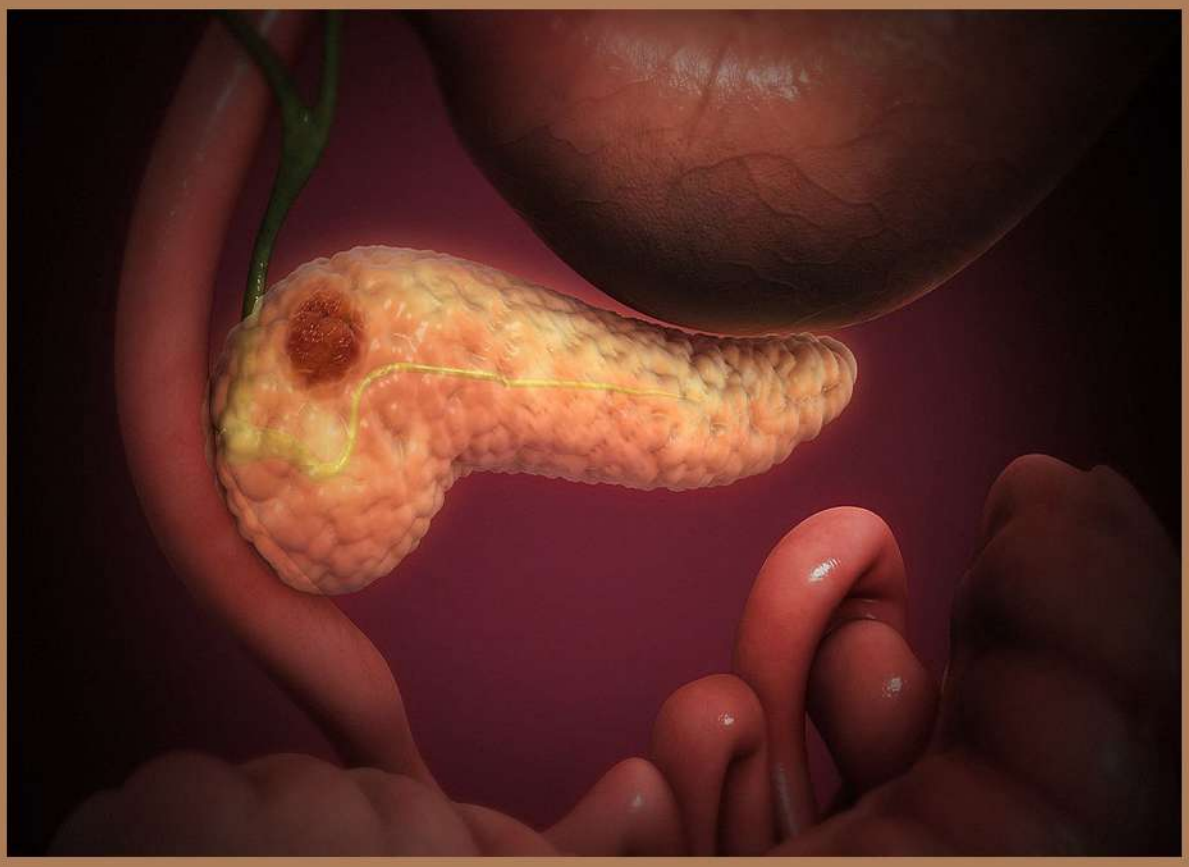
ISSN 2471-142X

Openventio
PUBLISHERS

PANCREAS

Open Journal

December, 2019. Volume 3 | Issue 1



www.openventio.org

ISSN 2471-142X

PANCREAS
Open Journal

Openventio
PUBLISHERS

CONTENTS

ISSN 2471-142X

PANCREAS
Open Journal

Openventio
PUBLISHERS

Original Research

Loss of Pancreatic β-cell Secretory Function During Disease Progression in Type 2 Diabetes Mellitus - A Small Cross-Sectional Study

George B. Kudolo, PhD, CPC, FAIC, FAACC*

Department of Health Sciences, Division of Medical Laboratory Sciences–MSC 6246, University of Texas Health Science Center at San Antonio, Texas 78229-3900, USA

****Corresponding author***
George B. Kudolo, PhD
UT Distinguished Teaching Professor and Chair, Department of Health Sciences, Division of Medical Laboratory Sciences–MSC 6246, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, Texas 78229-3900, USA; Tel. 1-(210) 567 4316; E-mail: kudolo@uthscsa.edu

Article information
Received: April 25th, 2019; **Revised:** June 29th, 2019; **Accepted:** June 29th, 2019; **Published:** July 12th, 2019

Cite this article
Kudolo GB. Loss of pancreatic β-cell secretory function during disease progression in type 2 diabetes mellitus:A small cross-sectional study. *Pancreas Open J*. 2019; 3(1): 1-8. doi: 10.17140/POJ-3-109

ABSTRACT

Introduction: Overt type 2 diabetes mellitus (T2DM) is a chronic progressive disease which is produced by the collusion of three metabolic defects-increased hepatic glucose production, impaired pancreatic β-cell insulin secretion and decreased insulin action. The measurement of plasma glucose 2 hours post-ingestion of 75 g of glucose during the oral glucose tolerance test (OGTT) may be used to classify individuals as normal glucose tolerant (NGT), impaired glucose tolerant, T2DM and T2DM with pancreatic β-cell failure.

Objectives: This study was undertaken primarily to show the importance of assessing the pancreatic β-cell function especially during the care of the diabetic patient.

Methods: A standard 75 g glucose tolerance test (OGTT) was administered to four groups of 8 subjects (4 male, 4 female). Blood was drawn every 15 minutes for 2 hours for the measurement of glucose, insulin and C-peptide and the measurement of the area under the curve ($AUC_{(0\rightarrow2)}$) over the 2-hour period.

Results: American Diabetes Association (ADA) criteria were used to classify the subjects. The normal glucose tolerant (NGT), had 2 h glucose 111±11 mg/dL, those with impaired glucose tolerance (IGT) had 2 h glucose 160±13 mg/dL. The 2 h glucose for the T2DM group was 258±27 mg/dL and those for the T2DM-PE group was 260±42 mg/dL. The $AUC_{(0\rightarrow2)}$ for NGT group were 254±40 mg/dL/h, 112±61 μU/mL/h and 10.2±4.6 ng/ml/h for glucose, insulin and C-peptide, respectively. The $AUC_{(0\rightarrow2)}$ for the IGT group were 394±32 mg/dL/h, 160±48 μU/mL/h and 19.8±7.7 ng/ml/h for glucose, insulin and C-peptide, respectively. The $AUC_{(0\rightarrow2)}$ for the T2DM group were 474±62 mg/dL/h, 194±40 μU/mL/h and 13.4±4.7 ng/mL/h for glucose and insulin, and C-peptide, respectively. The $AUC_{(0\rightarrow2)}$ for the T2DM-PE group were 481±80 mg/dL/h, 51±29 μU/mL/h and 7.2±2.8 ng/mL/h for glucose, insulin and C-peptide, respectively. There was no significant difference between the diabetic groups with respect to the glucose $AUC_{(0\rightarrow2)}$ but a significant difference existed in the insulin $AUC_{(0\rightarrow2)}$, ($p<0.0001$) mirrored by the fasting plasma insulin levels (30±8 μU/mL *vs* 14+8 μU/mL, for T2DM and T2DM-PE, respectively, $p<0.0005$). Although there was about a 300% increase in fasting insulin between the IGT and T2DM groups, the corresponding fasting C-peptide levels were only about 15%. This is probably due to differences in hepatic and renal functions in those two groups, the processes that control insulin and C-peptide levels in the body.

Conclusion: Although measurement of blood glucose appears adequate in the diagnosis of the diabetes, it seems that plasma insulin/C-peptide measurements could guide physicians in their choice of medications for the treatment of diabetic patients, especially when the pancreas begins to fail. To that end, larger studies are warranted to study the effects of hypoglycemic agents on hepatic insulin extraction and renal C-peptide excretion to ascertain the reliability of the plasma insulin and C-peptide levels.

Keywords
Type 2 diabetes mellitus; Pancreatic β-cells; Oral glucose tolerance test; Pancreatic exhaustion.

Pancreas Open J. 2019; 3(1): 1-8. doi: 10.17140/POJ-3-109

INTRODUCTION

To diagnose diseases of specific endocrine organs, it is common practice to measure directly hormones produced by those organs or measure the levels of the metabolites of those hormones. For example, for the diseases of the adrenal gland, the measurement of plasma cortisol (to assess the function of the *zona fasciculata* and diagnose either Addison's or Cushing's syndrome),[1] and androgens such as plasma dehydroepiandrosterone or urinary 17-ketosteroids (to assess the function of the *zona reticularis* for diagnosis of congenital adrenal hyperplasia),[2,3] or plasma renin and aldosterone (to assess the function of the *zona glomerulosa* and diagnose Conn's disease)[4] and plasma catecholamines and its metabolites plasma metanephrine and urinary vanillylmandelic acid (to assess the adrenal medulla and diagnose pheochromocytoma).[5] Insulin, produced by the pancreatic β-cell, is the only endogenous hormone that lowers blood glucose and the deficiency of insulin or its inability to adequately perform this task leads to the development of various types of diabetes mellitus. In all cases the hallmark of the disease is hyperglycemia. For example, type 1 diabetes (T1DM) is produced by loss of pancreatic β-cell function, leading to a deficiency of insulin and hyperglycemia, the onset of which is often seen in children and young adults, and accounting for less than 5% of the incidence of diabetes.[6] The development of type 2 diabetes mellitus (T2DM) takes several years and is the most common type. T2DM has now reached global epidemic status, affecting people in all socio-economic groups, all races and is found in both developed and developing countries.[7-9] This type of diabetes is also marked by defects of the pancreatic β-cell, swinging from hyperinsulinemia, a compensatory response to increasing peripheral insulin resistance, and progressing to loss of insulin production in later years due to pancreatic β-cell exhaustion.[10] It is well established that impaired pancreatic β-cell function, together with increased hepatic glucose production and increased peripheral insulin resistance, contributes to the development of T2DM.[11] The definitive diagnosis of T2DM uses only the plasma glucose at 2 hours post glucose challenge[6] and the care of the diabetic subject thereafter is based on predominantly blood glucose and hemoglobin A1c (HbA1c) measurements with only occasional tests of the pancreatic β-cell function during the care of the diabetic patient. The primary objective of this paper is to present some dataon the pancreatic β-cell function during T2DM in a cross-sectional study to stimulate more discussion, why pancreatic β-cell function (insulin/C-peptide) is not measured on a regular basis for the care of the T2DM patient.

RESEARCH DESIGN

Subjects

Study subjects were selected from prior studies[12-18] in which the subjects were enrolled in response to advertisements on bulletin boards around the University campus. The experimental protocolswere approved by the Institutional Review Board of the University of Texas Health Science Center, the General Clinical Research Center and the Research and Development Committee of the South Texas Veterans Health Care System, Audie L. Murphy Division. Written, informed, voluntary consent was obtained from all subjects. Body weights for all individuals had been stable for at least 3 months prior to study. None of the participants was on a dietary or exercise program for weight reduction. Potential subjects with significant medical problems (major cardiovascular, hepatic and other endocrine disease) other than elevated total cholesterol, as determined by routine medical history, physical examination, blood and urine tests were excluded. Diabetic subjects on oral hypoglycemic drugs were included but those on insulin injections were excluded. A pregnancy test was performed in all women of reproductive potential, and pregnant or nursing women were excluded. Subjects with anemia [Hb<12 g/dL (female) or <13 g/dL (male)] were also excluded.

