoradiation plus immunotherapy compared chemotherapy or chemoradiation without immunotherapy (HR: 1.01, CI: 0.75-1.37; p= 0.94) (Table 19). The adjuvant chemotherapy plus immunotherapy group was combined with chemoradiation plus immunotherapy due to a small sample size.

Discussion

To our knowledge, the current study is the most extensive study that has compared the impact of neoadjuvant immunotherapy vs. adjuvant immunotherapy on the OS of PDAC patients who received definitive surgery of the pancreatic tumor. There was no significant difference in the median OS of patients who received neoadjuvant immunotherapy compared to patients who received adjuvant immunotherapy. However, in the neoadjuvant subset analysis,

immunotherapy was associated with significantly improved OS compared to no immunotherapy in the univariate analysis though this significance was lost upon multivariable analysis.

The tumor cells use mechanisms such as the up-regulation of immune checkpoint signaling programmed death-ligand 1 (PD-L1), downregulation of cytotoxic T-lymphocyte-associated protein 4 (CTLA4), and the recruitment of MDSCs, to evade the immune system[125-127]. Immunotherapy, especially checkpoint inhibitors, down-regulates the PD-L1 pathway and upregulates anti-CTLA4[86,88]. The insignificant results of neoadjuvant immunotherapy compared to adjuvant immunotherapy may indicate that the impact of immunotherapy on the OS of PC patients who receive definitive surgery of the pancreatic tumor is not related to the sequence of immunotherapy with surgery. Our unpublished data found that immunotherapy was associated with improved OS compared to no immunotherapy indicating the potential benefit of immunotherapy in these patients. In that study, the sequence of the treatment was not studied. A small sample size of group comparisons in the neoadjuvant and adjuvant subsets analyses may be responsible for insignificant results.

Limitations

The large sample size for the comparison of neoadjuvant immunotherapy vs. adjuvant immunotherapy is the most important strength of the current study, which allowed us to adjust for patient and tumor characteristics. However, the study is not without limitations, most of which are inherent to NCDB and include selections bias, lack of information of the cause of death, lack of information about the type of immunotherapy and if a single or combined immunotherapy was administered, and lack of detailed information on the use of multi-agent chemotherapy. One other limitation was that due to the small sample size for immunotherapy plus RT, the sequence of immunotherapy with RT alone was not performed. Also, there were not enough cases for adjuvant comparison, and that maybe one of the reasons that we failed to

find any significant difference in the OS of patients who received adjuvant immunotherapy, chemotherapy plus immunotherapy, and chemoradiation plus immunotherapy compared to their counterparts without immunotherapy.

Nonetheless, in this study, a robust analysis of the impact of the timing of immunotherapy with surgery on the OS of PC patients who received definitive surgery of the pancreatic tumor using the NCDB was performed. The NCDB is the largest cancer database in the world which captures the majority of the annual cancer cases diagnosed in the U.S. It serves as an outstanding source for the investigation of the impact of novel cancer treatments on the OS of cancer patients

Conclusions

No difference in the OS between patients who received neoadjuvant immunotherapy and those who receive adjuvant immunotherapy was noticed. However, in the univariate analysis, neoadjuvant immunotherapy was associated with significantly improved OS compared to no immunotherapy. The findings warrant future studies with a large sample size for both neoadjuvant and adjuvant treatment comparisons of immunotherapy.

Table 16. Baseline characteristics of neoadjuvant vs. adjuvant immunotherapy

Variable		Neoadjuvant immunotherapy 408 (77.57%)	Adjuvant immunotherapy 118 (22.43%)	Total 526	P
Age at diagnosis (mean)		61.57	62.20	62.00 (29-88)	0.54
Sex	Male	238 (58.33)	62 (52.54)	300 (57.03)	
	Female	170 (41.67)	56 (47.46)	226 (42.97)	0.26
Race	White	359 (90.43)	113 (96.58)	472 (91.83)	0.049
	Black	21 (5.29)	4 (3.42)	25 (4.86)	
	Other	17 (4.28)	0 (0.00)	17 (3.31)	
	Unknown	11	1	12	
Education	>=13% HG	112 (27.72)	29 (24.58)	141 (27.01)	0.50
	<13%	292 (72.28)	89 (75.42)	381 (72.99)	
	Unknown	4	0	4	
Income	>=$35,000	292 (72.28)	89 (75.42)	381 (72.99)	
	<35,000	112 (27.72)	29 (24.58)	141 (27.01)	0.50
	Unknown	4	0	4	
Place of Living	Urban	384 (98.71)	115 (99.14)	499 (98.81)	
	Rural	5 (2.29)	1 (0.86)	6 (1.19)	0.71
	Unknown	19	2	21	
Hospital Type	Academic	318 (78.91)	95 (82.61)	413 (79.73)	
	Community	85 (21.09)	20 (17.29)	109 (20.27)	0.38

	Unknown	5	3	8	
Insurance Status	Insured	398 (98.51)	118 (100.00)	516 (98.85)	
	Not insured	6 (1.49)	0 (0.00)	6 (1.15)	0.18
	Unknown	4	0	4	
Charlson/Deyo Score	0	303 (74.26)	88 (74.58)	391 (74.33)	
	1	89 (21.81)	25 (21.19)	114 (21.67)	
	>=2	16 (3.92)	5 (4.24)	21 (3.99)	0.98
Year of Diagnosis	2004-2010	238 (58.33)	40 (33.90)	278 (52.85)	0.0001
	2011-2016	170 (41.67)	78 (66.10)	248 (47.15)	

Table 17. Univariate and multivariate Cox regression analysis of neoadjuvant immunotherapy vs. adjuvant immunotherapy

Variable		N (%)	Univariable analysis		Multivariable analysis	
			Hazard Ratio (95% CI)	P	Hazard Ratio (95% CI)	P
Immunotherapy	Neoadjuvant immunotherapy	408 (77.57)	1.06 (0.80-1.39)	0.703	1.06 (0.79-1.41)	0.71
	Adjuvant immunotherapy	118 (22.43)	Ref		Ref	

The multivariable analysis was adjusted for the age of diagnosis, sex, race, income, education, place of living, treatment facility type, insurance status, comorbidity score, and year of diagnosis

Table 18. Cox regression analysis of only neoadjuvant immunotherapy combinations

Variable		N (%)	Univariable analysis		Multivariable analysis	
			Hazard Ratio (95% CI)	P	Hazard Ratio (95% CI)	P
Immunotherapy	Neoadjuvant immunotherapy	373 (1.09)	0.88 (0.78-0.98)	0.026	0.93 (0.82-1.05)	0.220
	No immunotherapy	33,921 (98.91)	Ref		Ref	
CTx plus immunotherapy	Neoadjuvant CTx plus imm	95 (0.53)	0.84 (0.65-1.07)	0.15	0.93 (0.73-1.20)	0.57
	Adjuvant CTx only	17,868 (99.47)	Ref			
CTxRT plus immunotherapy	Neoadjuvant CTxRTx plus imm	258 (1.64)	0.90 (0.78-1.03)	0.12	0.94 (0.81-1.09)	0.43
	Adjuvant CTxRTx only	15,466 (98.36)	Ref			

CTx= chemotherapy CTxRTx=chemoradiation therapy imm=immunotherapy

Table 19. Cox regression analysis of only adjuvant immunotherapy combinations

Variable		N (%)	Univariable analysis		Multivariable analysis	
			Hazard Ratio (95% CI)	P	Hazard Ratio (95% CI)	P
Immunotherapy	Adjuvant immunotherapy	106 (0.96)	0.98 (0.76-1.28)	0.91	1.00 (0.76-1.32)	0.99
	No immunotherapy	10,950 (99.04)	Ref		Ref	
CTx or CTxRTx plus immunotherapy	Adjuvant CTx or CTxRTx plus imm	90 (0.88)	0.97 (0.73-1.30)	0.85	1.01 (0.75-1.37)	0.94
	Adjuvant CTx or CTxRTx	10,104 (99.12)	Ref		Ref	

We combined adjuvant chemotherapy plus immunotherapy with adjuvant chemoradiation plus immunotherapy due to a small sample size. When analyzed separately, the results were the same.

Figure 5: Overall survival of resectable PDAC patients for neoadjuvant immunotherapy (red) vs. adjuvant immunotherapy (blue)

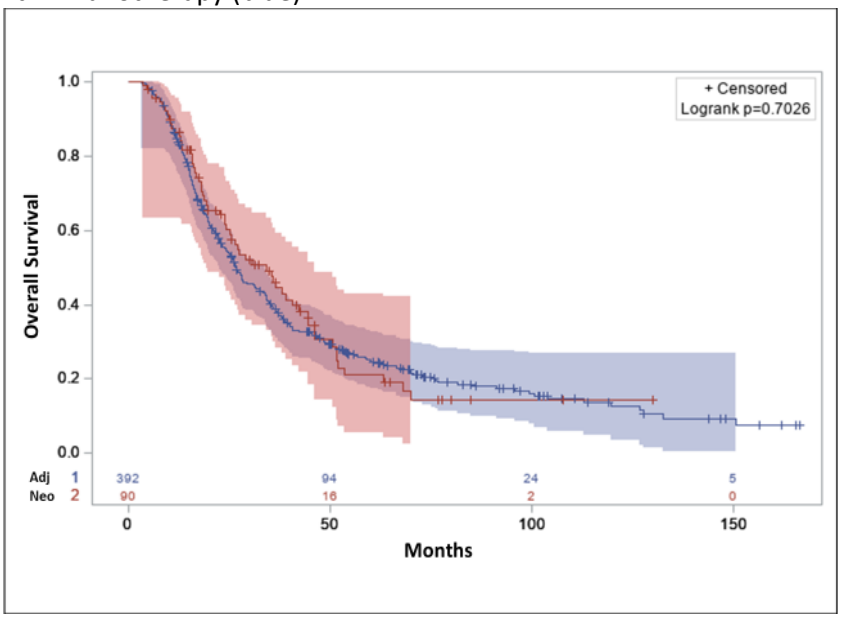


Figure 6a: Overall survival of resectable PDAC patients who received only neoadjuvant therapies with (blue) or without immunotherapy (red)

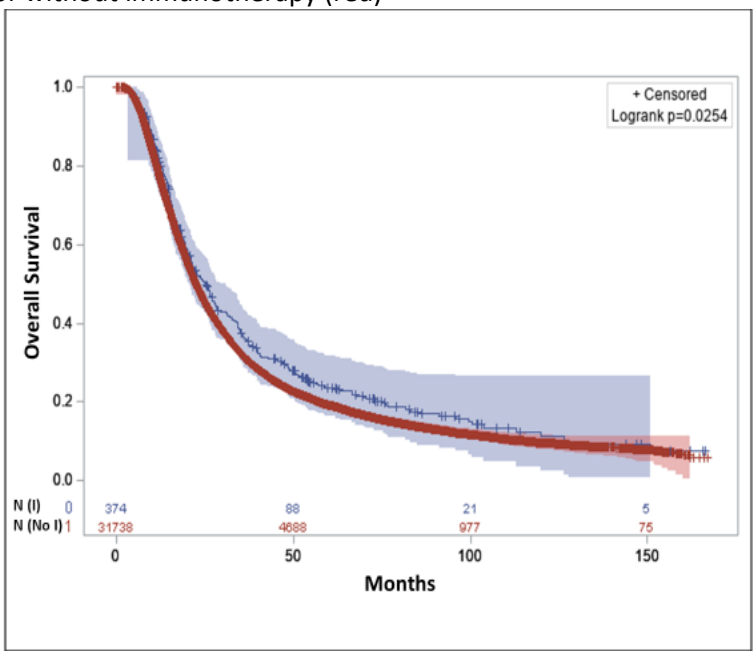


Figure 6b: Overall survival of resectable PDAC patients who received neoadjuvant chemotherapy with (blue) or without (red) neoadjuvant immunotherapy