Study Protocol

After an overnight fast (10-12 h) all subjects were asked to come to the Frederic C. Bartter General Clinical Research Center, where they were weighed, their heights taken (for determination of body mass indices), body composition profile performed using a bioelectrical impedance method (Spectrum Lightweight II, RJL Systems Instruments), electrocardiography performed, blood pressure checked and clinical examination performed. The subjects underwent the standard 75 g oral OGTT[7] to verify normal glucose tolerance and provide quantitative information about pancreatic β-cell secretion using established procedures. Three baseline blood samples (5 ml) were collected (time -30, -15 and -1-min) before the subjects ingested the glucose drink (Trutol® 75, NERL Diagnostics, East Providence, IR, USA) at time zero. Blood samples were obtained at 15 min intervals and analyzed for plasma glucose, insulin, and C-peptide. Blood samples were also collected during the basal state for the measurement of the basic metabolic panel, liver function tests and total lipid profile.

Analytical Techniques

All the clinical laboratory tests were performed in the Chemistry Laboratory of the South Texas Veterans Health Care System, Audie L. Murphy Division. LDL cholesterol was calculated using the Friedwald equation because fasting triglyceride levels did not exceed 400 mg/dL in any subject. Glycosylated hemoglobin was determined using high-pressure liquid chromatography using a Daimet Glycosylated Hemoglobin Analyzer (Bio-Rad Laboratories, Hercules, CA, USA). Plasma glucose concentrations during the OGTT was determined by the glucose oxidase method on a Beckman Glucose Analyzer II (Beckman, Fullerton, CA, USA). Plasma insulin and C-peptide levels were determined using radioimmunoassay kits (Diagnostic System Laboratories, Inc. Webster, TX, USA). At midcurve, the cross-reactivity of the C-peptide antiserum was less than 4% for proinsulin (and non-detectable with insulin) but the insulin antiserum may be as high as about 40% cross-reactivity with proinsulin. The fasting plasma levels of glucose, insulin and C-peptide were calculated as mean of the three baseline determinations whereas the fasting levels of free fatty acids were calculated as the mean of two baseline (-15 and -1 min) measurements. Intra-assay CV for plasma insulin and C-peptide were 3.5% and 2.6%, respectively, and inter-assay CV were 5.2%

Pancreas Open J. 2019; 3(1): 1-8. doi: 10.17140/POJ-3-109

and 4.2%, respectively.

Statistical Analysis

A post-hoc statistical power analysis was performed for sample size estimation, using G* Power v3.1 software, based on the fasting insulin levels of 7.5±2.8 μU/mL and 30.0±7.9 μU/mL for normal glucose tolerant (NGT) and T2DM subjects, respectively. The effect size (ES) was considered to be large using Cohen's criteria.[19] With an alpha=0.05 and power=0.8, the sample size of n=8 (4 males and 4 females) for each group (total 32) for this simple between group comparison was more than adequate for the main objective of this study. Each value is the mean±SD. Student's unpaired *t*-test comparison between NGT and IGT, T2DM and T2DM with pancreatic exhaustion (T2DM-PE) and between T2DM and T2DM-PE was performed using the statistical package on Microsoft Excel for Mac. Differences with $p<0.05$ were considered significant.

RESULTS

Subject Characteristics

Using the criteria of the American Diabetes Association (ADA) the subjects were divided into four groups based on their 2-hour plasma glucose levels after ingestion of a 75 g glucose load. The clinical characteristics of the subjects, with respect to the comprehensive metabolic panel, is presented in Table 1. Although this study was a cross-sectional one, it can be seen that in general, as an individual's tolerance to the glucose load deteriorated from an NGT state to impaired glucose tolerant (IGT), and full-blown T2DM and eventually into a T2DM-PE state. Full-blown T2DM was defined according to the American Diabetes Association criteria: 2-hour plasma glucose levels greater than 200 mg/dL after a 75 g glucose challenge. Whereas this is typically accompanied by a compensatory hyperinsulinemia, T2DM-PE patients exhibit hyperglycemia with reduced plasma insulin/C-peptide because of progressive failure of the pancreas to respond to rising blood glucose. The BMI increased from NGT to IGT to T2DM but subjects in the T2DM-PE state had significantly decreased BMI compared to the T2DM group. In all, several biochemical parameters of the body began to deteriorate significantly (compared to the NGT group) including the lipid panel. However, there was no significant difference between the T2DM and T2M-PE subjects, with respect to HbA1c (Table 2). The pancreatic response in the 4 groups in response to the glucose load is shown Figures 1-4. In Figures 1 and 2 it can be seen that using the 2-hour plasma glucose during the OGTT is an accurate reflection of the total plasma glucose area under the curve over the course of the 2-hour glucose challenge ($AUC_{(0\rightarrow2)}$). Importantly, although there was no difference between plasma glucose in T2DM and T2DM-PE, there was a very significant difference in the insulin $AUC_{(0\rightarrow2)}$, mirrored by the plasma C-peptide results (Figure 4), which reflects of the worsened state of the pancreas in T2DM-PE. One important observation was that although there was about a 300% increase in fasting insulin between the IGT and T2DM groups, the corresponding fasting C-peptide levels was only about 15%.

Table 1. *Summary of Selected Subject Clinical Characteristics in NGT, IGT, T2DM and T2DM-PE Subjects*

	Healthy n=8 (M/F; 4/4)	IGT n=8 (M/F; 4/4)	T2DM n=8 (M/F; 4/4)	T2DM-PE n=8 (M/F; 4/4)	Tests of Significance
Age (years)	45±11	54±7[a]	55±4[b]	51±9[c,d]	a. NGT *vs* IGT, $p<0.031$ b. NGT *vs* T2DM, $p<0.012$ c. NGT *vs* T2DM-PE, $p=0.127$ d.T2DM *vs* T2DM-PE, $p=0.125$
BMI (kg/m^2)	27.2±5.4	33.7±5.8[a]	36.6±5.6[b]	30.7±4.6[c,d]	a. NGT *vs* IGT, $p<0.018$ b. NGT *vs* T2DM, $p<0.002$ c. NGT *vs* T2DM-PE, $p=0.089$ d.T2DM *vs* T2DM-PE, $p<0.018$
Systolic BP (Hg mm)	129±14	132±13	135±16	137±17	Non-significant differences
Diastolic BP (Hg mm)	78±8	78±9	76±7	79±5	Non-significant differences
Cholesterol (mg/dL)	156±14	197±31[a]	185±38[b]	181±26[c,d]	a. NGT *vs* IGT, $p<0.002$ b. NGT *vs* T2DM, $p<0.029$ c. NGT *vs* T2DM-PE, $p<0.017$ d.T2DM *vs* T2DM-PE, $p=0.387$
Triglycerides (mg/dL)	114±47	131±46[a]	165±49[b]	196±103[c,d]	a. NGT *vs* IGT, $p=0.240$ b. NGT *vs* T2DM, $p<0.026$ c. NGT *vss* T2DM-PE, $p<0.030$ d.T2DM *vs* T2DM-PE, $p=0.228$
HDL (mg/dL)	53±16	47±9[a]	38±8[b]	37±9[c,d]	a. NGT *vs* IGT, $p=0.198$ b. NGT *vs*T2DM, $p<0.014$ c. NGT *vs* T2DM-PE, $p<0.019$ d.T2DM *vs* T2DM-PE, $p=0.407$
LDL (mg/dL)	93±12	125±27[a]	115±36[b]	105±28[c,d]	a. NGT *vs* IGT, $p=0.004$ b. NGT *vs* T2DM, $p<0.062$ c. NGT *vs* T2DM-PE, $p=0.138$ d.T2DM *vs* T2DM-PE, $p=0.277$

All values=mean±SD and unpaired t-test was used for statistical analysis and p-values<0.05 were considered significant.

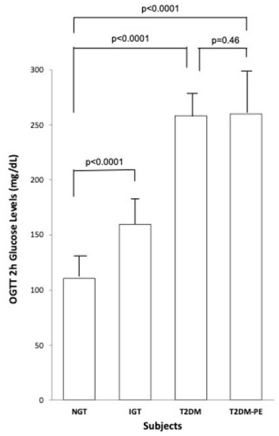
Pancreas Open J. 2019; 3(1): 1-8. doi: 10.17140/POJ-3-109

Table 2. *Summary of the Fasting Glucose and Insulin/C-peptide Characteristics of the NGT, IGT, T2DM and T2DM-PE Subjects*

	Healthy n=8 (M/F; 4/4)	IGT n=8 (M/F; 4/4)	T2DM n=8 (M/F; 4/4)	T2DM-PE n=8 (M/F; 4/4)	Tests of Significance
Fasting Plasma Glucose (mg/dL)	90±6	101±8[a]	121±17[b]	145±39[c,d]	a. NGT *vs* IGT, $p<0.003$ b. NGT *vs* T2DM, $p<0.001$ c. NGT *vs* T2DM-PE, $p<0.001$ d. T2DM *vs* T2DM-PE, $p<0.024$
HbA1c (%)	5.2±0.4	5.7±0.5[a]	6.8±1.3[b]	7.8±1.3[c,d]	a. NGT *vs* IGT, $p<0.021$ b. NGT *vs* T2DM, $p<0.002$ c. NGT *vs* T2DM-PE, $p<0.001$ d. T2DM *vs* T2DM-PE, $p=0.077$
Fasting Plasma Insulin (μU/mL)	7.5±2.8	10.8±5.2[a]	30.0v7.9[b]	13.9±7.8[c,d]	a. NGT *vs* IGT, $p=0.066$ b. NGT *vs* T2DM, $p<0.001$ c. NGT *vs* T2DM-PE, $p<0.022$ d. T2DM *vs* T2DM-PE, $p<0.001$
Fasting Plasma C-peptide (ng/mL)	1.1±0.5	3.4±1.5[a]	3.9±1.6[b]	2.4±1.1[c,d]	a. NGT *vs* IGT, $p<0.005$ b. NGT *vs* T2DM, $p<0.001$ c. NGT *vs* T2DM-PE, $p<0.001$ d. T2DM *vs* T2DM-PE, $p<0.024$

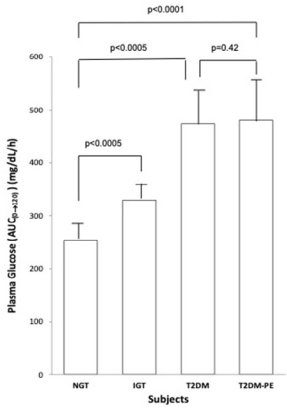
All values=mean±SD and unpaired t-test was used for statistical analysis and p-values<0.05 were considered significant.