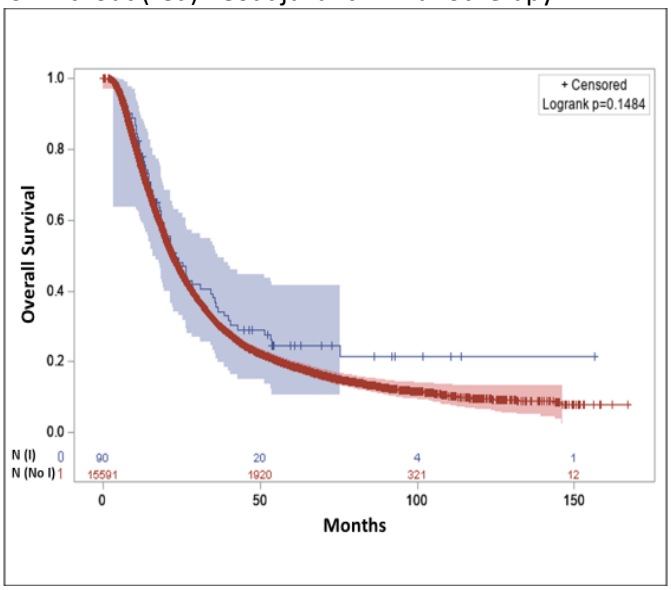


Figure 6c: Overall survival of resectable PDAC patients who received neoadjuvant chemoradiation with (blue) or without (red) neoadjuvant immunotherapy

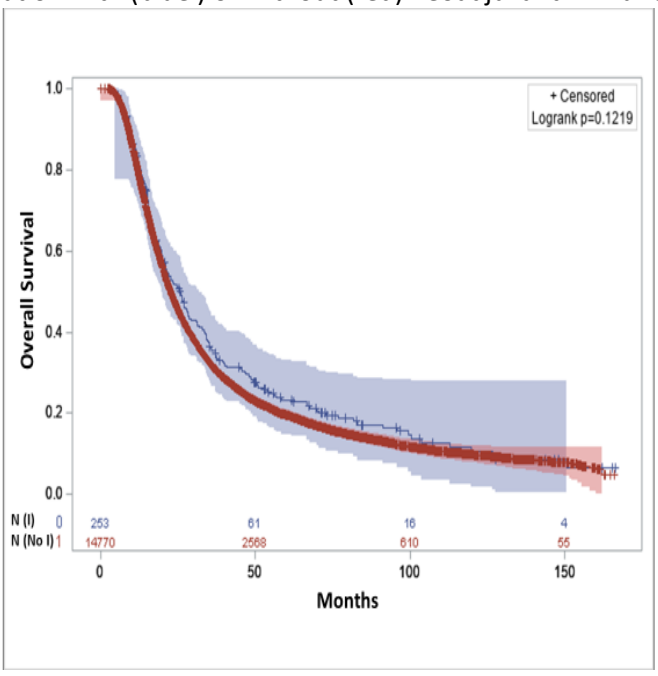


Figure 7a: Overall survival of resectable PDAC patients who received only adjuvant therapies with (blue) or without (red) immunotherapy

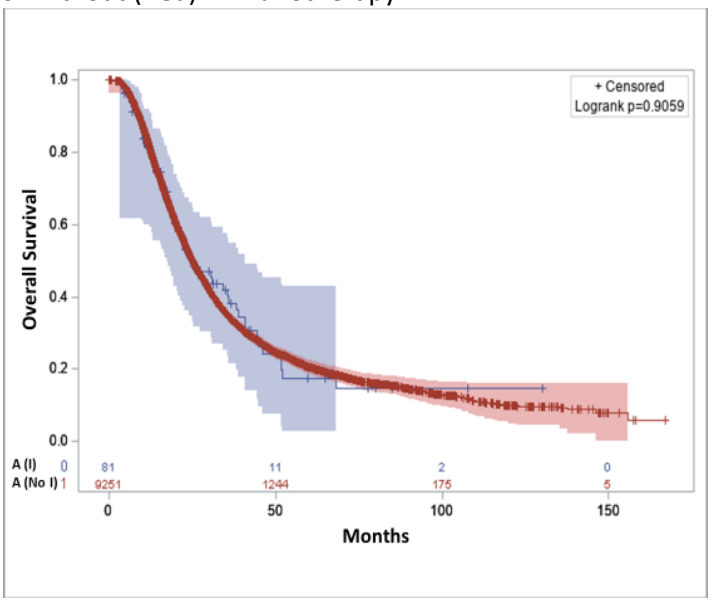
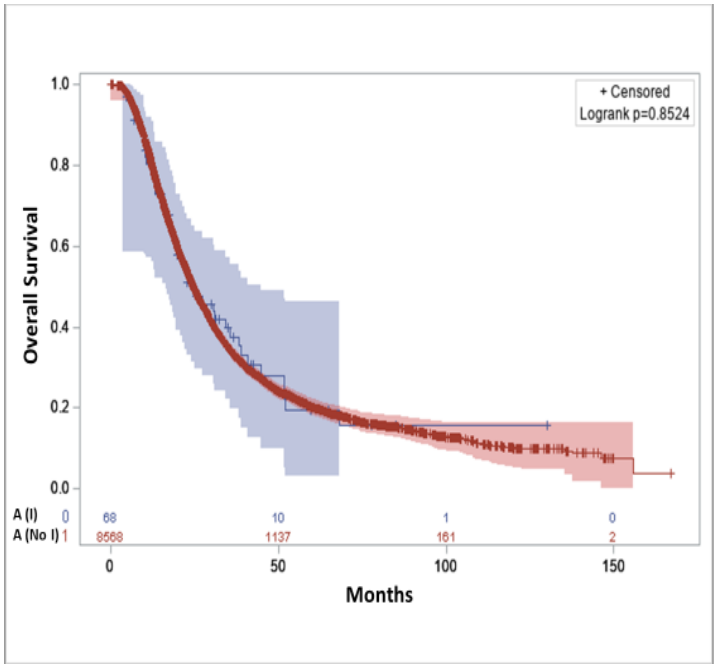


Figure 7b: Overall survival of resectable PDAC patients who received adjuvant chemotherapy or chemoradiation with (blue) or without (red) immunotherapy



Chapter 6

Discussion

Summary

The purpose of this dissertation was to use the NCDB, which captures 70% or more of newly diagnosed cancer cases nationwide to perform a robust analysis to investigate the impact of immunotherapy in the OS of PDAC patients. The overall goal of this research was to understand the potential role of immunotherapy in PC survival and determine how best to incorporate immunotherapy into the current standard-of-care PC treatment paradigms. The central hypothesis was that immunotherapy will improve the survival of patients with either resectable or unresectable PDAC and that combining immunotherapy with other treatments may differentially alter the effect of immunotherapy on patient outcomes. We conducted four studies to answer the research questions related to the specific aims of this dissertation, the findings of which are summarized here.

Manuscript 1: The impact of immunotherapy on the survival of pancreatic adenocarcinoma patients who do not receive definitive surgery of the tumor

Specific aim 1a: Identify the patients and disease characteristics associated with the use of immunotherapy in unresectable PDAC

Hypothesis: Certain patient and tumor-related factors are associated with the receipt of immunotherapy

In the multivariable logistic regression analysis treatment at an academic hospital, having a high school degree, and having insurance were positively associated with receiving immunotherapy. These patients were more likely to receive immunotherapy compared to their counterparts. For example, patients who were treated at the non-academic hospital were 62% (OR: 0.38, CI: 0.33-0.45) less likely to receive immunotherapy compared to patients treated at

an academic hospital (Table 4). Patients who did not have a high school degree were 23% (OR: 0.77, CI: 0.66-0.90) less likely to receive immunotherapy compared to patients who had a high school degree. Patients who did not have health insurance were 56% (OR: 0.44, CI: 0.27-0.78) less likely to receive immunotherapy compared to patients who had insurance (Table 4). These findings are critical in terms of access to care, improving awareness about decision making, and improving the education and learning experience of oncologists at non-academic hospitals.

Specific aim 1b: Evaluate the impact of immunotherapy in combination with other standard-of-care treatments on the survival of unresectable PDAC patients

Hypothesis: Combining immunotherapy with RT, chemotherapy, and chemoradiation has a superior impact on OS than these treatments without immunotherapy in unresectable PC

A multivariable analysis was performed to compare the survival outcomes of PDAC patients without surgery who received chemotherapy with and without immunotherapy and those who received chemoradiation with and without immunotherapy. The analysis demonstrated that adding immunotherapy to either chemotherapy or chemoradiation therapy led to a significant OS benefit in both univariate and multivariable Cox regression analysis (Table 6). What is unique about the findings of this study was that chemoradiation plus immunotherapy was associated with a significantly improved OS, which to our knowledge, has not been investigated yet. The findings of this study, together with early results of some clinical trials, warrant future large phase III clinical trials of immunotherapy combined with chemotherapy or chemoradiation in PAD patients who did not receive definitive surgery of the pancreatic tumor.

Manuscript 2: The impact of immunotherapy on the survival of pancreatic adenocarcinoma patients who received definitive surgery of the pancreatic tumor

Specific Aim 2a: Identify the predictors of receiving immunotherapy in resectable PDAC patients

Hypothesis: Treatment facility type and socioeconomic status are associated with the use of immunotherapy

In the multivariable logistic regression analysis, treatment at an academic hospital and having high school degrees were positively associated with receiving immunotherapy. For example, patients who were treated in non-academic hospitals were 74% (OR: 0.26, CI: 0.21-0.32) less likely to receive immunotherapy compared to their counterparts who were treated in an academic hospital. Patients who did not have a high school degree were 35% (OR: 0.65, CI: 0.54-0.78) less likely to receive immunotherapy compared to patients who had a high school degree (Table 7). The findings are similar to that of PDAC patients who did not undergo definitive surgery. These findings are critical for improving access to novel treatments such as immunotherapy in non-academic health institutions and people who are less educated.

Specific aim 2b: Examined the impact of immunotherapy with other standard-of-care treatments on the survival of resectable PDAC

Hypothesis: Combining immunotherapy with RT, chemotherapy, and chemoradiation has a superior impact on OS than these treatments without immunotherapy in resectable PC

In one of the first and largest studies with a robust analysis using the NCDB, the impact of immunotherapy in combination with chemotherapy, RT, and chemoradiation on the OS of PDAC patients who received definitive surgery of the tumor was investigated. In this study, combining chemoradiation therapy with immunotherapy was associated with significantly improved OS of the patients (Table 9). The findings of this study, together with the results of other previous studies of the use of immunotherapy with other standard-of-care cancer

treatments in PDAC patients who receive surgery, warrant the need for future clinical trials of investigating the impact of immunotherapy in this group of patients.

Manuscript 3: The impact of the sequence of immunotherapy on the survival of pancreatic adenocarcinoma patients: a retrospective analysis of the national cancer database

Specific Aim 3a: Identify the treatment sequence of immunotherapy with other standard-of-care treatments on the survival of unresectable PDAC patients

Hypothesis: The OS of patients who start immunotherapy within 30 days of RT or chemotherapy is superior to those who receive the treatments more than 30 days from each other in unresectable PDAC

In an extensive analysis using the NCDB, the impact of the timing of immunotherapy with chemotherapy and RT on the OS of PDAC patients who did not receive definitive surgery of the pancreatic tumor was investigated. There was no significant difference in the OS of patients who started immunotherapy within 30 days of chemotherapy, patients who started immunotherapy 31-90 days before chemotherapy, and patients who started immunotherapy 91-180 days before chemotherapy. (Table 12). There was also no difference in the OS of patients who started immunotherapy within 30 days of RT, patients who started RT > 30 days before immunotherapy, and patients who began immunotherapy > 30 days before RT (Table 15). These findings provide insight into the design of future clinical trials of immunotherapy in PDAC. Future clinical trials may allow the administration of immunotherapy with chemotherapy and RT regardless of the treatment sequence.

Manuscript 4: The impact of neoadjuvant and adjuvant immunotherapy on the survival of pancreatic cancer patients: a retrospective analysis

Specific Aim 3b: Identify the treatment sequence of immunotherapy with other standard-of-care treatments on the survival of resectable PDAC patients

Hypothesis: The OS of resectable PDAC patients who receive neoadjuvant immunotherapy is better than the OS patients who receive adjuvant immunotherapy

The OS between patients who received neoadjuvant immunotherapy and patients who received adjuvant immunotherapy was compared. There was no significant difference in the median OS of patients who received neoadjuvant immunotherapy compared to patients who received adjuvant immunotherapy (Table 17). In the adjuvant subset analysis, immunotherapy combined with chemotherapy or chemoradiation therapy was not associated with improved OS compared to chemotherapy or chemoradiation without immunotherapy (Table 19). The findings warrant future studies with a large sample size for both neoadjuvant and adjuvant treatment comparisons of immunotherapy in PDAC patients who receive definitive surgery of the pancreatic tumor.

Implications

This research is the most extensive and robust analysis that has used the NCDB and investigated the impact of immunotherapy on the OS of PDAC patients. The findings of the first study indicated that PDAC patients who were treated in an academic hospital and patients who had a high school degree were more likely to receive immunotherapy compared with their counterparts. This is important, especially that we found that immunotherapy is associated with significantly improved OS compared to no immunotherapy. The issue of access to healthcare, and the utilization of novel treatments by patients need to be addressed. Physicians and oncologists at nonacademic hospitals may need the training to get familiar with academic research and provide patients with the most up to date treatment options. In the current study, immunotherapy combined with chemotherapy and chemoradiation therapy was associated with

improved OS compared to these treatments without immunotherapy in PDAC patients who did not receive definitive surgery of pancreatic cancer.

In PDAC patients who received definitive surgery of the pancreatic tumor, immunotherapy combined with chemoradiation therapy was associated with improved OS compared to chemoradiation alone. These findings indicate the potential role of immunotherapy in combination with the current standard-of-care treatments in PDAC patients. The current study is the most extensive study with a robust analysis that investigated the impact of immunotherapy on the OS of PDAC patients in combination with other cancer treatments. The majority of previous studies that reported negative results were based on a small number of patients and included heavily pre-treated PDAC patients. The findings warrant future clinical trials of immunotherapy combined with chemotherapy, radiation therapy, and chemoradiation.

In the current research, we found that immunotherapy may interact differently with chemotherapy and chemoradiation in PDAC patients who receive surgical resection of the pancreatic tumor and patients who do not undergo surgical resection. Immunotherapy combined with chemotherapy alone or chemoradiation was associated with improved OS compared to chemotherapy without immunotherapy or chemoradiation without chemotherapy in patients who did not receive surgical resection. In patients who underwent surgical resection of the pancreatic tumor, immunotherapy was only associated with the improved OS when combined with chemoradiation but not chemotherapy. This is very critical for the design of future clinical trials of immunotherapy in PDAC, especially that immunotherapy interacts differently with chemotherapy in patients who receive surgery and in patients who do not undergo surgery of the pancreatic tumor. Future clinical trials that are investigating the impact of immunotherapy in the OS of PDAC patients with chemotherapy may need to separate

patients by surgical status as the two groups responded differently to immunotherapy in our study.

The mechanism of the immunostimulatory effect of chemotherapy is different in patients who receive surgery and patients who did not receive surgery and is a possible reason for the difference in the interaction of immunotherapy with chemotherapy in these two groups of patients. In PDAC patients who undergo surgical resection of the pancreatic tumor, the immunostimulatory effect of chemotherapy, especially in the adjuvant setting is through the inhibition of T regulatory cell and MDSCs rather than the stimulation and increase of T cells. The increase and stimulation of T cells are significant for immunotherapy to deliver optimal survival benefits. In patients who do not receive surgery, the stimulatory effect of chemotherapy is mainly through the stimulation and increase of T cells, which may be one of the reasons immunotherapies are associated with the improved OS when combined with chemotherapy.

Another critical point to be noticed for the design of the future clinical trials is the synergetic interaction of immunotherapy with chemoradiation in both patients who receive surgical resection and patients who do not undergo surgical resection of the pancreatic tumor. A synergetic interaction with a comparable and improved OS outcome may be achieved in these groups if both systemic and local immune response is produced. Chemotherapy induces a systemic immune response, and RT mainly induces a local immune response. Immunotherapy can work in a synergetic way with systemic and local immune responses and deliver optimal results. The findings indicate that an aggressive multimodality treatment approach is required to achieve the maximum benefit of immunotherapy in PDAC patients. The combination of immunotherapy and chemoradiation has not been studied yet, and the findings of the current study provide a glimpse of hope and foundation for future clinical trials to consider this combination while also keeping in mind the life expectancy and treatment toleration of patients.

The improved OS associated with the use of immunotherapy in combination with chemoradiation, especially in PDAC patients who received surgical resection of the tumor is critical. The use of immunotherapy in PDAC has been mainly in a metastatic setting. However, the findings of our study, together with some studies conducted in non-small cell lung cancer, provide some evidence that immunotherapy may be beneficial in resectable cancers. Future clinical trials can use these findings as initial evidence for investigating the efficacy and survival benefit of immunotherapy in combination with chemoradiation in early-stage or resectable PDAC. Trials of immunotherapy in resectable PDAC may be even more critical as the majority of patients eventually succumb to the disease due to the widespread of micrometastases that happen early in the disease. Immunotherapy may be an excellent therapeutic option for shrinking the tumor and eliminating occult micrometastases before surgery or eliminating occult micrometastases after the removal of cancer.

The findings of this dissertation can also be beneficial for the designs of the future clinical trials of immunotherapy that are investigating the treatment sequence of immunotherapy with chemotherapy and RT both in resectable and unresectable PDAC. In PDAC patients with no surgery, there was no difference in the OS of patients who started immunotherapy and chemotherapy or RT within 30 days of each other, and > 30 days of each other. There was also no difference in the OS of patients who received neoadjuvant immunotherapy compared to adjuvant immunotherapy. These findings are essential for future trials. This is critical as so far; there is no data available that favors a specific treatment sequence. The majority of the ongoing clinical trials assume that the concurrent use of immunotherapy with chemotherapy or radiation therapy is better than the sequential.

Another vital element of the findings of the current research is the pattern of care in the context of immunotherapy. The results indicated a significant difference in the probability of

receiving immunotherapy for certain groups in both resectable and unresectable PC. Female sex, Black race, living in an area with high education level, and receiving treatment at community hospitals were all negatively associated with receiving immunotherapy. It is significantly crucial given that immunotherapy was associated with improved OS in PDAC patients. These groups need to be targeted from the perspective of improving access to care and improving OS. Patients who did not receive surgical resection of the pancreatic tumor and treated at non-academic hospitals were 74% less likely to receive immunotherapy and 20% more likely to die compared to their counterparts who were treated at academic hospitals. Patients who underwent surgery of the tumor and were treated at non-academic hospitals were 62% less likely to receive immunotherapy and 17% more likely to die. This is an example of a lack of access to novel treatments translated to the disparity in the OS. Several factors may contribute to the survival disparity in these patients, and treatment at a non-academic hospital is one of the contributing factors.

Our findings also showed a trend toward some specific treatment patterns in combining immunotherapy with chemotherapy or RT. In PDAC patients who did not receive surgical resection of the pancreatic tumor, 88% of the patients started immunotherapy and chemotherapy with 30 days of each other, indicating a tendency of clinicians to recommend starting the two treatments close to each other. Among these patients, 76% of the patients began immunotherapy and chemotherapy on the same day, with 89% of the patients starting the two treatments within a week of each other. More than 63% of these patients were treated at academic hospitals. More than 46% of the patients who started chemotherapy and immunotherapy within 30 days of each other were diagnosed in 2011 or after, while 63% and 84% of the patients who started immunotherapy 31-90 days before chemotherapy and patients who started immunotherapy 91-180 days before chemotherapy were diagnosed in 2011 or

after. This indicates a trend toward starting immunotherapy before chemotherapy in recent years, however, the sample size for these two categories was small.

The sequence of immunotherapy with RT followed the same pattern. More than 78% of the patients received RT and immunotherapy within 30 days of each other. In the remaining patients, 15% started RT > 30 days before immunotherapy, and 6% started immunotherapy > 30 days before RT. Among those who began RT and immunotherapy within 30 days of each other, 60% started the two treatments on the same day, with 86% of the patients starting the two treatments within a week of each other. Among patients who began RT and immunotherapy within 30 days of each other, 78% were treated at academic hospitals, much higher than the proportion of patients who received chemotherapy and immunotherapy within 30 days of each other and were treated at academic hospitals (63%). Only a small percentage (16%) of the patients who started RT and immunotherapy within 30 days of each other were diagnosed in 2011 or after, while 84% of the patients who received Rt > 30 days before immunotherapy and 60% of patients who received immunotherapy > 30 days before RT were diagnosed in 2011 or after. These findings provide some indications that in recent years more patients are starting to take immunotherapy with chemotherapy within 30 days of each other (46%) compared to starting RT and immunotherapy within 30 days of each other.

In PDAC patients who received surgical resection of the pancreatic tumor, 78% of them received neoadjuvant immunotherapy compared to the 22% who received adjuvant immunotherapy. Among those who received neoadjuvant immunotherapy, 41% were diagnosed in 2011 or after, while among those who received adjuvant immunotherapy, 66% were diagnosed in 2011 or after that. It indicates that the majority of the patients diagnosed in recent years received adjuvant immunotherapy. The proportions of patients who received neoadjuvant or adjuvant immunotherapy and were treated at academic hospitals were the same (79% vs.

82%). An important take away from the treatment sequence patterns is that the majority of the patients who received neoadjuvant immunotherapy and started immunotherapy within 30 days of the start of chemotherapy or RT were diagnosed in 2010 or before, opposite to our assumption. We observed a trend and noticed that most patients diagnosed in 2010 or before received neoadjuvant immunotherapy and started immunotherapy within 30 days of starting chemotherapy or RT.

Limitations

The strength of the current research is the large sample size of the study. The study used the world's largest cancer database to investigate the impact of immunotherapy on the OS of PDAC patients. With a large sample size, we were able to adjust for some important patient and tumor characteristics. The large sample size also allowed to stratify the study by patients who received definitive surgery of pancreatic cancer and patients who did not undergo definitive surgery of pancreatic cancer. However, this research has several limitations. The limitations are mostly inherent to NCDB, which include incomplete data and ascertainment bias, lack of data about the cause of death, lack of detailed information on the use of multi-agent chemotherapy regimens, and lack of information on the type of immunotherapy and if a single or combined immunotherapy was used. For example, the NCDB does not collect information if a checkpoint inhibitor or vaccine therapy was given to the patients.

Only a small percentage of the patients received immunotherapy, which indicates that patients who received immunotherapy represent a particular cohort. These patients may have characteristics that are different from the rest of the cohort, and the findings may be biased. It is also possible that these patients have some other confounding characteristics that we were not able to account for in the NCDB database. It is also possible that the PDAC patients who received immunotherapy were positive for microsatellite-instability status who responds better to

immunotherapy compared to other patients. The NCDB does not provide data on the microsatellite-instability status for PDAC patients. However, testing for microsatellite-instability status is not part of the routine clinical test of PDAC patients. There was not enough sample size for the analysis of comparing the impact of RT plus immunotherapy vs. RT alone.

Future Directions

The current retrospective analysis provided the most extensive research about the impact of immunotherapy on the OS of PDAC patients stratified by definitive surgery of pancreatic cancer. This research used the NCDB, which is the best resource for cancer research outside of the multi-institutional clinical trials. The findings provide insights about the potential role of immunotherapy in the OS of PDAC patients. The findings also provide information about access to novel treatments and patterns of care. The results of this study warrant future clinical trials of immunotherapy in PDAC. The clinical trials should be stratified by the definitive surgery of pancreatic cancer as the interaction of immunotherapy with chemotherapy and chemoradiation was different in patients who received surgery and in those who did not undergo surgery. The unique finding in the current study was that combining immunotherapy with chemoradiation therapy is associated with improved OS compared to chemoradiation alone in both patients who did not receive definitive surgery and patients who received definitive surgery of pancreatic cancer. Future clinical trials need to focus on this finding, especially in resectable PDAC. Immunotherapy has been used mainly in the metastatic setting, but recent research, including the results of the current study, provide some evidence that it may work in resectable PDAC.