Figure 1. *Plasma Glucose at 2 hours after the Ingestion of a 75 g Glucose Beverage*



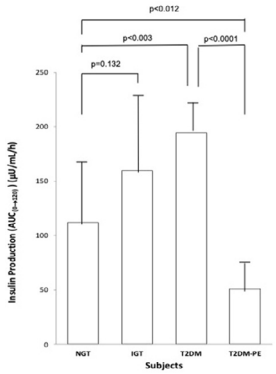
Each bar represents the mean of the subjects' plasma glucose for NGT (n=8, 4 females and 4 males), IGT (n=8, 4 females and 4 males) and T2DM (n=8, 4 females and 4 males) and T2DM-PE (n=8, 4 females and 4 males) groups. SD bars have been omitted for clarity. *p* values<0.05 were considered significant.

Figure 2. *Plasma Glucose Over the Course of 2 hours after the Ingestion of a 75 g Glucose Beverage*



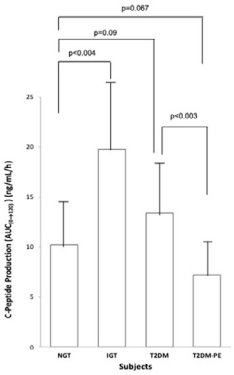
Each bar represents the mean of the subjects' plasma glucose for NGT (n=8, 4 females and 4 males), IGT (n=8, 4 females and 4 males) and T2DM (n=8, 4 females and 4 males) and T2DM-PE (n=8, 4 females and 4 males) groups. *p* values<0.05 were considered significant.

Figure 3. *Plasma Insulin Production Over the Course of 2 hours after the Ingestion of a 75 g Glucose Beverage*



Each bar represents the mean of the subjects' plasma glucose for NGT (n=8, 4 females and 4 males), IGT (n=8, 4 females and 4 males) and T2DM (n=8, 4 females and 4 males) and T2DM-PE (n=8, 4 females and 4 males) groups. *p* values<0.05 were considered significant.

Figure 4. *Plasma Insulin Production Over the Course of 2 hours after the Ingestion of a 75 g Glucose Beverage*



Each bar represents the mean of the subjects' plasma glucose for NGT (n=8, 4 females and 4 males), IGT (n=8, 4 females and 4 males) and T2DM (n=8, 4 females and 4 males) and T2DM-PE (n=8, 4 females and 4 males) groups. *p* values<0.05 were considered significant.

Pancreas Open J. 2019; 3(1): 1-8. doi: 10.17140/POJ-3-109

DISCUSSION

In 2011, the International Federation of Diabetes (IDF)[7] estimated that globally, there would be about 234 million people with diabetes by the year 2030 while the World Health Organization's (WHO) estimate was 366 million.[8] Both of these estimates appeared to be wrong as the global estimate of diabetes in 2015 was actually 415 million.[9] In the United States, an estimated 30 million people of all ages had diabetes, with the risk of developing T2DM increasing with age (reaching 25.2% among those aged 65 years or older), among several other factors.[20] Measurement of glucose in the laboratory, whether in plasma, serum or whole blood, is relatively easy and reliable and clearly self-monitoring blood glucose system is advantageous to patients.[21] Although several factors contribute to the failure to maintain normal blood glucose (euglycemia), it can be narrowed down to primarily insulin, the only endogenous hormone that lowers blood glucose.

The results of the present study illustrate and confirm the well-known facts about the development of T2DM. In the NGT individuals, plasma glucose was at euglycemia requiring relatively less insulin secretion when challenged with 75 g of glucose, because of enhanced whole-body insulin sensitivity. As insulin resistance increased, increased pancreatic β-cell function was required to keep blood glucose under control as seen in the IGT (pre-diabetic) individuals. In the overt T2DM subjects, there was significantly increased fasting insulin and increased insulin production in response to the 75 g glucose load. This compensatory hyperinsulinemia may initially produce euglycemia and may explain the substantial number of people who are generally aware of having diabetes. In this study, one important observation was that although there was about a 300% increase in fasting insulin between the IGT and T2DM groups the corresponding fasting C-peptide levels was only about 15%. Equimolar amounts of insulin and C-peptide are expected during synthesis from proinsulin. In this study, plasma levels of fasting insulin increased by nearly 3-fold (10.8 μU/ml *vs* 30 μU/ml, IGT and T2DM, respectively) probably due to increased peripheral insulin resistance and compensatory hyperinsulinemia by the pancreatic β-cells. However, the fasting plasma C-peptide outputs did not appear to match the insulin production (3.4 ng/ml *vs* 3.9 ng/mL in IGT and T2DM groups, respectively), accounting for only about 15% increase. This disparity could be related to the pharmacokinetics of these hormones - either decreased hepatic extraction of insulin or enhanced renal excretion of C-peptide. The seminal work of Matthews and colleagues[22] showed that whereas insulin levels are extracted from the peripheral circulation by the liver, the C-peptide levels were determined predominantly by renal clearance and therefore determined by kidney function.[23] In fact, subsequently, attempts to predict the actual insulin levels from the C-peptide levels have been described.[24] An extensive review of the clinical utility of C-peptide measurements in the care of diabetic patients has been published.[25] It is well known that diabetes confers increased risk for kidney diseases. Therefore, further studies are warranted to determine the effects of diabetes medications on hepatic and renal functions as they relate specifically to the insulin and C-peptide levels.

With entrenched insulin resistance and years of excessive secretory activity the pancreatic β-cells begin to fail as seen in the T2DM-PE, exhibiting increased fasting plasma glucose, with corresponding worsening of the HbA1c and decreased fasting plasma insulin/C-peptide (Table 2) and the insulin and C-peptide $AUC_{(0\rightarrow 2)}$ (Figures 3 and 4). It has long been known that the progression of NGT to IGT and ultimately to T2DM is typically hastened by obesity[26-28] which increases insulin resistance and is seen here by the increased BMI from NGT to T2DM. The decreased BMI in T2DM-PE compared to the overt T2DM is expected because T2DM diabetic patients are known to become leaner during pancreatic exhaustion.

The pancreas is a very unique organ performing both endocrine and exocrine functions in the body. The endocrine function of the pancreas controls predominantly carbohydrate metabolism. The pancreatic α-cells constitutes about 30-40% of the islet population, producing glucagon that elevates blood glucose by stimulating glycogenolysis and gluconeogenesis and adipose tissue lipolysis. On the other hand, the pancreatic β-cells, constituting about 50-60% of the islet population produces insulin, the only hormone that lowers blood glucose. Insulin acts by increasing glucose uptake by the liver, skeletal muscle and adipose tissue, stimulating glycogenesis and inhibiting gluconeogenesis. Impaired insulin secretion and/or insulin action may lead to the failure to maintain glucose at euglycemia, leading to hyperglycemia.

The age of diagnosis of the disease in the T2DM group was 47±3 years which agrees with the Centers for Disease Detection and Prevention (CDC) report indicating that adults in the United States typically receive new diagnosis of the disease between 45 and 64 years of age.[29] The age of diagnosis of diabetes for the T2DM-PE group was lower (41±5 years of age) because two patients were diagnosed at 34 and 35 years of age.

Although several derangements have now been shown to contribute to the pathogenesis of T2DM, the three most common are increased endogenous (hepatic) glucose production, impaired insulin secretion and impaired/decreased skeletal muscle insulin action (or decreased peripheral insulin sensitivity).[11] Correspondingly, the most common targeted oral hypoglycemic agents to address these defects are biguanides (e.g. metformin) to treat the increased hepatic glucose production,[30] sulfonylureas (e.g. glipizide) to treat the impaired pancreatic β-cell secretory function[31] and the thiazolidinediones (TZD) (e.g. pioglitazone) to increase insulin sensitivity.[32]

All the subjects in the two diabetes groups were on combination therapy. In the T2DM group, 4 out of 8 subjects were taking glipizide (5 mg daily), all were on metformin (2 subjects, 250 mg daily; 4 subjects, 500 mg daily; and 2 (500 mg×2 daily) and all were on 15 mg of pioglitazone. In the T2DM-PE group, all the subjects were on glipizide (10 mg daily), perhaps a recognition of decreased pancreatic β-cell function, all of them were metformin (4 subjects, 500 mg daily; one subject, 850 mg daily and 3 subjects were prescribed, 850 mg×3 daily), and all the 8 T2DM-PE subjects were on 15 mg pioglitazone. The prescription regimens

indicate that the patients were all deemed to have all three defects – impaired pancreatic β-cell function, increased hepatic glucose production and increased peripheral insulin resistance. So, even though all the diabetic patients were managed by combination oral hypoglycemic agents (sulfonylureas, biguanides and thiazolidinediones, respectively), it can be seen that the fasting blood glucose was worse in patients with T2DM-PE. The decrease in the insulin secretory function at this stage is thought to be due predominantly to an actual loss of pancreatic β-cell mass with the mechanism being increased pancreatic β-cell apoptosis and dediffrentiation.[23,33-35]

Even though several new antidiabetic drugs are now available, sulfonylureas which target the pancreas, remain the most prescribed class of drugs but metformin, the recommended first line of treatment, is the most common prescribed individual drug among the oral hypoglycemic agents.[36,37] The guidelines endorsed by the American Diabetes Association for pharmacological treatment of T2DM recommends metformin as an initial treatment, in addition to lifestyle changes. If glycemic targets are not achieved in 3 months, then other drugs like the sulfonylureas and thiazolidinediones may be added. The euglycemic clamp that may be used to determine hepatic glucose production and peripheral insulin resistance is available only in a clinical research setting. On the other hand, pancreatic β-cell function for routine patient care could be assessed easily by measurement of plasma insulin and C-peptide but Quest Diagnostics, a leading laboratory testing organization in the United States and others like it, do not have plasma insulin in their test guide for "Laboratory testing for diabetes and management." This is probably because assessment of pancreatic function is not a recommendation in the American Diabetes Association guidelines for the pharmacologic management of the diabetic patient. So, insulin and C-peptide tests are not ordered. This is unlike the case of hyperlipidemia where blood cholesterol levels are routinely ordered to guide doctors in treatment options. There must be some value to the physician and the patient knowing about the status of the pancreas.

CONCLUSION

Impaired hepatic glucose production, increased peripheral insulin and impaired pancreatic β-cell are major players that collude to produce T2DM.

The OGTT is very useful for the diagnosis of the disease because it assesses both the secretory capacity of the pancreatic β-cell as well as the tissue response to insulin (peripheral tissue insulin sensitivity). Using 2-hour plasma glucose as a diagnostic cutoff, fasting glucose or HbA1c levels are reliable for diagnosis of T2DM, but it is also clear that progressive loss of pancreatic β-cell function is inevitable in the disease progression. Just like plasma cholesterol measurements are relied upon to guide medication prescriptions in the care of patients with hyperlipidemia, we must start discussions on why measurement of insulin/C-peptide plasma levels could not be relied upon in the care of the diabetic patient. That knowledge of the pancreatic β-cell secretory capacity may be useful to the physician for choosing sulfonylureas in the case of patients approaching T2DM-PE.

ACKNOWLEDGEMENT

Nursing and dietetic care was provided by the staff of the Frederic C. Bartter General Clinical Research Center at the South Texas Veterans Health Care Systems - Audie Murphy Division. This study was a part of a larger study on *Ginkgo biloba* extract which was supported by funds from NIH grants M01-RR-01346 and R01-AT-00832 (National Center for Complementary and Alternative Medicine, NCCAM). My gratitude to Janet Blodgett, MD who supervised our patients and to all my research assistants and graduate students over the years.