References

1. Columbia University Irving Medical Center. The pancreas and its functions. https://columbiasurgery.org/pancreas/pancreas-and-its-functions. Accessed 05/07, 2020.

2. JOHN HOPKINS MEDICINE PATHOLOGY. The Sol Goldman Pancreatic Cancer Research Center. The function of the pancreas. http://pathology.jhu.edu/pancreas/basicoverview3.php?area=ba. Accessed 05/07, 2020.

3. Hidalgo M, Cascinu S, Kleeff J, et al. Addressing the challenges of pancreatic cancer: Future directions for improving outcomes. *Pancreatology*. 2015;15(1):8-18.

4. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2018;68(6):394-424.

5. Ferlay J EM, Lam F, Colombet M, Mery L, Pineros M, Znaor A, Soerjomataram. Global cancer observatory: Cancer today. http://gco.iarc.fr/today/online-analysis-table. Accessed 05/07, 2020.

6. National Cancer Institute (NIH). Surveillance, Epidemiology, and End Result Program (SEER). Cancer stat facts: Pancreatic cancer. https://seer.cancer.gov/statfacts/html/pancreas.html. Updated 2019. Accessed 01/10, 2019.

7. Rahib L, Smith BD, Aizenberg R, Rosenzweig AB, Fleshman JM, Matrisian LM. Projecting cancer incidence and deaths to 2030: The unexpected burden of thyroid, liver, and pancreas cancers in the united states. *Cancer Res*. 2014;74(11):2913-2921.

8. McGuigan A, Kelly P, Turkington RC, Jones C, Coleman HG, McCain RS. Pancreatic cancer: A review of clinical diagnosis, epidemiology, treatment, and outcomes. *World J Gastroenterol*. 2018;24(43):4846-4861.

9. Iodice S, Gandini S, Maisonneuve P, Lowenfels AB. Tobacco and the risk of pancreatic cancer: A review and meta-analysis. *Langenbecks Arch Surg*. 2008;393(4):535-545.

10. Vrieling A, Bueno-de-Mesquita HB, Boshuizen HC, et al. Cigarette smoking, environmental tobacco smoke exposure, and pancreatic cancer risk in the European prospective investigation into cancer and nutrition. *Int J Cancer*. 2010;126(10):2394-2403.

11. Yadav D, Lowenfels AB. The epidemiology of pancreatitis and pancreatic cancer. *Gastroenterology*. 2013;144(6):1252-1261.

12. Genkinger JM, Spiegelman D, Anderson KE, et al. Alcohol intake and pancreatic cancer risk: A pooled analysis of fourteen cohort studies. *Cancer Epidemiol Biomarkers Prev*. 2009;18(3):765-776.

13. Rawla P, Sunkara T, Gaduputi V. Epidemiology of pancreatic cancer: Global trends, etiology, and risk factors. *World J Oncol*. 2019;10(1):10-27.

14. Skelton RA, Javed A, Zheng L, He J. Overcoming the resistance of pancreatic cancer to immune checkpoint inhibitors. *J Surg Oncol*. 2017;116(1):55-62.

15. Melstrom LG, Salazar MD, Diamond DJ. The pancreatic cancer microenvironment: A true double agent. *J Surg Oncol*. 2017;116(1):7-15.

16. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. *CA Cancer J Clin*. 2017;67(1):7-30.

17. Sideras K, Biermann K, Yap K, et al. Tumor cell expression of immune inhibitory molecules and tumor-infiltrating lymphocyte count predict cancer-specific survival in pancreatic and ampullary cancer. *Int J Cancer*. 2017;141(3):572-582.

18. Oettle H, Neuhaus P, Hochhaus A, et al. Adjuvant chemotherapy with gemcitabine and long-term outcomes among patients with resected pancreatic cancer: The CONKO-001 randomized trial. *JAMA*. 2013;310(14):1473-1481.

19. Li D, Xie K, Wolff R, Abbruzzese JL. Pancreatic cancer. *Lancet*. 2004;363(9414):1049-1057.

20. Paez D, Labonte MJ, Lenz HJ. Pancreatic cancer: Medical management (novel chemotherapeutics). *Gastroenterol Clin North Am*. 2012;41(1):189-209.

21. Conroy T, Hammel P, Hebbar M, et al. FOLFIRINOX or gemcitabine as adjuvant therapy for pancreatic cancer. *N Engl J Med*. 2018;379(25):2395-2406.

22. Young K, Hughes DJ, Cunningham D, Starling N. Immunotherapy, and pancreatic cancer: Unique challenges and potential opportunities. *Ther Adv Med Oncol*. 2018;10:1758835918816281.

23. Cancer.Net. Pancreatic cancer. types of treatment. https://www.cancer.net/cancer-types/pancreatic-cancer/types-treatment. Updated 2018. Accessed 02, 2020.

24. Oberstein PE, Olive KP. Pancreatic cancer: Why is it so hard to treat? *Therap Adv Gastroenterol*. 2013;6(4):321-337.

25. Disis ML. Mechanism of action of immunotherapy. *Semin Oncol*. 2014;41 Suppl 5:S3-13.

26. Oiseth, S., Aziz, M. Cancer immunotherapy: A brief review of the history, possibilities, and challenges ahead. Journal of Cancer Metastasis and Treatment 2017;3:250-261.

27. Kitamura T, Qian BZ, Pollard JW. Immune cell promotion of metastasis. *Nat Rev Immunol*. 2015;15(2):73-86.

28. McAllister SS, Weinberg RA. The tumor-induced systemic environment as a critical regulator of cancer progression and metastasis. *Nat Cell Biol*. 2014;16(8):717-727.

29. Yang Y. Cancer immunotherapy: Harnessing the immune system to battle cancer. *J Clin Invest*. 2015;125(9):3335-3337.

30. Cancer.Net. Understanding immunotherapy. https://www.cancer.net/navigating-cancer-care/how-cancer-treated/immunotherapy-and-vaccines/understanding-immunotherapy. Updated 2018. Accessed 03/12, 2018.

31. Skelton RA, Javed A, Zheng L, He J. Overcoming the resistance of pancreatic cancer to immune checkpoint inhibitors. *J Surg Oncol*. 2017;116(1):55-62.

32. American Cancer Society. What is cancer immunotherapy? https://www.cancer.org/treatment/treatments-and-side-effects/treatment-types/immunotherapy/what-is-immunotherapy.html. Updated 2018. Accessed 03/14, 2018.

33. Chiaravalli M, Reni M, O'Reilly EM. Pancreatic ductal adenocarcinoma: State-of-the-art 2017 and new therapeutic strategies. *Cancer Treat Rev*. 2017;60:32-43.

34. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer*. 2012;12(4):252-264.

35. Alsaab HO, Sau S, Alzhrani R, et al. PD-1 and PD-L1 checkpoint signaling inhibition for cancer immunotherapy: Mechanism, combinations, and clinical outcome. *Front Pharmacol*. 2017;8:561.

36. Dine J, Gordon R, Shames Y, Kasler MK, Barton-Burke M. Immune checkpoint inhibitors: An innovation in immunotherapy for the treatment and management of patients with cancer. *Asia Pac J Oncol Nurs*. 2017;4(2):127-135.

37. Terme M, Ullrich E, Aymeric L, et al. IL-18 induces PD-1-dependent immunosuppression in cancer. *Cancer Res*. 2011;71(16):5393-5399.

38. Sharabi AB, Nirschl CJ, Kochel CM, et al. Stereotactic radiation therapy augments antigen-specific PD-1-mediated antitumor immune responses via cross-presentation of tumor antigen. *Cancer Immunol Res*. 2015;3(4):345-355.

39. Ceeraz S, Nowak EC, Noelle RJ. B7 family checkpoint regulators in immune regulation and disease. *Trends Immunol*. 2013;34(11):556-563.

40. Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med*. 2010;363(8):711-723.

41. Robert C, Schachter J, Long GV, et al. Pembrolizumab versus ipilimumab in advanced melanoma. *N Engl J Med*. 2015;372(26):2521-2532.

42. Hilmi M, Bartholin L, Neuzillet C. Immune therapies in pancreatic ductal adenocarcinoma: Where are we now? *World J Gastroenterol*. 2018;24(20):2137-2151.

43. Thind K, Padrnos LJ, Ramanathan RK, Borad MJ. Immunotherapy in pancreatic cancer treatment: A new frontier. *Therap Adv Gastroenterol*. 2017;10(1):168-194.

44. Rittmeyer A, Barlesi F, Waterkamp D, et al. Atezolizumab versus docetaxel in patients with previously treated non-small-cell lung cancer (OAK): A phase 3, open-label, multicentre randomised controlled trial. *Lancet*. 2017;389(10066):255-265.

45. Fehrenbacher L, Spira A, Ballinger M, et al. Atezolizumab versus docetaxel for patients with previously treated non-small-cell lung cancer (POPLAR): A multicentre, open-label, phase 2 randomised controlled trial. *Lancet*. 2016;387(10030):1837-1846.

46. Johansson H, Andersson R, Bauden M, Hammes S, Holdenrieder S, Ansari D. Immune checkpoint therapy for pancreatic cancer. *World J Gastroenterol*. 2016;22(43):9457-9476.

47. Zheng L. Does vaccine-primed pancreatic cancer offer better candidates for immune-based therapies? *Immunotherapy*. 2014;6(10):1017-1020.

48. Sideras K, Biermann K, Yap K, et al. Tumor cell expression of immune inhibitory molecules and tumor-infiltrating lymphocyte count predict cancer-specific survival in pancreatic and ampullary cancer. *Int J Cancer*. 2017;141(3):572-582.

49. Kang J, Demaria S, Formenti S. Current clinical trials testing the combination of immunotherapy with radiotherapy. *J Immunother Cancer*. 2016;4:51-016-0156-7. eCollection 2016.

50. Royal RE, Levy C, Turner K, et al. Phase 2 trial of single agent ipilimumab (anti-CTLA-4) for locally advanced or metastatic pancreatic adenocarcinoma. *J Immunother*. 2010;33(8):828-833.

51. Brahmer JR, Tykodi SS, Chow LQ, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. *N Engl J Med*. 2012;366(26):2455-2465.

52. Patnaik A, Kang SP, Rasco D, et al. Phase I study of pembrolizumab (MK-3475; anti-PD-1 monoclonal antibody) in patients with advanced solid tumors. *Clin Cancer Res*. 2015;21(19):4286-4293.

53. Herbst RS, Soria JC, Kowanetz M, et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. *Nature*. 2014;515(7528):563-567.

54. N.H. Segal, O. Hamid, W. Hwu, C. Massard, M. Butler, S. Antonia, A. Blake-Haskins, P.B. Robbins7 X. Li, J. Vasselli, S. Khleif. A phase I multi-arm dose-expansion study of the anti-programmed cell death-ligand-1 (PD-l1) antibody medi4736: Preliminary data. 2014;25(4):361-372.

55. Geynisman DM, Zha Y, Kunnavakkam R, et al. A randomized pilot phase I study of modified carcinoembryonic antigen (CEA) peptide (CAP1-6D)/montanide/GM-CSF-vaccine in patients with pancreatic adenocarcinoma. *J Immunother Cancer*. 2013;1:8-1426-1-8. eCollection 2013.

56. Schuetz T, Kaufman H, Marshall J, Safran H. Extended survival in second-line pancreatic cancer after therapeutic vaccination. 2005.

57. Gilliam AD, Broome P, Topuzov EG, et al. An international multicenter randomized controlled trial of G17DT in patients with pancreatic cancer. *Pancreas*. 2012;41(3):374-379.

58. Brett BT, Smith SC, Bouvier CV, et al. Phase II study of anti-gastrin-17 antibodies, raised to G17DT, in advanced pancreatic cancer. *J Clin Oncol*. 2002;20(20):4225-4231.