REFERENCES

1. Guignat L, Bertherat J. The diagnosis of Cushing's syndrome: An European society clinical practice guideline: Commentary from a European perspective. *Eur J Endocrinol.* 2010; 163: 9-13. doi: 10.1530/EJE-09-0627

2. Merke DP. Approach to the adult with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *J Clin Endocrinol Metab.* 2008; 93: 653-660. doi: 10.1210/jc.2007-2417

3. Witchel SF. Nonclassic congenital adrenal hyperplasia. *Curr Opin Endocrinol Diabetes Obes.* 2012; 19: 151-158. doi: 10.1097/MED.0b013e3283534db2

4. Nikhikumar OH, Bakulesh DR. Primary hyperaldosteronism presenting as hypokalemic paralysis. *Int J Med Public Health.* 2019; 9: 31-33. doi: 10.5530/ijmedph.2019.1.8

5. Young MJ. Dmuchowski C, Wallis JW, et al. Biochemical tests for pheochromocytoma: strategies in hypertensive patients. *J Gen Intern Med.* 1989; 4: 273-276. doi: 10.1007/bf02597394

6. American Diabetes Association. Classification and diagnosis of diabetes: Standards of medical care in diabetes-2019. *Diabetes Care.* 2019; 42 (Suppl.1): S13-S28. doi: 10.2337/dc19-S002

7. Zimmet PZ. Diabetes and its drivers: The largest epidemic in human history? *Clinical Diabetes and Endocrinology.* 2017; 3: 1-8. doi: 10.1186/s40842-016-0039-3

8. International Diabetes Federation. IDF Diabetes. 1st ed. Brussels: International Diabetes Federation; 2000. http://www.diabetesatlas.org. Accessed June 20, 2019.

9. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes–estimates for the year 2000 and projections for 2030. *Diabetes Care.* 2004; 27: 1047-1053. doi: 10.2337/diacare.27.5.1047

10. Buchanan TA. Pancreatic beta-cell loss and preservation in type 2 diabetes. *Clin Ther.* 2003; 25(Suppl. B): B32-B46. doi: 10.1016/S0149-2918(03)80241-2

11. DeFronzo RA. Lilly lecture 1987. The triumvirate: B-cell, muscle, liver. A collision responsible for NIDDM. *Diabetes.* 1988; 37:

667-687. doi: 10.2337/diab.37.6.667

12. Kudolo GB. The effect of 3-month ingestion of Ginkgo biloba extract on pancreatic β-cell function in response to glucose loading in healthy volunteers. *J Clin Pharm.* 2000; 40: 647-654. doi: 10.1002/j.1552-4604.2000.tb05991.x

13. Kudolo GB. The effect of 3-month ingestion of Ginkgo biloba extract (EGb 761) on pancreatic β-cell function in response to glucose loading in individuals with non-insulin dependent diabetes mellitus. *Journal of Clinical Pharmacology.* 2001; 41: 600-611. doi: 10.1177/009127000104100602

14. Kudolo GB, Dorsey S, Blodgett J. Effect of the ingestion of Ginkgo biloba extract on platelet aggregation and urinary prostanoid excretion in healthy and type 2 diabetic subjects. *Thromb Res.* 2003; 108: 151-160. doi: 10.1016/S0049-3848(02)00394-8

15. Kudolo GB, Wang W, Dorsey S, Blodgett J. Oral ingestion of Ginkgo biloba extract reduces thiobarbituric acid reacting (TBAR) substances in washed platelets of healthy subjects. *J Herb Pharmacother.* 2003; 3: 1-15. doi: 10.1300/J157v03n04_01

16. Kudolo GB, Wang W, Barrientos J, Elrod R, Blodgett J. The ingestion of ginkgo biloba extract inhibits arachidonic acid-mediated platelet aggregation and thromboxane B_2 production in healthy subjects volunteers. *J Herb Pharmacother.* 2004; 4: 13-26. doi: 10.1080/J157v04n04_02

17. Kudolo GB, Delaney D, Blodgett J. Short-term oral ingestion of Ginkgo biloba extract (EGb 761) reduces malondialdehyde levels in washed platelets of type 2 diabetic subjects. *Diabetes Res Clin Pract.* 205; 68: 29-38. doi: 10.1016/j.diabres.2004.08.007

18. Kudolo GB, Wang W, Elrod R, et al. Short-term ingestion of ginkgo biloba extract (EGb 761) does not alter whole body insulin resistance in the non-diabetic, pre-diabetic or type 2 diabetic Subjects–a short randomized double-blind placebo-controlled crossover study. *Clin Nutr.* 2006; 25: 123-134. doi: 10.1016/j.clnu.2005.10.001

19. Cohen J. *Statistical Power Analysis for the Behavioral Sciences.* New Jersey, USA: Hillsdale, Lawrence Erlbaum Associates; 1988.

20. Centers for Disease Control and Prevention. National diabetes statistics report, 2017. Estimates of diabetes and its burden in the United States. Web site. https://www.cdc.gov/features/diabetes-statistic-report/index.html. Accessed June 20, 2019.

21. Murata GH, Duckworth WC, Shah JH. et al. Blood glucose monitoring is associated with better glycemic control in type 2 diabetes: A database study. *J Gen Intern Med.* 2009; 24: 48-52. doi: 10.1007/s11606-008-0830-7

22. Matthews DR, Rudenski AS, Burnett MA, et al. The half-life of endogenous insulin and C-peptide in man assessed by somatostatin suppression. *Clin Endocrinol (Oxf).* 1985; 23: 71-79. doi: 10.1111/j.1365-2265.1985.tb00185.x

23. Puri S, Folias AE, Hebrok M. Plasticity and dedifferentiation within the pancreas: Development, hemostasis, and disease. *Cell Stem Cell.* 2015; 16: 18-31. doi: 10.1016/j.stem.2014.11.001

24. Van Cauter E, Mesterz F, Stursi J, Polonsky KS. Estimation of insulin secretion rates from C-peptide levels. Comparison of individual and standard kinetic parameters for C-peptide clearance. *Diabetes.* 1992; 41: 368-377. doi: 10.2337/diab.41.3.368

25. Jones AG, Hattersley AT. The clinical utility of C-peptide measurement in the care of patients with diabetes. *Diabet Med.* 2013; 30: 803-817. doi: 10.1111/dme.12159

26. National Diabetes Data Group. Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. *Diabetes.* 1979; 28: 1039-1057. doi: 10.2337/diab.28.12.1039

27. World Health Organization (WHO). Diabetes mellitus: Report of a WHO study group. *World Health Organ Tech Rep Ser.* 1985; 17: 45.

28. American Diabetes Association. Report of an expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care.* 1998; 21(Suppl. 1): S5-S19.

29. National Diabetes Statistics Report, 2017. Estimates of Diabetes and its burden in the United States. Web site. https://www.cdc.gov/diabetes/pdfs/data/statistics/national-diabetes-statistics-report.pdf. Accessed June 20, 2019.

30. Cicero AFG, Tartagni E, Ertek S. Metformin and its clinical use: New insights for an old drug in clinical practice. *Arch Med Sci.* 2012; 8:907-917. doi: 10.5114/aoms.2012.31622

31. Sola D, Rossi L, Schianca GPC, et al. Sulfonylureas and their use clinical in clinical practice. *Arch Med Sci.* 2015; 4: 840-848. doi: 10.5114/aoms.2015.53304

32. Yasmin S, Jayaprakash V. Thiazolidinediones and PPAR orchestra as antidiabetic agents: From past to present. *Eur J Med Chem.* 2017; 126: 879-893. doi: 10.1016/j.ejmech.2016.12.020

33. Butler AE, Janson J, Bonner-Weir S, et al. Beta-cell deficit and increased beta-cell apoptosis in humans with type diabetes. *Diabetes.* 2003; 52: 102-110. doi: 10.2337/diabetes.52.1.102

34. Dor Y, Glasser B. Beta-cell dedifferentiation and type 2 diabetes. *N Engl J Med.* 2013; 368: 572-573. doi: 10.1056/NEJMcibr1214034

35. Clinti F, Bouchi R, Kim-Muller JY, et al. Evidence of β-cell dedifferentiation in human type 2 diabetes. *J Clin Endocrinol Metab.* 2016; 101: 1044-1054. doi: 10.1210/jc.2015-2860

Pancreas Open J. 2019; 3(1): 1-8. doi: 10.17140/POJ-3-109

36. Agarwal AA, Jadhav PR, Deshmukh YA. Prescribing pattern and efficacy of anti-diabetic drugs in maintaining optimal glycemic levels in diabetic patients. *J Basic Clin Pharma.* 2014; 5: 79-83. doi: 10.4103/0976-0105.139731

37. Qaseem A, Barry MJ, Humphrey LL, Forciea MA. Oral pharmacologic treatment of type 2 diabetes mellitus: A clinical practice guideline update from the American College of Physicians. *Ann Intern Med.* 2017; 166: 279-290. doi: 10.7326/M16-1860

ISSN 2471-142X

PANCREAS
Open Journal

Openventio
PUBLISHERS

Review

The Evolving Field of Stereotactic Body Radiation Therapy in Pancreatic Cancer

Maged Ghaly, MD[1*]; Emile Gogineni, DO[1]; Muhammad W. Saif, MD[2]

[1]*Department of Radiation Medicine, Northwell Health Cancer Institute, Lake Success, NY, USA*
[2]*Department of Medical Oncology, Northwell Health Cancer Institute, Lake Success, NY, USA*

[*]***Corresponding author***
Maged Ghaly, MD
Associate Professor, Department of Radiation Medicine, Northwell Health Cancer Institute, Lake Success, NY, USA; Phone. +1-516-321-3122; Fax. +1-516-470-8445; E-mail: Mghaly@northwell.edu

Article information
Received: October 2nd, 2019; **Revised:** October 30th, 2019; **Accepted:** October 31st, 2019; **Published:** November 6th, 2019

Cite this article
Ghaly M, Gogineni E, Saif MW. The evolving field of stereotactic body radiation therapy in pancreatic cancer. *Pancreas Open J*. 2019; 3(1): 9-14. doi: 10.17140/POJ-3-110

ABSTRACT

Pancreatic cancer remains a devastating disease with dismal outcomes despite the development of novel chemotherapeutic regimens and radiation techniques. Stereotactic body radiation therapy (SBRT) offers an advantage both in image guidance and radiation dose delivery to direct ablative doses to tumors with acceptable toxicity compared to conventional techniques. Recent literature is clustered with data pertaining to SBRT in patients with resectable, borderline resectable and locally advanced pancreatic tumors. We here present a summary of the current data and highlight the limitations and potential for future growth. Further clinical study in the form of multi-institutional trials is warranted to establish the role of SBRT in combination with new chemotherapeutic agents as well as a non-invasive alternative to surgery.

Keywords
Pancreatic neoplasms; Pancreas cancer; Radiosurgery; Stereotactic; Stereotactic body radiation therapy (SBRT); Radiation; Radiotherapy.

INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is an aggressive malignancy with limited effective therapeutic options and exceedingly high mortality. Currently, a cure may be achieved through resection; recent evidence suggests that neoadjuvant therapy can increase R0 (pathologically negative margin) resection rates with effective local control.[1] Stereotactic body radiation therapy (SBRT) has garnered significant interest for pancreatic cancer patients as it is completed quickly over 1-5 fractions, requires less time away from full doses of chemotherapy, and is generally much better tolerated than conventional radiographic testing (RT) as a result of more limited target volumes. Favorable results of SBRT for locally advanced pancreatic cancer (LAPC) patients are now leading to the exploration of SBRT for other pancreatic cancer patients.[2]

SBRT FOR LOCALLY ADVANCED PANCREAS

The utility of pancreatic SBRT was established in the locally advanced patient population. With the advent of gemcitabine-based (GEM) chemotherapy, the role of RT for LAPC has become more precarious.[3] The European Fédération Francophone de Cancérologie Digestive (FFCD)/The Société Francophone de Radiothérapie Oncologique (SFRO) Phase III trial compared GEM alone *versus* induction 5 Fluorouracil (FU) and cisplatin chemoradiation (CRT), followed by maintenance gem.[4] Overall survival (OS) was shortened in the CRT arm from 13 to 8.6-months. Higher grade 3 toxicities with CRT were observed during both induction (36% *vs.* 22%) and maintenance (32% *vs.* 18%) phases. Notably, the trial utilized a higher than normal conventionally fractionated 60 Gy dose. The recent success of more aggressive, but increasingly toxic, chemotherapy regimens such as FOLFIRINOX and gem plus nab-paclitaxel have spurred re-examination of local therapy.[5,6] With improved systemic control, local progression may become a more serious issue for survival and quality of life. However, local control rates from standard external beam radiotherapy (EBRT) have been disappointing with 1-year local progression rates of around 50%.[7] Furthermore, with two-thirds of patients failing distantly within 1 year, a shorter course approach with minimal interruption to systemic therapy is desirable.[7] These factors paved the way for the use of SBRT in pancreatic cancer patients, and initially those with LAPC.

Pancreas Open J. 2019; 3(1): 9-14. doi: 10.17140/POJ-3-110

The inception of SBRT for pancreatic cancers began at Stanford with a phase I dose escalation study in a LAPC cohort.[8] The trial was stopped at a dose of 25 Gy since all patients achieved local control with distant metastasis as the first site of failure. The median survival for all patients was 11-months, with 100% local control. However, despite smaller margins and less acute toxicity, patients treated on the Stanford single-fraction SBRT protocol experienced a high degree of late toxicities (25% grade ≥2).[9] Hypofractionated studies showed reduced 1-year grade 2 toxicity to 7.8%. This reduction came without a compromise in disease control. The 1-year local control was 91.5% *vs.* 88.3% (p=0.8) for single *vs.* 5-fraction SBRT with median OS of 13.6-months for all patients. More contemporary SBRT series have also largely employed a fractionated approach.[10-14] These institutional studies reveal a median survival of 14-15-months, 1-year local control rates of about 80%, and grade 3 toxicities below 10%.[15]

Very recently, a few groups have reported that LAPC patients may have an increased likelihood of undergoing resection after aggressive induction chemotherapy regimens. Recently, the group from Hopkins reported on 88 patients treated from 2010-14 with SBRT using gem-based or FOLFIRINOX regimens.[16] SBRT doses ranged from 25-33 Gy in 5 fractions. The 1-year local control rate was 61%, but with a median OS of 18.4-months for LAPC patients. Notably, 20% of LAPC patients underwent surgery. Resected patients had a median OS of 20.2-months, compared to 12.3-months for unresected cases. Grade 3 toxicity was below 6%. Similar to the study from Hopkins, SBRT data from Moffitt also shows the possibility of downstaging for surgery.[14] They reported a 24% surgical conversion rate for LAPC patients receiving FOLFIRINOX chemotherapy. All converted patients achieved an R0 (microscopic negative margin) resection. Any grade 3 or higher toxicity was 7%. Median OS was 34.2-months for patients who underwent resection, and 11.3-months for those who did not. See Table 1 for a list of SBRT studies for LAPC.

SBRT FOR BORDERLINE RESECTABLE PANCREAS

While pancreatic SBRT has been most extensively evaluated in LAPC patients, there is emerging data that SBRT may also benefit patients with borderline resectable pancreas (BRPC) (Table 2). The SBRT literature for BRPC largely comes from the Moffitt Cancer Center. Chuong et al reported on a larger series of 73 patients (57 BRPC, 16 LAPC) who received induction gem, docetaxel, and capecitabine (GTX) followed by SBRT.[12] SBRT was delivered using 5 consecutive daily fractions targeting the primary tumor with a median dose of 30 Gy (range, 25-30 Gy), the region of vasculature involvement was prescribed a median dose of 35 Gy (range, 35-50 Gy) using a simultaneous integrated boost (SIB) to further increase the likelihood of tumor regression and R0 resection. After restaging, 56.1% of the BRPC patients underwent surgical resection with all except for one (96.9%) having negative margins. Resected patients had significantly improved median OS (19.3 *vs.* 12.3 months; p=0.03) and median progression-free survival (PFS) (12.7 *vs.* 5-months; p<0.0001). No acute grade 3 toxicities were reported and the most common acute toxicities were grade 1-2 fatigue and nausea. Their subsequent study of 159 patients (110 BRPC, 49 LAPC), surgical resection was performed on 51% of the BRPC patients and R0 resection was achieved in 96%. Portal vein (PV) or superior mesenteric vein (SMV) resection and reconstruction was performed in 34% of BRPC patients. Median OS was significantly higher among patients who had surgery compared to those who did not (34.2 *vs.* 14.0-months; p<0.001). Finally, while the prescription doses generally increased compared to the previous publication (primary tumor: median 30 *vs.* 35 Gy; tumor-vessel interface: median 35 *vs.* 40 Gy), the incidence of late grade 3 radiation-related toxicity remained consistently low (~5%).[14]

The feasibility of using SBRT for BRPC is also supported by other studies with more limited numbers of BRPC patients. A study from Johns Hopkins included 88 patients (74 LAPC, 14

Table 1. *SBRT for Locally Advanced Pancreatic Cancer*

Study	n	Dose Fractionation	Chemo	Local control	Survival	Toxicity
Koong et al[8]	6	25Gy in 1fx (73 Gy2)	None	100% @ 1 year	Median 8 mo	33% acute G3+
Chang et al[9]	77	25Gy in 1fx (73 Gy2)	Gemcitabine	84% @ 1 year	Median 12 mo	25% G2+ @ 1yr
Mahadevan et al[11]	39	24-36Gy in 5fx (30-50 Gy2)	Gemcitabine	85% crude	Median 20 mo	9% late G3+
Herman et al[13]	49	33Gy in 5fx (46 Gy2)	Gemcitabine	78% @ 1 year	Median 13.9 mo	12% acute G3+ 11% late G2+
Moningi et al[17]	88	25-33Gy in 5fx (31-46 Gy2)	Gemcitabine or FOLFIRINOX	61% @ 1 year	Median 18.4 mo	3% acute G3+ 6% late G2+

Table 2. *SBRT for Borderline Resectable Pancreatic Cancer*

Study	n	Dose Fractionation	Chemo	Survival	Conversion rate	R0	pCR	Toxicity
Chuong et al[12]	73 (78% BRPC)	25-50Gy in 5fx (31-83 Gy2)	GTX	Median 16.4m 72% @ 1 year	56%	97%	Not reported	0% acute G3+ 5% late G3+
Mellon et al[14]	159 (69% BRPC)	30-40 Gy in 5fx (40-60 Gy2	GTX	Median 19.2 m	51%	96%	7%	7% acute & late G3+
Rajagopalan et al[18]	12 (58% BRPC)	36 Gy in 3fx (66 Gy2) 24 Gy in 1fx (68 Gy2)	Gemcitabine-Capecitabine	Median 47.2 m 92% @ 1 year	100%	92%	25%	0% acute G3+

Pancreas Open J. 2019; 3(1): 9-14. doi: 10.17140/POJ-3-110

BRPC) who received 5-fraction SBRT and reported favorable surgical and SBRT-related toxicity outcomes.[17]

Investigators from the University of Pittsburgh published their experience of 12 patients (7 BRPC, 5 LAPC) who received chemotherapy followed by SBRT prescribed to 36 Gy in 3 fractions (n=7) or 24 Gy in a single fraction (n=5) and then had surgery.[18] A high rate of R0 resection was achieved (92%) with minimal toxicity. Pathologic complete response (pCR) was achieved in 25%, which is higher than would be expected with standard EBRT and perhaps signaling that SBRT may have unique histopathologic effects. It is plausible that a higher rate of pCR may be achieved using dose fractionation schedules with a higher biologically effective dose. He et al compared surgical outcomes among BRPC/LAPC patients who received SBRT (n=29), CRT (n=82), or chemotherapy alone (n=26) and reported R0 resection rates of 90%, 84% and 62%, respectively (p=0.02).[19] The PCR rate was notably higher among patients who received SBRT (21% *vs.* 4% *vs.* 0%; p<0.001).

In conclusion, while various neoadjuvant treatment regimens are commonly used for BRPC including standard fractionation CRT, increasing consideration should be given to SBRT based on its clear advantage in increasing R0 resectability with higher PCR rates, and providing improved OS in these patients.

SBRT FOR RESECTABLE PANCREAS

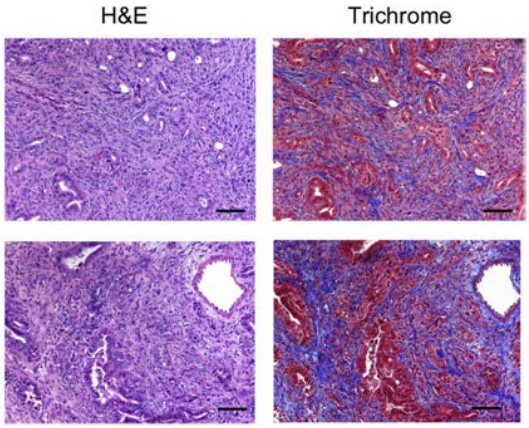
The significance of microscopic margin involvement on survival is a controversial topic, with some studies claiming an impact on survival and others finding no such correlation.[20] Recent studies based on rigorous pathological examination protocols report R1 rates of well over 70%.[21-25] Several studies have shown that residual cancer cells are frequently present in the resection bed even in appropriately staged patients after surgery that is properly performed,[26] where even with R0 resections nearly 80% of patients were found to have evidence of microscopic cells left *in situ* at the surgical site.[27] In a recent phase III adjuvant chemotherapy trial in patients with resected pancreatic cancer in which many patients had positive margins (0-60%) and nodal involvement (63-80%), local recurrence rates were 18-41%, suggesting the presence of residual disease may benefit from local therapy in addition to systemic therapy.[28] Early data from MD Anderson Cancer Center included 86 patients who received gemcitabine-based X-ray telescope (XRT) radiation (30 Gy); 75% of patients were resected, 95% had R0 resections and the median OS for those who completed all therapy was 34-months.[29] Their subsequent study of cisplatin and gemcitabine followed by gemcitabine-based chemoradiation in 90 patients with remote procedure call (RPC) revealed an R0 resection rate of 96% and median OS of 31-months.[30] Cloyd et al published a unique retrospective study utilizing propensity score weighted methodologies. The authors queried MD Anderson database to identify all patients who received pre-operative chemotherapy or CRT before pancreatectomy for anatomically resectable PDAC between 1999 and 2014. They concluded that the receipt of pre-operative CRT alone was associated with a higher rate of margin-negative resection (91% *vs.* 79%, p<0.01), lower rate of positive lymph nodes (53% *vs.* 23%, p<0.01), greater treatment effect, reduced incidence of locoregional recurrence (LR) (LR; 16% *vs.* 33%, p<0.01) but similar median overall survival (OS; 33.6 *vs.* 26.4-months, p=0.09) compared with systemic chemotherapy alone.[31] Katz et al, reported wider special memorandum account (SMA) margin distance on histological examination on patients who receive pre-operative CRT.[32] This suggests that the local effect of CRT may occur primarily through sterilization of the retroperitoneum.