59. Shapiro J, Marshall J, Karasek P, et al. G17DT+gemcitabine [gem] versus placebo+Gem in untreated subjects with locally advanced, recurrent, or metastatic adenocarcinoma of the pancreas: Results of a randomized, double-blind, multinational, multicenter study. *JCO*. 2005;23(16):LBA4012-LBA4012.

60. Aglietta M, Barone C, Sawyer MB, et al. A phase I dose-escalation trial of tremelimumab (CP-675,206) in combination with gemcitabine in chemotherapy-naive patients with metastatic pancreatic cancer. *Ann Oncol*. 2014;25(9):1750-1755.

61. Kalyan A, Kircher SM, Mohindra NA, et al. Ipilimumab and gemcitabine for advanced pancreas cancer: A phase ib study. *JCO*. 2016;34(15):e15747-e15747.

62. Wainberg ZA, Hochster HS, Kim EJ, et al. Phase I study of nivolumab (nivo) + nab-paclitaxel (nab-P) + gemcitabine (gem) in advanced pancreatic cancer (APC). *JCO*. 2019;37(4):298-298.

63. Nakamura M, Wada J, Suzuki H, Tanaka M, Katano M, Morisaki T. Long-term outcome of immunotherapy for patients with refractory pancreatic cancer. *Anticancer Res*. 2009;29(3):831-836.

64. Lepisto AJ, Moser AJ, Zeh H, et al. A phase I/II study of a MUC1 peptide pulsed autologous dendritic cell vaccine as adjuvant therapy in patients with resected pancreatic and biliary tumors. *Cancer Ther*. 2008;6(B):955-964.

65. Kondo H, Hazama S, Kawaoka T, et al. Adoptive immunotherapy for pancreatic cancer using MUC1 peptide-pulsed dendritic cells and activated T lymphocytes. *Anticancer Res*. 2008;28(1B):379-387.

66. Pecher G, Haring A, Kaiser L, Thiel E. Mucin gene (MUC1) transfected dendritic cells as vaccine: Results of a phase I/II clinical trial. *Cancer Immunol Immunother*. 2002;51(11-12):669-673.

67. Kawaoka T, Oka M, Takashima M, et al. Adoptive immunotherapy for pancreatic cancer: Cytotoxic T lymphocytes stimulated by the MUC1-expressing human pancreatic cancer cell line YPK-1. *Oncol Rep*. 2008;20(1):155-163.

68. Yamamoto K, Ueno T, Kawaoka T, et al. MUC1 peptide vaccination in patients with advanced pancreas or biliary tract cancer. *Anticancer Res*. 2005;25(5):3575-3579.

69. Ramanathan RK, Lee KM, McKolanis J, et al. Phase I study of a MUC1 vaccine composed of different doses of MUC1 peptide with SB-AS2 adjuvant in resected and locally advanced pancreatic cancer. *Cancer Immunol Immunother*. 2005;54(3):254-264.

70. Kimura Y, Tsukada J, Tomoda T, et al. Clinical and immunologic evaluation of dendritic cell-based immunotherapy in combination with gemcitabine and/or S-1 in patients with advanced pancreatic carcinoma. *Pancreas*. 2012;41(2):195-205.

71. Gjertsen MK, Buanes T, Rosseland AR, et al. Intradermal ras peptide vaccination with granulocyte-macrophage colony-stimulating factor as adjuvant: Clinical and

immunological responses in patients with pancreatic adenocarcinoma. *Int J Cancer*. 2001;92(3):441-450.

72. Abou-Alfa GK, Chapman PB, Feilchenfeldt J, et al. Targeting mutated K-ras in pancreatic adenocarcinoma using an adjuvant vaccine. *Am J Clin Oncol*. 2011;34(3):321-325.

73. Muscarella P, Wilfong LS, Ross SB, et al. A randomized, placebo-controlled, double blind, multicenter phase II adjuvant trial of the efficacy, immunogenicity, and safety of GI-4000 plus gem versus gem alone in patients with resected pancreas cancer with activating RAS mutations/survival and immunology analysis of the R1 subgroup. *JCO*. 2012;30(15):e14501-e14501.

74. Weden S, Klemp M, Gladhaug IP, et al. Long-term follow-up of patients with resected pancreatic cancer following vaccination against mutant K-ras. *Int J Cancer*. 2011;128(5):1120-1128.

75. Middleton G, Silcocks P, Cox T, et al. Gemcitabine and capecitabine with or without telomerase peptide vaccine GV1001 in patients with locally advanced or metastatic pancreatic cancer (TeloVac): An open-label, randomised, phase 3 trial. *Lancet Oncol*. 2014;15(8):829-840.

76. BioSpace. Pharmexa A/S stops one of two phase III trials. https://www.biospace.com/article/releases/pharmexa-a-s-stops-one-of-two-phase-iii-trials-/. Updated 2018. Accessed 05/08, 2020.

77. Buanes T, Maurel J, Liauw W, Hebbar M, Nemunaitis J. A randomized phase III study of gemcitabine (G) versus GV1001 in sequential combination with G in patients with unresectable and metastatic pancreatic cancer (PC). *JCO*. 2009;27(15):4601-4601.

78. Bernhardt SL, Gjertsen MK, Trachsel S, et al. Telomerase peptide vaccination of patients with non-resectable pancreatic cancer: A dose escalating phase I/II study. *Br J Cancer*. 2006;95(11):1474-1482.

79. Jaffee EM, Hruban RH, Biedrzycki B, et al. Novel allogeneic granulocyte-macrophage colony-stimulating factor-secreting tumor vaccine for pancreatic cancer: A phase I trial of safety and immune activation. *J Clin Oncol*. 2001;19(1):145-156.

80. Lutz E, Yeo CJ, Lillemoe KD, et al. A lethally irradiated allogeneic granulocyte-macrophage colony stimulating factor-secreting tumor vaccine for pancreatic adenocarcinoma. A phase II trial of safety, efficacy, and immune activation. *Ann Surg*. 2011;253(2):328-335.

81. Laheru D, Lutz E, Burke J, et al. Allogeneic granulocyte macrophage colony-stimulating factor-secreting tumor immunotherapy alone or in sequence with cyclophosphamide for metastatic pancreatic cancer: A pilot study of safety, feasibility, and immune activation. *Clin Cancer Res*. 2008;14(5):1455-1463.

82. Zhang Y, Zhang X, Zhang A, Li K, Qu K. Clinical applications of dendritic cells-cytokine-induced killer cells mediated immunotherapy for pancreatic cancer: An up-to-date meta-analysis. *Onco Targets Ther*. 2017;10:4173-4192.

83. Chen L, Zhang X. Primary analysis for clinical efficacy of immunotherapy in patients with pancreatic cancer. *Immunotherapy*. 2016;8(2):223-234.

84. Weden S, Klemp M, Gladhaug IP, et al. Long-term follow-up of patients with resected pancreatic cancer following vaccination against mutant K-ras. *Int J Cancer*. 2011;128(5):1120-1128.

85. Laheru D, Lutz E, Burke J, et al. Allogeneic granulocyte macrophage colony-stimulating factor-secreting tumor immunotherapy alone or in sequence with cyclophosphamide for metastatic pancreatic cancer: A pilot study of safety, feasibility, and immune activation. *Clin Cancer Res*. 2008;14(5):1455-1463.

86. Rosenberg A, Mahalingam D. Immunotherapy in pancreatic adenocarcinoma-overcoming barriers to response. *J Gastrointest Oncol*. 2018;9(1):143-159.

87. Chang JH, Jiang Y, Pillarisetty VG. Role of immune cells in pancreatic cancer from bench to clinical application: An updated review. *Medicine (Baltimore)*. 2016;95(49):e5541.

88. Torphy RJ, Zhu Y, Schulick RD. Immunotherapy for pancreatic cancer: Barriers and breakthroughs. *Ann Gastroenterol Surg*. 2018;2(4):274-281.

89. Beatty GL, Chiorean EG, Fishman MP, et al. CD40 agonists alter tumor stroma and show efficacy against pancreatic carcinoma in mice and humans. *Science*. 2011;331(6024):1612-1616.

90. Byrne KT, Vonderheide RH. CD40 stimulation obviates innate sensors and drives T cell immunity in cancer. *Cell Rep*. 2016;15(12):2719-2732.

91. Siva S, MacManus MP, Martin RF, Martin OA. Abscopal effects of radiation therapy: A clinical review for the radiobiologist. *Cancer Lett*. 2015;356(1):82-90.

92. Barker HE, Paget JT, Khan AA, Harrington KJ. The tumour microenvironment after radiotherapy: Mechanisms of resistance and recurrence. *Nat Rev Cancer*. 2015;15(7):409-425.

93. Vatner RE, Cooper BT, Vanpouille-Box C, Demaria S, Formenti SC. Combinations of immunotherapy and radiation in cancer therapy. *Front Oncol*. 2014;4:325.

94. Mittal D, Gubin MM, Schreiber RD, Smyth MJ. New insights into cancer immunoediting and its three component phases--elimination, equilibrium and escape. *Curr Opin Immunol*. 2014;27:16-25.

95. Beatty GL, Torigian DA, Chiorean EG, et al. A phase I study of an agonist CD40 monoclonal antibody (CP-870,893) in combination with gemcitabine in patients with advanced pancreatic ductal adenocarcinoma. *Clin Cancer Res*. 2013;19(22):6286-6295.

96. Nywening TM, Wang-Gillam A, Sanford DE, et al. Targeting tumour-associated macrophages with CCR2 inhibition in combination with FOLFIRINOX in patients with borderline resectable and locally advanced pancreatic cancer: A single-centre, open-label, dose-finding, non-randomised, phase 1b trial. *Lancet Oncol*. 2016;17(5):651-662.

97. Antonia SJ, Villegas A, Daniel D, et al. Durvalumab after chemoradiotherapy in stage III non-small-cell lung cancer. *N Engl J Med*. 2017;377(20):1919-1929.

98. Grass GD, Krishna N, Kim S. The immune mechanisms of abscopal effect in radiation therapy. *Curr Probl Cancer*. 2016;40(1):10-24.

99. Ng J, Dai T. Radiation therapy and the abscopal effect: A concept comes of age. *Ann Transl Med*. 2016;4(6):118.

100. Ngwa W, Irabor OC, Schoenfeld JD, Hesser J, Demaria S, Formenti SC. Using immunotherapy to boost the abscopal effect. *Nat Rev Cancer*. 2018;18(5):313-322.

101. Owen D, Chaft JE. Immunotherapy in surgically resectable non-small cell lung cancer. *J Thorac Dis*. 2018;10(Suppl 3):S404-S411.

102. Forde PM, Chaft JE, Smith KN, et al. Neoadjuvant PD-1 blockade in resectable lung cancer. *N Engl J Med*. 2018;378(21):1976-1986.

103. Markowitz GJ, Havel LS, Crowley MJ, et al. Immune reprogramming via PD-1 inhibition enhances early-stage lung cancer survival. *JCI Insight*. 2018;3(13):10.1172/jci.insight.96836.

104. Chiari R, Sidoni A, Metro G. Early stage resectable non-small cell lung cancer: Is neoadjuvant immunotherapy the right way forward? *J Thorac Dis*. 2018;10(Suppl 33): S3890-S3894.

105. Ghysen K, Vansteenkiste J. Immunotherapy in patients with early stage resectable nonsmall cell lung cancer. *Curr Opin Oncol*. 2019;31(1):13-17.

106. Tsai S, Evans DB. Therapeutic advances in localized pancreatic cancer. *JAMA Surg*. 2016;151(9):862-868.

107. Du L, Wang-Gillam A. Trends in neoadjuvant approaches in pancreatic cancer. *J Natl Compr Canc Netw*. 2017;15(8):1070-1077.

108. Castellanos JA, Merchant NB. Intensity of follow-up after pancreatic cancer resection. *Ann Surg Oncol*. 2014;21(3):747-751.

109. Iacobuzio-Donahue CA, Fu B, Yachida S, et al. DPC4 gene status of the primary carcinoma correlates with patterns of failure in patients with pancreatic cancer. *J Clin Oncol*. 2009;27(11):1806-1813.

110. Toesca DAS, Koong AJ, Poultsides GA, et al. Management of borderline resectable pancreatic cancer. *Int J Radiat Oncol Biol Phys*. 2018;100(5):1155-1174.

111. Paez D, Labonte MJ, Lenz HJ. Pancreatic cancer: Medical management (novel chemotherapeutics). *Gastroenterol Clin North Am*. 2012;41(1):189-209.

112. Cancer.Net. Pancreatic cancer. types of treatment. https://www.cancer.net/cancer-types/pancreatic-cancer/types-treatment. Updated 2019. Accessed 01/09, 2019.

113. Kabacaoglu D, Ciecielski KJ, Ruess DA, Algul H. Immune checkpoint inhibition for pancreatic ductal adenocarcinoma: Current limitations and future options. *Front Immunol*. 2018;9:1878.

114. Dovedi SJ, Adlard AL, Lipowska-Bhalla G, et al. Acquired resistance to fractionated radiotherapy can be overcome by concurrent PD-L1 blockade. *Cancer Res*. 2014;74(19):5458-5468.

115. Deng L, Liang H, Burnette B, et al. Irradiation and anti-PD-L1 treatment synergistically promote antitumor immunity in mice. *J Clin Invest*. 2014;124(2):687-695.

116. Demaria S, Kawashima N, Yang AM, et al. Immune-mediated inhibition of metastases after treatment with local radiation and CTLA-4 blockade in a mouse model of breast cancer. *Clin Cancer Res*. 2005;11(2 Pt 1):728-734.

117. Vincent A, Herman J, Schulick R, Hruban RH, Goggins M. Pancreatic cancer. *Lancet*. 2011;378(9791):607-620.

118. Gong J, Tuli R, Shinde A, Hendifar AE. Meta-analyses of treatment standards for pancreatic cancer. *Mol Clin Oncol*. 2016;4(3):315-325.

119. Tsai S, Evans DB. Therapeutic advances in localized pancreatic cancer. *JAMA Surg*. 2016;151(9):862-868.

120. Konstantinidis IT, Warshaw AL, Allen JN, et al. Pancreatic ductal adenocarcinoma: Is there a survival difference for R1 resections versus locally advanced unresectable tumors? what is a "true" R0 resection? *Ann Surg*. 2013;257(4):731-736.

121. Koido S, Homma S, Takahara A, et al. Current immunotherapeutic approaches in pancreatic cancer. *Clin Dev Immunol*. 2011;2011:267539.

122. Chen L. Co-inhibitory molecules of the B7-CD28 family in the control of T-cell immunity. *Nat Rev Immunol*. 2004;4(5):336-347.

123. Chambers, CA, Kuhns, MS, Egen JG, Allison JP. CTLA-4-mediated inhibition in regulation of T cell responses: Mechanisms and manipulation in tumor immunotherapy. *Annu Rev Immunol*. 2001;19:565-594.

124. Flies DB, Chen L. The new B7s: Playing a pivotal role in tumor immunity. *J Immunother*. 2007;30(3):251-260.

125. Ohaegbulam KC, Assal A, Lazar-Molnar E, Yao Y, Zang X. Human cancer immunotherapy with antibodies to the PD-1 and PD-L1 pathway. *Trends Mol Med*. 2015;21(1):24-33.

126. Umansky V, Sevko A. Tumor microenvironment and myeloid-derived suppressor cells. *Cancer Microenviron*. 2013;6(2):169-177.

127. Chanmee T, Ontong P, Konno K, Itano N. Tumor-associated macrophages as major players in the tumor microenvironment. *Cancers (Basel)*. 2014;6(3):1670-1690.

128. Young K, Hughes DJ, Cunningham D, Starling N. Immunotherapy and pancreatic cancer: Unique challenges and potential opportunities. *Ther Adv Med Oncol*. 2018;10:1758835918816281.

129. Chang JH, Jiang Y, Pillarisetty VG. Role of immune cells in pancreatic cancer from bench to clinical application: An updated review. *Medicine (Baltimore)*. 2016;95(49):e5541.

130. Young K, Hughes DJ, Cunningham D, Starling N. Immunotherapy and pancreatic cancer: Unique challenges and potential opportunities. *Ther Adv Med Oncol*. 2018;10:1758835918816281.

131. Blair AB, Zheng L. Rational combinations of immunotherapy for pancreatic ductal adenocarcinoma. *Chin Clin Oncol*. 2017;6(3):31.

132. Kershaw MH, Devaud C, John LB, Westwood JA, Darcy PK. Enhancing immunotherapy using chemotherapy and radiation to modify the tumor microenvironment. *Oncoimmunology*. 2013;2(9):e25962.

133. Golden EB, Pellicciotta I, Demaria S, Barcellos-Hoff MH, Formenti SC. The convergence of radiation and immunogenic cell death signaling pathways. *Front Oncol*. 2012;2:88.

134. Demaria S, Ng B, Devitt ML, et al. Ionizing radiation inhibition of distant untreated tumors (abscopal effect) is immune mediated. *Int J Radiat Oncol Biol Phys*. 2004;58(3):862-870.

135. Siva S, MacManus MP, Martin RF, Martin OA. Abscopal effects of radiation therapy: A clinical review for the radiobiologist. *Cancer Lett*. 2015;356(1):82-90.

136. Vatner RE, Cooper BT, Vanpouille-Box C, Demaria S, Formenti SC. Combinations of immunotherapy and radiation in cancer therapy. *Front Oncol*. 2014;4:325.

137. Haynes NM, van der Most RG, Lake RA, Smyth MJ. Immunogenic anti-cancer chemotherapy as an emerging concept. *Curr Opin Immunol*. 2008;20(5):545-557.

138. Ma Y, Conforti R, Aymeric L, et al. How to improve the immunogenicity of chemotherapy and radiotherapy. *Cancer Metastasis Rev*. 2011;30(1):71-82.

139. Germano G, Lamba S, Rospo G, et al. Inactivation of DNA repair triggers neoantigen generation and impairs tumour growth. *Nature*. 2017;552(7683):116-120.

140. Schumacher TN, Schreiber RD. Neoantigens in cancer immunotherapy. *Science*. 2015;348(6230):69-74.

141. Demaria S, Formenti SC. Role of T lymphocytes in tumor response to radiotherapy. *Front Oncol*. 2012;2:95.

142. National Cancer Institute (NIH). Surveillance, Epidemiology, and End Result Program (SEER). Cancer stat facts: Pancreatic cancer. https://seer.cancer.gov/statfacts/html/pancreas.html. Updated 2018. Accessed 02, 2020.

143. Wolfgang CL, Herman JM, Laheru DA, et al. Recent progress in pancreatic cancer. *CA Cancer J Clin*. 2013;63(5):318-348.

144. Du L, Wang-Gillam A. Trends in neoadjuvant approaches in pancreatic cancer. *J Natl Compr Canc Netw*. 2017;15(8):1070-1077.

145. Tsai S, Evans DB. Therapeutic advances in localized pancreatic cancer. *JAMA Surg*. 2016;151(9):862-868.

146. Gnerlich JL, Luka SR, Deshpande AD, et al. Microscopic margins and patterns of treatment failure in resected pancreatic adenocarcinoma. *Arch Surg*. 2012;147(8):753-760.

147. Barugola G, Falconi M, Bettini R, et al. The determinant factors of recurrence following resection for ductal pancreatic cancer. *JOP*. 2007;8(1 Suppl):132-140.

148. Iacobuzio-Donahue CA, Fu B, Yachida S, et al. DPC4 gene status of the primary carcinoma correlates with patterns of failure in patients with pancreatic cancer. *J Clin Oncol*. 2009;27(11):1806-1813.

149. Menon S, Shin S, Dy G. Advances in cancer immunotherapy in solid tumors. *Cancers (Basel)*. 2016;8(12):10.3390/cancers8120106.

150. Brahmer JR, Tykodi SS, Chow LQ, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. *N Engl J Med*. 2012;366(26):2455-2465.

151. Patnaik A, Kang SP, Rasco D, et al. Phase I study of pembrolizumab (MK-3475; anti-PD-1 monoclonal antibody) in patients with advanced solid tumors. *Clin Cancer Res*. 2015;21(19):4286-4293.

152. Herbst RS, Soria JC, Kowanetz M, et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. *Nature*. 2014;515(7528):563-567.

153. Nummer D, Suri-Payer E, Schmitz-Winnenthal H, et al. Role of tumor endothelium in CD4+ CD25+ regulatory T cell infiltration of human pancreatic carcinoma. *J Natl Cancer Inst*. 2007;99(15):1188-1199.

154. Panni RZ, Sanford DE, Belt BA, et al. Tumor-induced STAT3 activation in monocytic myeloid-derived suppressor cells enhances stemness and mesenchymal properties in human pancreatic cancer. *Cancer Immunol Immunother*. 2014;63(5):513-528.

155. Flies DB, Chen L. The new B7s: Playing a pivotal role in tumor immunity. *J Immunother*. 2007;30(3):251-260.

156. Tran TB, Maker VK, Maker AV. Impact of immunotherapy after resection of pancreatic cancer. *J Am Coll Surg*. 2019;229(1):19-27.e1.

157. Ma Y, Conforti R, Aymeric L, et al. How to improve the immunogenicity of chemotherapy and radiotherapy. *Cancer Metastasis Rev*. 2011;30(1):71-82.

158. Sondak VK, McArthur GA. Adjuvant immunotherapy for cancer: The next step. *Lancet Oncol*. 2015;16(5):478-480.

159. Katz MHG, Varadhachary GR, Bauer TW, et al. Preliminary safety data from a randomized multicenter phase ib/II study of neoadjuvant chemoradiation therapy (CRT) alone or in combination with pembrolizumab in patients with resectable or borderline resectable pancreatic cancer. *JCO*. 2017;35(15):4125-4125.

160. Aguilar LK, Shirley LA, Chung VM, et al. Gene-mediated cytotoxic immunotherapy as adjuvant to surgery or chemoradiation for pancreatic adenocarcinoma. *Cancer Immunol Immunother*. 2015;64(6):727-736.

161. Hardacre JM, Mulcahy M, Small W, et al. Addition of algenpantucel-L immunotherapy to standard adjuvant therapy for pancreatic cancer: A phase 2 study. *J Gastrointest Surg*. 2013;17(1):94-100; discussion p. 100-1.

162. Fridlender ZG, Sun J, Singhal S, et al. Chemotherapy delivered after viral immunogene therapy augments antitumor efficacy via multiple immune-mediated mechanisms. *Mol Ther*. 2010;18(11):1947-1959.

163. Vincent J, Mignot G, Chalmin F, et al. 5-fluorouracil selectively kills tumor-associated myeloid-derived suppressor cells resulting in enhanced T cell-dependent antitumor immunity. *Cancer Res*. 2010;70(8):3052-3061.

164. Shevchenko I, Karakhanova S, Soltek S, et al. Low-dose gemcitabine depletes regulatory T cells and improves survival in the orthotopic Panc02 model of pancreatic cancer. *Int J Cancer*. 2013;133(1):98-107.

165. Yanagimoto H, Takai S, Satoi S, et al. Impaired function of circulating dendritic cells in patients with pancreatic cancer. *Clin Immunol*. 2005;114(1):52-60.

166. Rock KL, Rothstein L, Gamble S, Fleischacker C. Characterization of antigen-presenting cells that present exogenous antigens in association with class I MHC molecules. *J Immunol*. 1993;150(2):438-446.

167. Tseng JF, Willett CG, Fernandez-del Castillo C, et al. Patients undergoing treatment for pancreatic adenocarcinoma can mount an effective immune response to vaccinations. *Pancreatology*. 2005;5(1):67-74.