THE IMPACT OF SBRT ON THE TUMOR MICROENVIRONMENT

Both SBRT and SRS have been used effectively for the treatment of lung, liver, brain, prostate, and recurrent head and neck cancers, among others.[33-37] Damage to tumor cell deoxyribonucleic acid (DNA) is thought to account for only part of the efficacy of hypofractionated regimens.[38] Many studies indicate that in addition to the direct impact on DNA, the effects of high-dose radiation on the tumor microenvironment (TME) may play a role in tumor control by SBRT and stereotactic radiosurgery (SRS).[38-41] Many studies indicate the effect of a single fraction or hypofractionated radiation therapy in the treatment of pancreatic tumor xenografts.

In the stroma of human carcinomas, cancer-associated fibroblasts (CAFs) are the most abundant cell types and play a significant role in tumor cell growth, angiogenesis, and invasiveness (Figure 1).[42-46] CAFs are also responsible for the deposition of key extracellular matrix (ECM) proteins (e.g., collagen, fibronectin, and laminin) as well as secreting ECM-degrading enzymes (e.g., matrix metalloproteinases),[42,43] which promotes migration of CAFs and degradation of the ECM, allowing the invasion of tumor cells.[47]

Figure 1. *The Paradoxical Web of Pancreatic Cancer Tumor Microenvironment*



Hematoxylin and eosin (H&E) and trichrome staining of pancreatic tumors arising in two KPC mice recapitulating the dense collagen-rich stroma seen in human pancreatic adenocarcinoma tumors. Scale bars Z 100 mm.

In vitro studies have shown that fibroblasts develop an irreversible senescent phenotype when exposed to a dose>10 Gy of radiation, whereas low doses of radiation induce reversible DNA damage without growth arrest. Senescent fibroblasts release proteolytic enzymes, cytokines, growth factors, and reactive oxygen

species, creating a protumorigenic environment.[48] Radiation doses higher than 10 Gy per fraction are associated with severe vascular damage leading to the deterioration of the TME.[39,49] Although endothelial cell damage has been shown to be a major factor in the biological mechanism of SBRT and SRS, this phenomenon is sometimes transient and may lead to neovasculogenesis *via* hypoxia-inducible factor (HIF)-1 induction.[49] Baird et al reported pancreatic tumor regression through activation of type 1 interferon-dependent responses with a single dose of 10 Gy and co-treatment with *cGAMP* or *STING* (simulator of interferon genes) agonists that amplify the radiation-induced antitumor immune response.[50,51] Type 1 interferons (interferon (IFN)-α and IFN-β) are important for activation of both innate and adaptive immune responses and are well-known for their role in viral immunity.[52]

Treatment of pancreatic tumor xenografts with radiation given as 4 Gy in 2 fractions resulted in a switchin tumor-infiltrating macrophages from a protumorigenic M2 phenotype to an antitumorigenic M1 phenotype.[53] Likewise, increased infiltration of T-cells into tumors and tumor killing mediated by iNOS+M1 macrophages through the expression of Type 1 T helper (TH1) cytokines have been reported in murine models of pancreatic cancer and melanoma after low-dose radiation treatment.[53,54] Moreover, many studies have demonstrated M2 polarization after treatment with single high-dose and hypofractionated radiation regimens.[55-57] Several clinical trials are underway to determine the effects of combination therapy with radiation and immune checkpoint inhibitors (Table 3).[58-60]

Table 3. *Ongoing Pancreatic Trials*

Unresectable pancreatic cancer	NCT01926197
Borderline resectable pancreatic cancer	NCT01992705, NCT02308722, NCT01446458
Resectable pancreatic cancer	NCT03704662,NCT02347618, NCT02318095,NCT02208024, NCT01446458

CONCLUSION

SBRT has been shown to be safe and effective in pancreatic cancer patients. It offers several advantages over standard EBRT including increased patient convenience, reduced toxicities, and the ability to minimize delays in modern multi-agent chemotherapy. The ability of SBRT to convert patients with borderline and locally advanced tumors to resectable disease with higher percentage of negative resection margins may improve survival. Favorable SBRT outcomes for LAPC patients have paved the way for exploration of SBRT for resectable pancreatic cancer patients, with promising early results. The immunotherapeutic approach has very limited clinical activity to date in pancreatic cancer, it is still unclear how to optimally combine ablative radiation and immunotherapy, including optimal sequencing, radiation dose to effectively overcome the immunosuppressive pancreatic tumor microenvironment.

REFERENCES

1. Heestand GM, Murphy JD, Lowy AM. Approach to patients with pancreatic cancer without detectable metastases. *J Clin Oncol.* 2015; 33: 1770-1778. doi: 10.1200/JCO.2014.59.7930

2. Pollom EL, Alagappan M, von Eyben R, et al. Single- versus multifraction stereotactic body radiation therapy for pancreatic adenocarcinoma: Outcomes and toxicity. *Int J RadiatOncol Biol Phys.* 2014; 90(4): 918-925. doi: 10.1016/j.ijrobp.2014.06.066

3. Burris HA, Moore MJ, Andersen J, et al. Improvements line therapy for patients with advanced pancreas cancer: A randomized trial. *J Clin Oncol.* 1997; 15: 2403-2413. doi: 10.1200/JCO.1997.15.6.2403

4. Chauffert B, Mornex F, Bonnetain F, et al. Phase III trial comparing intensive induction chemoradiotherapy (60 Gy, infusional 5-FU and intermittent cisplatin) followed by maintenance gemcitabine with gemcitabine alone for results of the 2000-01 FFCD/SFRO study. *Ann Oncol.* 2008; 19: 1592-1599. doi: 10.1093/annonc/mdn281

5. Von Hoff DD, Ervin T, Arena FP, et al. Increased survival in pancreatic cancer with nab-paclitaxel plus gemcitabine. *N Engl J Med.* 2013; 369: 1691-1703. doi: 10.1056/NEJMoa1304369

6. Conroy T, Desseigne F, Ychou M, et al. FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. *N Engl J Med.* 2011; 364: 1817-1825. doi: 10.1056/NEJMoa1011923

7. Mukherjee S, Hurt CN, Bridgewater J, et al. Gemcitabinebased or capecitabine-based chemoradiotherapy for locally advanced pancreatic cancer (SCALOP): A multicentre, randomised, phase 2 trial. *Lancet Oncol.* 2013; 14: 317-326. doi: 10.1016/S1470-2045(13)70021-4

8. Koong AC, Le QT, Ho A, et al. Phase I study of stereotactic radiosurgery in patients with locally advanced pancreatic cancer. *Int J Radiat Oncol Biol Phys.* 2004; 58: 1017-1021. doi: 10.1016/j.ijrobp.2003.11.004

9. Chang DT, Schellenberg D, Shen J, et al. Stereotactic radiotherapy for unresectable adenocarcinoma of the pancreas. *Cancer.* 2009; 115: 665-672. doi: 10.1002/cncr.24059

10. Mahadevan A, Jain S, Goldstein M, et al. Stereotactic body radiotherapy and gemcitabine for locally advanced pancreatic cancer. *Int J Radiat Oncol Biol Phys.* 2010; 78: 735-742. doi: 10.1016/j.ijrobp.2009.08.046

11. Mahadevan A, Miksad R, Goldstein M, et al. Induction gemcitabine and stereotactic body radiotherapy for locally advanced nonmetastatic pancreas cancer. *Int J Radiat Oncol Biol Phys.* 2011; 81: e615-622. doi: 10.1016/j.ijrobp.2011.04.045

12. Chuong MD, Springett GM, Freilich JM, et al. Stereotactic body radiation therapy for locally advanced and borderline resectable pancreatic cancer is effective and well tolerated. *Int J Radiat Oncol Biol Phys.* 2013; 86: 516-522. doi: 10.1016/j.ijrobp.2013.02.022

13. Herman JM, Chang DT, Goodman KA, et al. Phase 2 multi-institutional trial evaluating gemcitabine and stereotactic body radiotherapy for patients with locally advanced unresectable pancreatic adenocarcinoma. *Cancer.* 2015; 121: 1128-1137. doi: 10.1002/cncr.29161

14. Mellon EA, Hoffe SE, Springett GM, et al. Long-term outcomes of induction chemotherapy and neoadjuvant stereotactic body radiotherapy for borderline resectable and locally advanced pancreatic adenocarcinoma. *Acta Oncol.* 2015; 54: 979-985. doi: 10.3109/0284186X.2015.1004367

15. Chhabra A, Kaiser A, Regine WF, Chuong MD. The expanding role of stereotactic body radiation therapyfor pancreatic cancer: A review of the literature. *Transl Cancer Res.* 2015; 4(6): 659-670. doi: 10.3978/j.issn.2218-676X.2015.11.01

16. Moningi S, Marciscano AE, Rosati LM, et al. Stereotactic body radiation therapy in pancreatic cancer: The new frontier. *Expert Rev Anticancer Ther.* 2014; 14: 1461-1475. doi: 10.1586/14737140.2014.952286

17. Moningi S, Dholakia AS, Raman SP, et al. The role of stereotactic body radiation therapy for pancreatic cancer: A single-institution experience. *Ann Surg Oncol.* 2015; 22: 2352-2358. doi: 10.1245/s10434-014-4274-5

18. Rajagopalan MS, Heron DE, Wegner RE, et al. Pathologic response with neoadjuvant chemotherapy and stereotactic body radiotherapy for borderline resectable and locallyadvanced pancreatic cancer. *Radiat Oncol.* 2013; 8: 254. doi: 10.1186/1748-717X-8-254

19. He J, Moningi S, Blair AB, et al. Surgical outcomes of patients with pancreatic cancer treated with stereotactic body radiation therapy. *J Clin Oncol.* 2015; 33: abstr 341. doi: 10.1200/jco.2015.33.3_suppl.341

20. Verbeke CS, Gladhaug IP. Resection margin involvement and tumour origin in pancreatic head cancer. *Br J Surg.* 2012; 99(8): 1036-1049. doi: 10.1002/bjs.8734

21. Verbeke CS, Leitch D, Menon KV, McMahon MJ, Guillou PJ, Anthoney A. Redefining the R1 resection inpancreatic cancer. *Br J Surg.* 2006; 93: 1232-1237. doi: 10.1002/bjs.5397

22. Menon KV, Gomez D, Smith AM, Anthoney A, Verbeke CS. Impact of margin status on survival following pancreatoduodenectomy for cancer: The Leeds Pathology Protocol (LEEPP). *HPB (Oxford).* 2009; 11: 18-24. doi: 10.1111/j.1477-2574.2008.00013.x