168. Rahib L, Smith BD, Aizenberg R, Rosenzweig AB, Fleshman JM, Matrisian LM. Projecting cancer incidence and deaths to 2030: The unexpected burden of thyroid, liver, and pancreas cancers in the united states. *Cancer Res*. 2014;74(11):2913-2921.

169. Melstrom LG, Salazar MD, Diamond DJ. The pancreatic cancer microenvironment: A true double agent. *J Surg Oncol*. 2017;116(1):7-15.

170. Mahmood J, Shukla HD, Soman S, et al. Immunotherapy, radiotherapy, and hyperthermia: A combined therapeutic approach in pancreatic cancer treatment. *Cancers (Basel)*. 2018;10(12):10.3390/cancers10120469.

171. Du L, Wang-Gillam A. Trends in neoadjuvant approaches in pancreatic cancer. *J Natl Compr Canc Netw*. 2017;15(8):1070-1077.

172. Anagnostou VK, Brahmer JR. Cancer immunotherapy: A future paradigm shift in the treatment of non-small cell lung cancer. *Clin Cancer Res*. 2015;21(5):976-984.

173. Drake CG, Lipson EJ, Brahmer JR. Breathing new life into immunotherapy: Review of melanoma, lung and kidney cancer. *Nat Rev Clin Oncol*. 2014;11(1):24-37.

174. June CH, O'Connor RS, Kawalekar OU, Ghassemi S, Milone MC. CAR T cell immunotherapy for human cancer. *Science*. 2018;359(6382):1361-1365.

175. Sahin U, Tureci O. Personalized vaccines for cancer immunotherapy. *Science*. 2018;359(6382):1355-1360.

176. Ribas A, Wolchok JD. Cancer immunotherapy using checkpoint blockade. *Science*. 2018;359(6382):1350-1355.

177. Hoseini SS, Cheung NV. Immunotherapy of hepatocellular carcinoma using chimeric antigen receptors and bispecific antibodies. *Cancer Lett*. 2017;399:44-52.

178. Mellman I, Coukos G, Dranoff G. Cancer immunotherapy comes of age. *Nature*. 2011;480(7378):480-489.

179. Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoediting: From immunosurveillance to tumor escape. *Nat Immunol*. 2002;3(11):991-998.

180. Marciscano AE, Walker JM, McGee HM, et al. Incorporating radiation oncology into immunotherapy: Proceedings from the ASTRO-SITC-NCI immunotherapy workshop. *J Immunother Cancer*. 2018;6(1):6-018-0317-y.

181. Aliru ML, Schoenhals JE, Venkatesulu BP, et al. Radiation therapy and immunotherapy: What is the optimal timing or sequencing? *Immunotherapy*. 2018;10(4):299-316.

182. Young KH, Baird JR, Savage T, et al. Optimizing timing of immunotherapy improves control of tumors by hypofractionated radiation therapy. *PLoS One*. 2016;11(6):e0157164.

183. Postow MA, Callahan MK, Barker CA, et al. Immunologic correlates of the abscopal effect in a patient with melanoma. *N Engl J Med*. 2012;366(10):925-931.

184. Silk AW, Bassetti MF, West BT, Tsien CI, Lao CD. Ipilimumab and radiation therapy for melanoma brain metastases. *Cancer Med*. 2013;2(6):899-906.

185. Alsuwaigh R, Lee J, Chan G, Chee CE, Choo SP. Response to targeted therapy or chemotherapy following immunotherapy in patients with gastrointestinal cancers - a case series. *J Immunother Cancer*. 2019;7(1):162-019-0637-6.

186. Dwary AD, Master S, Patel A, et al. Excellent response to chemotherapy post immunotherapy. *Oncotarget*. 2017;8(53):91795-91802.

187. Park SE, Lee SH, Ahn JS, Ahn MJ, Park K, Sun JM. Increased response rates to salvage chemotherapy administered after PD-1/PD-L1 inhibitors in patients with non-small cell lung cancer. *J Thorac Oncol*. 2018;13(1):106-111.

188. Leger PD, Rothschild S, Castellanos E, Pillai RN, York SJ, Horn L. Response to salvage chemotherapy following exposure to immune checkpoint inhibitors in patients with non-small cell lung cancer. *JCO*. 2017;35(15):9084-9084.

189. Simon A, Kourie HR, Kerger J. Is there still a role for cytotoxic chemotherapy after targeted therapy and immunotherapy in metastatic melanoma? A case report and literature review. *Chin J Cancer*. 2017;36(1):10-017-0179-6.

190. Schoenfeld JD, Mahadevan A, Floyd SR, et al. Ipilmumab and cranial radiation in metastatic melanoma patients: A case series and review. *J Immunother Cancer*. 2015;3:50-015-0095-8. eCollection 2015.

191. Cohen-Inbar O, Shih HH, Xu Z, Schlesinger D, Sheehan JP. The effect of timing of stereotactic radiosurgery treatment of melanoma brain metastases treated with ipilimumab. *J Neurosurg*. 2017;127(5):1007-1014.

192. Kiess AP, Wolchok JD, Barker CA, et al. Stereotactic radiosurgery for melanoma brain metastases in patients receiving ipilimumab: Safety profile and efficacy of combined treatment. *Int J Radiat Oncol Biol Phys*. 2015;92(2):368-375.

193. Qian JM, Yu JB, Kluger HM, Chiang VL. Timing and type of immune checkpoint therapy affect the early radiographic response of melanoma brain metastases to stereotactic radiosurgery. *Cancer*. 2016;122(19):3051-3058.

194. Spaas M, Lievens Y. Is the combination of immunotherapy and radiotherapy in non-small cell lung cancer a feasible and effective approach? *Front Med (Lausanne)*. 2019;6:244.

195. Meng X, Feng R, Yang L, Xing L, Yu J. The role of radiation oncology in immuno-oncology. *Oncologist*. 2019;24(Suppl 1): S42-S52.

196. Shaverdian N, Lisberg AE, Bornazyan K, et al. Previous radiotherapy and the clinical activity and toxicity of pembrolizumab in the treatment of non-small-cell lung

cancer: A secondary analysis of the KEYNOTE-001 phase 1 trial. *Lancet Oncol*. 2017;18(7):895-903.

197. Liniker E, Menzies AM, Kong BY, et al. Activity and safety of radiotherapy with anti-PD-1 drug therapy in patients with metastatic melanoma. *Oncoimmunology*. 2016;5(9):e1214788.

198. Ahmed KA, Stallworth DG, Kim Y, et al. Clinical outcomes of melanoma brain metastases treated with stereotactic radiation and anti-PD-1 therapy. *Ann Oncol*. 2016;27(3):434-441.

199. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. *CA Cancer J Clin*. 2017;67(1):7-30.

200. Seufferlein T, Ettrich TJ. Treatment of pancreatic cancer-neoadjuvant treatment in resectable pancreatic cancer (PDAC). *Transl Gastroenterol Hepatol*. 2019;4:21.

201. Yeo CJ, Abrams RA, Grochow LB, et al. Pancreaticoduodenectomy for pancreatic adenocarcinoma: Postoperative adjuvant chemoradiation improves survival. A prospective, single-institution experience. *Ann Surg*. 1997;225(5):621-33; discussion 633-6.

202. Yeo CJ, Cameron JL, Lillemoe KD, et al. Pancreaticoduodenectomy for cancer of the head of the pancreas. 201 patients. *Ann Surg*. 1995;221(6):721-31; discussion 731-3.

203. Neoptolemos JP, Stocken DD, Bassi C, et al. Adjuvant chemotherapy with fluorouracil plus folinic acid vs. gemcitabine following pancreatic cancer resection: A randomized controlled trial. *JAMA*. 2010;304(10):1073-1081.

204. Lopez NE, Prendergast C, Lowy AM. Borderline resectable pancreatic cancer: Definitions and management. *World J Gastroenterol*. 2014;20(31):10740-10751.

205. Roth MT, Berlin JD. Current concepts in the treatment of resectable pancreatic cancer. *Curr Oncol Rep*. 2018;20(5):39-018-0685-y.

206. Evans DB, Varadhachary GR, Crane CH, et al. Preoperative gemcitabine-based chemoradiation for patients with resectable adenocarcinoma of the pancreatic head. *J Clin Oncol*. 2008;26(21):3496-3502.

207. Varadhachary GR, Wolff RA, Crane CH, et al. Preoperative gemcitabine and cisplatin followed by gemcitabine-based chemoradiation for resectable adenocarcinoma of the pancreatic head. *J Clin Oncol*. 2008;26(21):3487-3495.

208. Herman JM, Swartz MJ, Hsu CC, et al. Analysis of fluorouracil-based adjuvant chemotherapy and radiation after pancreaticoduodenectomy for ductal adenocarcinoma of the pancreas: results of a large, prospectively collected database at the Johns Hopkins Hospital. *J Clin Oncol*. 2008;26(21):3503-3510.

209. Corsini MM. Adjuvant radiotherapy and chemotherapy for pancreatic carcinoma: The mayo clinic experience (1975-2005). *Journal of clinical oncology*. 07;26(21):3511-3516.

210. Parmar AD, Vargas GM, Tamirisa NP, Sheffield KM, Riall TS. Trajectory of care and use of multimodality therapy in older patients with pancreatic adenocarcinoma. *Surgery*. 2014;156(2):280-289.

211. Versteijne E, Suker M, Groothuis K, et al. Preoperative chemoradiotherapy versus immediate surgery for resectable and borderline resectable pancreatic cancer: Results of the dutch randomized phase III PREOPANC trial. *J Clin Oncol*. 2020:JCO1902274.

212. Motoi F, Kosuge T, Ueno H, et al. Randomized phase II/III trial of neoadjuvant chemotherapy with gemcitabine and S-1 versus upfront surgery for resectable pancreatic cancer (prep-02/JSAP05). *Jpn J Clin Oncol*. 2019;49(2):190-194.

213. de Geus SW, Evans DB, Bliss LA, et al. Neoadjuvant therapy versus upfront surgical strategies in resectable pancreatic cancer: A markov decision analysis. *Eur J Surg Oncol*. 2016;42(10):1552-1560.

214. Mokdad AA, Minter RM, Zhu H, et al. Neoadjuvant therapy followed by resection versus upfront resection for resectable pancreatic cancer: A propensity score matched analysis. *J Clin Oncol*. 2017;35(5):515-522.

215. Dhir M, Malhotra GK, Sohal DPS, et al. Neoadjuvant treatment of pancreatic adenocarcinoma: A systematic review and meta-analysis of 5520 patients. *World J Surg Oncol*. 2017;15(1):183-017-1240-2.

216. Tsai S, Christians KK, Ritch PS, et al. Multimodality therapy in patients with borderline resectable or locally advanced pancreatic cancer: Importance of locoregional therapies for a systemic disease. *J Oncol Pract*. 2016;12(10):915-923.

217. Sohal DP, Walsh RM, Ramanathan RK, Khorana AA. Pancreatic adenocarcinoma: Treating a systemic disease with systemic therapy. *J Natl Cancer Inst*. 2014;106(3):dju011.

218. Lee YS, Lee JC, Yang SY, Kim J, Hwang JH. Neoadjuvant therapy versus upfront surgery in resectable pancreatic cancer according to intention-to-treat and per-protocol analysis: A systematic review and meta-analysis. *Sci Rep*. 2019;9(1):15662-019-52167-9.

219. Wolff RA. Adjuvant or neoadjuvant therapy in the treatment in pancreatic malignancies: Where are we? *Surgical Clinics of North America*. 2018;98(1):95-111.

220. Lennard TW, Shenton BK, Borzotta A, et al. The influence of surgical operations on components of the human immune system. *Br J Surg*. 1985;72(10):771-776.

221. Zhang P, Cote AL, de Vries VC, Usherwood EJ, Turk MJ. Induction of postsurgical tumor immunity and T-cell memory by a poorly immunogenic tumor. *Cancer Res*. 2007;67(13):6468-6476.