23. Esposito I, Kleeff J, Bergmann F, et al. Most pancreatic cancer resections are R1 resections. *Ann Surg Oncol.* 2008; 15: 1651-1660. doi: 10.1245/s10434-008-9839-8

24. Campbell F, Smith RA, Whelan P, et al. Classification of R1 resections forpancreatic cancer: The prognostic relevance of tumour involvement within 1 mm of a resection margin. *Histopathology.* 2009; 55: 277-283. doi: 10.1111/j.1365-2559.2009.03376.x

25. Jamieson NB, Foulis AK, Oien KA, et al. Positive immobilization margins alone donot influence survival following pancreatico-duodenectomy for pancreatic ductal adenocarcinoma. *Ann Surg.* 2010; 251: 1003-1010. doi: 10.1097/SLA.0b013e3181d77369

26. Verbeke CS, Gladhaug IP. Resection margin involvement and tumour origin in pancreatic head cancer. *Br J Surg.* 2012; 99: 1036-1049. doi: 10.1002/bjs.8734

27. Colbert LE, Hall WA, Nickleach D, Switchenko J, Kooby DA, Landry JC. Chemoradiation therapy sequencing for resected pancreatic adenocarcinoma in the National Cancer Data Base. *Cancer.* 2014; 120(4): 499-506. doi: 10.1002/cncr.28530

28. Neoptolemos JP, Palmer DH, Ghaneh P, et al. Comparison of adjuvant gemcitabine and capecitabine with gemcitabine monotherapy in patientswith resected pancreatic cancer (ESPAC-4): A multicentre, open-label, randomised, phase 3 trial. *Lancet.* 2017; 389(10073): 1011-1024. doi: 10.1016/S0140-6736(16)32409-6

29. 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: 3496-3502. doi: 10.1200/JCO.2007.15.8634

30. 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: 3487-3495. doi: 10.1200/JCO.2007.15.8642

31. Cloyd JM, Chen HC, Wang X, et al. Chemotherapy versus chemoradiation as preoperative therapy for resectable pancreatic ductal adenocarcinoma: A propensity score adjusted analysis. *Pancreas.* 2019; 48(2): 216-222. doi: 10.1097/MPA.0000000000001231

32. Katz MH, Wang H, Balachandran A, et al. Effect of neoadjuvant chemoradiation and surgical technique on recurrence of localizedpancreatic cancer. *J Gastrointest Surg.* 2012; 16: 68-78; discussion 78-79. doi: 10.1007/s11605-011-1748-7

33. Mahajan A, Ahmed S, McAleer MF, et al. Post-operative stereotactic radiosurgery versus observation for completely resected brain metastases: A single-centre, randomised, controlled, phase 3 trial. *Lancet Oncol.* 2017; 18: 1040-1048. doi: 10.1016/S1470-2045(17)30414-X

34. Aridgides P, Bogart J. Stereotactic body radiation therapy for stage I non-small cell lung cancer. *Thorac Surg Clin.* 2016; 26: 261-269. doi: 10.1016/j.thorsurg.2016.04.008

35. Qiu H, Moravan MJ, Milano MT, Usuki KY, Katz AW. SBRT for hepatocellular carcinoma: 8-year experience from a regional transplant center. *J Gastrointest Cancer.* 2018; 49(4): 463-469. doi: 10.1007/s12029-017-9990-1

36. Kishan AU, King CR. Stereotactic body radiotherapy for low- and intermediate-risk prostate cancer. *Semin Radiat Oncol.* 2017; 27: 268-278. doi: 10.1016/j.semradonc.2017.02.006

37. Baliga S, Kabarriti R, Ohri N, et al. Stereotactic body radiotherapy for recurrent head and neck cancer: A critical review. *Head Neck.* 2017; 39: 595-601. doi: 10.1002/hed.24633

38. Kim MS, Kim W, Park IH, et al. Radiobiological mechanisms of stereotactic body radiation therapy and stereotactic radiation surgery. *Radiat Oncol J.* 2015; 33: 265-275. doi: 10.3857/roj.2015.33.4.265

39. Song CW, Lee YJ, Griffin RJ, et al. Indirect tumor cell death after high-dose hypofractionated irradiation: Implications for stereotactic body radiation therapy and stereotactic radiation surgery. *Int J Radiat Oncol Biol Phys.* 2015; 93: 166-172. doi: 10.1016/j.ijrobp.2015.05.016

40. Park HJ, Griffin RJ, Hui S, Levitt SH, Song CW. Radiation-induced vascular damage in tumors: implications of vascular damage in ablative hypofractionated radiotherapy (SBRT and SRS). *Radiat Res.* 2012; 177: 311-327. doi: 10.1667/rr2773.1

41. Brown JM, Carlson DJ, Brenner DJ. The tumor radiobiology of SRS and SBRT: Are more than the 5 R's involved? *Int J Radiat Oncol Biol Phys.* 2014; 88: 254-262. doi: 10.1016/j.ijrobp.2013.07.022

42. Bhowmick NA, Neilson EG, Moses HL. Stromal fibroblasts in cancer initiation and progression. *Nature.* 2004; 432: 332-337. doi: 10.1038/nature03096

43. Kalluri R, Zeisberg M. Fibroblasts in cancer. *Nat Rev Cancer.* 2006; 6: 392-401. doi: 10.1038/nrc1877

44. Orimo A, Weinberg RA. Stromal fibroblasts in cancer: a novel tumor-promoting cell type. *Cell Cycle.* 2006; 5: 1597-1601. doi: 10.4161/cc.5.15.3112

45. Shimoda M, Mellody KT, Orimo A. Carcinoma-associated fibroblasts are a rate-limiting determinant for tumour progression. *Semin Cell Dev Biol.* 2010; 21: 19-25. doi: 10.1016/j.semcdb.2009.10.002

46. Mishra P, Banerjee D, Ben-Baruch A. Chemokines at the crossroads of tumor-fibroblast interactions that promote malignancy. *J Leukoc Biol.* 2011; 89: 31-39. doi: 10.1189/jlb.0310182

47. Gaggioli C, Hooper S, Hidalgo-Carcedo C, et al. Fibroblast-led collective invasion of carcinoma cells with differing roles for RhoGTPases in leading and following cells. *Nat Cell Biol.* 2007; 9: 1392-1400. doi: 10.1038/ncb1658

48. Arnold KM, Flynn NJ, Raben A, et al. The impact of radiation on the tumor microenvironment: Effect of dose and fractionation schedules. *Cancer Growth Metastasis.* 2018; 11: 1179064418761639. doi: 10.1177/1179064418761639

49. Maeda A, Chen Y, Bu J, Mujcic H, Wouters BG, DaCosta RS. In vivo imaging reveals significant tumor vascular dysfunction and increased tumor hypoxia-inducible factor-1α expression induced by high single-dose irradiation in a pancreatic tumor model. *Int J Radiat Oncol Biol Phys.* 2017; 97: 184-194. doi: 10.1016/j.ijrobp.2016.09.005

50. Deng L, Liang H, Xu M, et al. STING-dependent cytosolic DNA sensing promotes radiation-induced type I interferon-dependent antitumor immunity in immunogenic tumors. *Immunity.* 2014; 41: 843-852. doi: 10.1016/j.immuni.2014.10.019

51. Baird JR, Friedman D, Cottam B, et al. Radiotherapy combined with novel STING-targeting oligonucleotides results in regression of established tumors. *Cancer Res.* 2016; 76: 50-61. doi: 10.1158/0008-5472.CAN-14-3619

52. Perry AK, Chen G, Zheng D, Tang H, Cheng G. The host type I interferon response to viral and bacterial infections. *Cell Res.* 2005; 15: 407-422. doi: 10.1038/sj.cr.7290309

53. Prakash H, Klug F, Nadella V, Mazumdar V, Schmitz-Winnenthal H, Umansky L. Low doses of gamma irradiation potentially modifies immunosuppressive tumor microenvironment by retuning tumor-associated macrophages: Lesson from insulinoma. *Carcinogenesis.* 2016; 37: 301-313. doi: 10.1093/carcin/bgw007

54. Klug F, Prakash H, Huber PE, et al. Low-dose irradiation programs macrophage differentiation to an INOS(+)/M1 phenotype that orchestrates effective T cell immunotherapy. *Cancer Cell.* 2013; 24: 589-602. doi: 10.1016/j.ccr.2013.09.014

55. Chiang CS, Fu SY, Wang SC, et al. Irradiation promotes an m2 macrophage phenotype in tumor hypoxia. *Front Oncol.* 2012; 2: 89. doi: 10.3389/fonc.2012.00089

56. Okubo M, Kioi M, Nakashima H, et al. M2-polarized macrophages contribute to neovasculogenesis, leading to relapse of oral cancer following radiation. *Sci Rep.* 2016; 6: 27548. doi: 10.1038/srep27548

57. Seifert L, Werba G, Tiwari S, et al. Radiation therapy induces macrophages to suppress T-cell responses against pancreatic tumors in mice. *Gastroenterology.* 2016; 150: 1659.e5-1672.e5. doi: 10.1053/j.gastro.2016.02.070

58. Kang J, Demaria S, Formenti S. Current clinical trials testing the combination of immunotherapy with radiotherapy. *J Immunother Cancer.* 2016; 4: 51. doi: 10.1186/s40425-016-0156-7

59. Berberat PO, Kunzli BM, Gulbinas A, et al. An audit of outcomes of a series of periampullary carcinomas. *Eur J SurgOncol.* 2009; 35: 187-191. doi: 10.1016/j.ejso.2008.01.030

60. Lafaro KJ, Melstrom LG. The paradoxical web of pancreatic cancer tumor microenvironment. *Am J Pathol.* 2019; 189(1): 44-57. doi: 10.1016/j.ajpath.2018.09.009

ISSN 2471-142X

PANCREAS
Open Journal

Openventio
PUBLISHERS

Editorial

Why HALO 301 Failed and Implications for Treatment of Pancreatic Cancer

Nausheen Hakim, DO; Rajvi Patel, MD; Craig Devoe MD; Muhammad W. Saif, MD*

Northwell Health Cancer Institute and Donald and Barbara Zucker School of Medicine, Hofstra/Northwell, NY, USA

****Corresponding author***
Muhammad W. Saif, MD
Professor, Deputy Physician-in-Chief, Northwell Health Cancer Institute and Donald and Barbara Zucker School of Medicine, Hofstra/Northwell, NY, USA;
Tel. (516) 321-2238; E-mail: wsaif@northwell.edu

Article information
Received: December 3rd, 2019; **Revised:** December 11th, 2019; **Accepted:** December 17th, 2019; **Published:** December 20th, 2019

Cite this article
Hakim N, Patel R, Devoe C, Saif MW. Why HALO-301 failed and implications for treatment of pancreatic cancer. *Pancreas Open J.* 2019; 3(1): e1-e4. doi: 10.17140/POJ-3-e010

ABSTRACT

Survival rates for pancreatic cancer (PC) remain dismal. Current standard of care treatment regimens provide transient clinical benefit but eventually chemoresistance develops leading to poor outcomes. PC is a relatively chemoresistant tumor and one of the explanations for this is attributed to desmoplasia that impedes drug delivery. Based on this, stromal modifying agent such as Pegvorhyaluronidase alfa (PEGPH20) was developed and investigated in phase I-III studies. Although phase I-II studies showed promising results in patients with high hyaluronic acid (HA) expressing tumors, the phase III HALO 301 study failed to miss it's primary endpoint and further development of PEHPH20 is halted. This failure implies that targeting desmoplasia alone is not sufficient and other intrinsic factors such as lack of significant neoantigens, low tumor mutational burden, and epithelial to mesenchymal transition may be at play. It is also important to consider that although the tumor stroma may be a physical barrier hampering drug delivery, it may also have protective effects in restraining tumor growth and progression. Further studies in molecular biology to better characterize the complex interaction between the microenvironment and cancer cells are warranted.