Supplemental Tables

Supplemental Table 1. The odds ratio for logistic original and bootstrap models in PDAC patients who did not receive surgery of the pancreatic tumor

Variables		Original Model	Bootstrap Model	P
		Odds Ratio (OR)	Odds Ratio (OR)	
Age		0.97	0.97	0.0001
Sex	Male	Ref	Ref	
	Female	0.94	0.93	0.35
Race	White	Ref		
	Black	0.65	0.65	0.0008
	Non-White non-Black	1.12	1.14	0.51
Insurance	Yes	Ref		
	No	0.49	0.49	0.009
Dscore	0	Ref	Ref	
	1	0.76	0.76	0.003

	2	0.59	0.59	0.002
Place of living	Urban	Ref	Ref	
	Rural	1.18	1.20	0.53
Hospital type	Academic	Ref	Ref	
	Community	0.38	0.38	0.0001
Income	>=$35,000	Ref	Ref	
	<$35,000	0.95	0.95	0.54
Education	<13% No HSD	Ref	Ref	
	>=13% No HSD	0.79	0.79	0.007
Year of diagnosis	2004-2010	Ref	Ref	
	2011-2016	1.04	1.04	0.63
Chemotherapy	Yes	Ref	Ref	
	No	0.10	0.10	0.0001
RT	Yes	Ref	Ref	
	No	0.59	0.59	0.0001

P: The P-value is for the comparison of the odds ratio between the categories of each variable for the original model not for the comparison of OR between the original model and the bootstrap model

Supplemental Table 2. The hazard ratio of the original and bootstrap model for (immunotherapy no vs. yes) in PDAC patients who did not receive surgery of the pancreatic tumor

Variables		Original Model	Bootstrap Model	P
		Hazard Ratio (HR)	Hazard Ratio (HR)	
Age		1.01	1.01	0.0001
Sex	Male	Ref	Ref	
	Female	0.94	0.94	0.0001
Race	White	Ref		
	Black	0.99	0.99	0.66
	Non-White non-Black	0.88	0.88	0.0001
Insurance	Yes	Ref	Ref	
	No	1.06	1.06	0.0001
Dscore	0	Ref	Ref	
	1	1.12	1.12	0.0001
	2	1.35	1.35	0.0001

Place of living	Urban	Ref	Ref	
	Rural	1.04	1.04	0.033
Hospital type	Academic	Ref	Ref	
	Community	1.21	1.20	0.0001
Income	>=$35,000	Ref	Ref	
	<$35,000	1.06	1.06	0.0001
Education	<13% No HSD	Ref	Ref	
	>=13% No HSD	0.99	0.99	0.0001
Year of diagnosis	2004-2010	1.14	1.14	0.0001
	2011-2016	Ref	Ref	
Chemotherapy	Yes	Ref	Ref	
	No	1.85	1.85	0.0001
RT	Yes	Ref	Ref	
	No	1.41	1.41	0.0001
Immunotherapy	Yes	0.88	0.88	
	No	Ref		0.008

Supplemental Table 3. The hazard ratio of the original and bootstrap model for (chemotherapy plus immunotherapy vs. chemotherapy alone) in PDAC patients who did not receive surgery of the pancreatic tumor

Variables		Original Model	Bootstrap Model	P
		Hazard Ratio (HR)	Hazard Ratio (HR)	
Age		1.01	1.01	0.0001
Sex	Male	Ref	Ref	
	Female	0.93	0.93	0.0001
Race	White	Ref		
	Black	0.97	0.97	0.017

	Non-White non-Black	0.88	0.88	0.0001
Insurance	Yes	Ref	Ref	
	No	1.13	1.13	0.0001
Dscore	0	Ref	Ref	
	1	1.09	1.09	0.0001
	2	1.27	1.27	0.0001
Place of living	Urban	Ref	Ref	
	Rural	1.04	1.04	0.20
Hospital type	Academic	Ref	Ref	
	Community	1.23	1.23	0.0001
Income	>=$35,000	Ref	Ref	
	<$35,000	1.06	1.06	0.0001
Education	<13% No HSD	Ref	Ref	
	>=13% No HSD	1.01	1.01	0.26
Year of diagnosis	2004-2010	1.31	1.31	0.0001
	2011-2016	Ref	Ref	
Chemoimmunotherapy combination	Chemotherapy plus immunotherapy	0.86	0.86	0.003
	Chemotherapy	Ref		

Supplemental Table 4. The hazard ratio of the original and bootstrap model for (chemoradiation plus immunotherapy vs. chemoradiation alone) in PDAC patients who did not receive surgery of the pancreatic tumor

Variables		Original Model	Bootstrap Model	P
		Hazard Ratio (HR)	Hazard Ratio (HR)	
Age		1.01	1.01	0.0001
Sex	Male	Ref	Ref	

	Female	0.97	0.97	0.031
Race	White	Ref		
	Black	0.93	0.93	0.0001
	Non-White non-Black	0.93	0.94	0.076
Insurance	Yes	Ref	Ref	
	No	1.04	1.04	0.40
Dscore	0	Ref	Ref	
	1	1.10	1.10	0.0001
	2	1.19	1.19	0.0001
Place of living	Urban	Ref	Ref	
	Rural	1.10	1.10	0.034
Hospital type	Academic	Ref	Ref	
	Community	1.16	1.16	0.0001
Income	>=$35,000	Ref	Ref	
	<$35,000	1.10	1.10	0.0005
Education	<13% No HSD	Ref	Ref	
	>=13% No HSD	1.10	1.10	0.001
Year of diagnosis	2004-2010	1.34	1.34	0.0001
	2011-2016	Ref	Ref	
Chemoimmunotherapy combination	Chemotherapy plus immunotherapy	0.80	0.81	0.002
	Chemotherapy	Ref		

Supplemental Table 5. Concordance Index of models for PDAC patients who did not receive definitive surgery of the pancreatic tumor

Variables	Original Model	Bootstrap Model	Concordance Index Bias

	Concordance Index	Concordance Index	
Logistic regression analysis	0.81	0.81	0.000
Cox analysis immunotherapy Yes vs. No	0.685	0.685	0.000
Cox analysis chemo plus immunotherapy	0.572	0.572	0.000
Cox analysis chemoradiation plus immunotherapy	0.566	0.567	0.001

Supplemental Table 6. The odds ratio for logistic original and bootstrap models in PDAC patients who received surgery of the pancreatic tumor

Variables		Original Model	Bootstrap Model	P
		Odds Ratio (OR)	Odds Ratio (OR)	
Age		0.97	0.97	0.0001
Sex	Male	Ref	Ref	
	Female	0.83	0.84	0.031
Race	White	Ref		
	Black	0.48	0.85	0.0003
	Non-White non-Black	0.86	0.87	0.53
Insurance	Yes	Ref		
	No	0.50	0.51	0.074
Dscore	0	Ref	Ref	
	1	0.74	0.74	0.004
	2	0.54	0.55	0.004
Place of living	Urban	Ref	Ref	
	Rural	0.43	0.44	0.099
Hospital type	Academic	Ref	Ref	
	Community	0.26	0.26	0.0001
Income	>=$35,000	Ref	Ref	
	<$35,000	0.86	0.86	0.16

Education	<13% No HSD	Ref	Ref	
	>=13% No HSD	0.71	0.71	0.002
Year of diagnosis	2004-2010	1.27	1.27	0.005
	2011-2016	Ref	Ref	
Chemotherapy	Yes	Ref	Ref	
	No	0.21	0.21	0.0001
RT	Yes	Ref	Ref	
	No	0.35	0.35	0.0001

Supplemental Table 7. The hazard ratio of the original and bootstrap model for (immunotherapy no vs. yes) in PDAC patients who received surgery of the pancreatic tumor

Variables		Original Model	Bootstrap Model	P
		Hazard Ratio (HR)	Hazard Ratio (HR)	
Age		1.01	1.01	0.0001
Sex	Male	Ref	Ref	
	Female	0.92	0.92	0.0001
Race	White	Ref		
	Black	1.04	1.04	0.022
	Non-White non-Black	0.87	0.87	0.0001
Insurance	Yes	Ref	Ref	
	No	1.07	1.08	0.039
Dscore	0	Ref	Ref	
	1	1.06	1.06	0.0001
	2	1.23	1.23	0.0001
Place of living	Urban	Ref	Ref	

	Rural	1.05	1.05	0.16
Hospital type	Academic	Ref	Ref	
	Community	1.21	1.21	0.0001
Income	>=$35,000	Ref	Ref	
	<$35,000	1.09	1.09	0.0001
Education	<13% No HSD	Ref	Ref	
	>=13% No HSD	1.07	1.07	0.0001
Year of diagnosis	2004-2010	1.16	1.16	0.0001
	2011-2016	Ref	Ref	
Chemotherapy	Yes	Ref	Ref	
	No	1.13	1.13	0.0001
RT	Yes	Ref	Ref	
	No	1.06	1.06	0.0001
Immunotherapy	Yes	0.90	0.90	0.038
	No	Ref	Ref	

Supplemental Table 8. The hazard ratio of the original and bootstrap model for (chemotherapy plus immunotherapy vs. chemotherapy alone) in PDAC patients who received surgery of the pancreatic tumor

Variables		Original Model	Bootstrap Model	P
		Hazard Ratio (HR)	Hazard Ratio (HR)	
Age		1.01	1.01	0.0001
Sex	Male	Ref	Ref	
	Female	0.95	0.95	0.002
Race	White	Ref		
	Black	1.09	1.09	0.008

	Non-White non-Black	0.91	0.91	0.055
Insurance	Yes	Ref	Ref	
	No	0.93	1.93	0.23
Dscore	0	Ref	Ref	
	1	1.07	1.07	0.0007
	2	1.19	1.18	0.0001
Place of living	Urban	Ref	Ref	
	Rural	1.07	1.07	0.23
Hospital type	Academic	Ref	Ref	
	Community	1.22	1.22	0.0001
Income	>=$35,000	Ref	Ref	
	<$35,000	1.07	1.08	0.001
Education	<13% No HSD	Ref	Ref	
	>=13% No HSD	1.05	1.05	0.0133
Year of diagnosis	2004-2010	1.20	1.20	0.0001
	2011-2016	Ref	Ref	
Chemoimmunotherapy combination	Chemotherapy plus immunotherapy	0.93	0.93	0.48
	Chemotherapy	Ref		

Supplemental Table 9. The hazard ratio of the original and bootstrap model for (chemoradiation plus immunotherapy vs. chemoradiation alone) in PDAC patients who received surgery of the pancreatic tumor

Variables		Original Model	Bootstrap Model	P
		Hazard Ratio (HR)	Hazard Ratio (HR)	
Age		1.01	1.01	0.0001
Sex	Male	Ref	Ref	

	Female	0.93	0.93	0.0001
Race	White	Ref		
	Black	0.97	0.97	0.32
	Non-White non-Black	0.86	0.86	0.003
Insurance	Yes	Ref	Ref	
	No	1.07	1.07	0.25
Dscore	0	Ref	Ref	
	1	1.07	1.08	0.0002
	2	1.19	1.19	0.0001
Place of living	Urban	Ref	Ref	
	Rural	1.02	1.02	0.80
Hospital type	Academic	Ref	Ref	
	Community	1.15	1.15	0.0001
Income	>=$35,000	Ref	Ref	
	<$35,000	1.07	1.07	0.001
Education	<13% No HSD	Ref	Ref	
	>=13% No HSD	1.08	1.08	0.0002
Year of diagnosis	2004-2010	1.17	1.18	0.0001
	2011-2016	Ref	Ref	
Chemoimmunotherapy combination	Chemotherapy plus immunotherapy	0.85	0.85	0.005
	Chemotherapy	Ref		

Supplemental Table 10. Concordance Index of models for PDAC patients who received definitive surgery of the pancreatic tumor

Variables	Original Model	Bootstrap Model	Concordance Index Bias
	Concordance Index	Concordance Index	

Logistic regression analysis	0.799	0.801	0.002
Cox analysis immunotherapy Yes vs. No	0.587	0.587	0.000
Cox analysis chemo plus immunotherapy	0.558	0.558	0.000
Cox analysis chemoradiation plus immunotherapy	0.557	0.557	0.000