Keywords
Pegvorhyaluronidase alfa (PEGPH20); Desmoplasia; Pancreatic cancer; Chemoresistance; HALO.

Pancreatic cancer (PC) remains one of the deadliest cancers in the United States with very poor outcomes. In 2019, it is estimated that 56,770 Americans will be diagnosed with pancreatic cancer in the US and more than 45,750 will die of the disease. It is expected to become the second leading cause of cancer-related deaths by 2020 and currently has a 5-year overall survival rate of 8.5% for all stages combined.[1] PC is a relatively chemoresistant tumor with limited treatment options, which can include surgery if identified early in the disease process, radiation, chemotherapy, and some targeted therapies. Most patients present with unresectable or metastatic disease resulting in a 5-year-overall survival (OS) rate of only 7%. Even when surgery is feasible in 15-20% of the patients, the 5-year survival remains only about 10%.[2]

Chemotherapy, which can be used in the neoadjuvant setting in order to decrease tumor size in the borderline resectable or resectable patients, in the adjuvant setting after surgery, or first line in the metastatic/advanced setting, is the forefront of systemic therapy. Two chemotherapy combination regimens have shown superiority in patients with metastatic disease. In the Partenariat de Recherche en Oncologie Digestive (PRODIGE)/Actions Concertées dans les Cancers Colorectaux et Digestifs (ACCORD) and Metastatic Pancreatic Adenocarcinoma Clinical Trial (MPACT) trials, folinic acid fluorouracil irinotecan oxaliplatin (FOLFIRINOX) and gemcitabine plus nab-paclitaxel, respectively, showed an overall survival (OS) benefit at the cost of increased toxicity.[3,4] In 2007, the Food and Drug Administration (FDA) approved erlotinib (epidermal growth factor receptor tyrosine kinase inhibitor) based on a study that showed survival benefit with combination of gemcitabine plus erlotinib, however, clinical benefit is limited and erlotinib is not widely accepted or used.[5] A recent study using olaparib (poly (adenosine diphosphate [ADP]–ribose) polymerase-PARP inhibitor) in the maintenance setting in patients with germline *BRCA* mutated metastatic pancreatic cancer that had not progressed on first line platinum based therapy showed significant improvement in median progression free survival (PFS) but no improvement in OS.[6] However, this only applies to a small subgroup of patients with pancreatic cancer. Despite these advances, there remains much room for improvement and one of the explanations for resistance to conventional chemotherapy can be attributed to

Pancreas Open J. 2019; 3(1): e1-e4. doi: 10.17140/POJ-3-e010

desmoplasia that impedes drug delivery.

Pancreatic tumor cells have a thick and poorly perfused stroma, which includes pancreatic stellate cells (PSCs). These cells, in turn, produce stromal elements, including collagens, laminin, fibronectin, and hyaluronic acid (HA). The dense, collagen-rich extracellular matrix and stroma creates high interstitial pressure. This can potentially constrict blood vessels leading to a hypovascular environment that impairs drug delivery rendering tumor cells chemoresistant.[7] This fundamental characteristic of significant overproduction of extracellular matrix proteins and extensive proliferation of myofibroblast-like cells is termed desmoplastic reaction and can decrease the efficacy of chemotherapy.

Tumors with high-levels of HA have shown to be poor prognostic indicators in patients with PC.[8] Stromal modifying agents, such as Pegvorhyaluronidase alfa (PEGPH20), an enzyme that temporarily degrades HA, should decrease tumor pressure and vascular compression thereby penetrating the "halo" surrounding the tumor cells. PEGPH20 showed promising results in pre-clinical and early phase studies. Based on this the FDA granted orphan drug designation to PEGPH20 for treatment of PC.

Encouraged by the preliminary data from phase I/II studies, the phase III HALO-109-301 was conducted which was a randomized, double-blind, multicenter trial.[9,10] This study compared PEGPH20 in combination with nab-paclitaxel/gemcitabine to nab-paclitaxel/gemcitabine alone in previously untreated patients with stage IV PC.[11] Patients had to have tumors that were high expressors of HA, a clear difference from SWOG1313. Patients were randomized in 2:1 manner to receive the experimental arm. The primary endpoint was overall survival, and the originally planned interim analysis was not done. Five hundred patients were enrolled and estimated completion date was to be December of 2019. However, on November 4, 2019, Halozyme announced that HALO 301 did not meet its primary endpoint of OS (11.2 months compared to 11.5 months (hazard ratio of 1.00 and p=0.096).[12] Per Halozyme, there was a higher response rate in the experimental arm but unfortunately no improvement in duration of response, PFS, or OS was seen. Since this announcement, all future development of PEGPH20 has been halted.

Earlier in 2019, Ramanathan et al, presented similar negative data of Southwest Oncology Group (SWOG) 1313 that was conducted to determine the safety and efficacy of PEGPH20 in combination with modified version of FOLFIRINOX. Since 2011, when an exceptional improvement in overall survival with FOLFIRINOX compared with single agent gemcitabine (HR for death=0.57) was published,[3] a substantial interest had developed to use this new triplet chemotherapy as the backbone for a randomized clinical trial with PEGPH20. The SWOG set out to determine the safety and efficacy of PEGPH20 in combination with modified version of FOLFIRINOX in a phase Ib/II clinical trial.[13] Of note, eligibility of subjects was not dependent upon tumor expression status of HA by immunohistochemistry. The standard 3+3 dose escalation phase Ib component demonstrated that biweekly dosing of PEGPH20 was poorly tolerated, and the RP2D was 3 μg/kg every 2 weeks. The open-label, phase 2 portion randomly assigned 114 patients to mFOLFIRINOX+/-PEGPH20. However, accrual was suspended and trial was terminated based on the results of a pre-planned interim futility analysis. In terms of safety, treatment-related adverse events ≥ grade 3 were substantially higher for the investigational arm (45%) *versus* control (9%).[8]

EXPERT OPINION

Unfortunately, we now have two failed clinical trials for PEGPH20. It is of both academic and clinical importance to consider possible explanations for these failed trials. In SWOG 1313, the answer may lie more within the toxicity of the entire regimen. As a consequence, the overall treatment exposure in the experimental arm was inferior. The study group received half the number of cycles of chemotherapy and more dose reductions than the control group. This substantial imbalance in treatment exposure is likely the most relevant factor contributing to the very poor outcomes of the study group. Dosing of PEGPH20 could be another factor that contributed to the negative results of SWOG 1313. The recommended phase II dose (RP2D) schedule for PEGPH20 was q2 weeks, whereas in the positive HALO-202 trial it was administered weekly. Additionally, the lack of subject selection based on the HA high biomarker likely played a role.

However, this was rectified in the HALO 301 study, which only included patients that had tumors with high-levels of HA expression. The phase II study showed promising results, especially in this subset of patients. The R2PD with the twice weekly dosing for PEGPH20 was also applied on this trial. Patients were also given enoxaparin to avoid the venous thromboembolism risk. Despite these corrections, HALO 301 now failed. Halozyme announced that although response rate was similar to the control arm, no significant difference was seen. Could it be perhaps that our theory of targeting the desmoplastic response is simply not enough?

Perhaps it is not solely the desmoplastic reaction that is the cause of chemoresistance of pancreatic cells, but additional intrinsic factors at play. The pancreatic cancer microenvironment is also filled with immunosuppressive cell types, such as myeloid derived suppressor cells (MDSCs) and regulatory T-cells (Tregs), which in turn alter the effector function of cytotoxic T-cells, leading to evasion of the immune system. It has also been shown that the amount of Tregs in the pancreatic microenvironment corresponds to the Tregs found peripherally, which could result in systemic chemotherapy resistance.[14] The location of the cytotoxic T-cells also is problematic; they are located at the front of pancreatic cancer and not so much in the center where they can target malignant cells. Also, when they do cluster next to malignant cells they are unable to act upon them due to the dense stromal tissue.[15] This has explained the poor response to immunotherapies in pancreatic cancer as well, along with the low mutational burden, and lack of significant neoantigens. Although preclinical studies have shown promise in the area of suppressing Tregs, clinical studies have not followed suit. The ECLIPSE trial, which depleted Tregs with cyclophosphamide and the GVAX vaccine has not shown any

Pancreas Open J. 2019; 3(1): e1-e4. doi: 10.17140/POJ-3-e010

Table 1. *Key Trials in Treatment of Pancreatic Cancer*

Trial (year)	Number of Patients	Disease	Treatment	Median Survival (months)	*p*-value
Chemotherapy					
Burris et al[17]	126	Metastatic	Gemcitabine Fluorouracil	5.6 4.4	0.0025
Conroy et al[3]	342	Metastatic	FOFLIRINOX Gemcitabine	11.1 6.8	<0.001
Von Hoff et al[4]	861	Metastatic	Gemcitabine Gemcitabine+ nab-paclitaxel	8.5 6.7	<0.001
Ueno et al[18]	834	Locally advanced or metastatic	S1 alone Gemcitabine alone	9.7 8.8	<0.001 for non-inferiority
Targeted Therapy					
Moore et al[5]	569	Locally advanced unresectable or metastatic	Gemcitabine+Erlotinib Gemcitabine+Placebo	6.2 5.9	0.038
Golan et al[6]	154	Metastatic with germline *BRCA* mutation	Maintenance Olaparib Placebo	18.9 18.1	0.68
Immunotherapy					
O'Reilly et al[19]	65	Metastatic	Durvalumab+ Tremelimumab Duravlumab	3.1 3.6	Not Available
Le et al[16]	213	Metastatic	Cy/GVAX+CRS207 Physician's choice chemo	3.7 4.6	Not Significant

Cy=cyclophosphamide; FOLFIRINOX=5-fluorouracil/leucovorin, irinotecan, oxaliplatin;GVAX=Vaccine consisting of two human allogeneic pancreatic tumor cells lines irradiated to release antigen and transfected with DNA to release granulocyte-macrophage colony-stimulating factor, CRS207=double deleted Listeria Monocytegenes, engineered to secrete mesothelin into antigen presenting cells.

significant clinical outcomes.[16]

The epithelial to mesenchymal transition (EMT) is the conversion of epithelial cells into mesenchymal like cells in cell culture conditions, which is seen in the pancreatic tumor cell.[20] However, the inhibition of this has been not proven to have an effect on pancreatic metastasis. Paradoxically, it does seem to affect chemoresistance of the pancreatic tumor cell. The theory behind this is the cell has seemed to have compensatory mechanisms to overcome the inhibition in terms of metastasis, but may suppress drug transporter and concentrating proteins, which could affect resistance to chemotherapies such as gemcitabine. Therefore, perhaps adding epithelial–mesenchymal transition (EMT) inhibitors to standard backbone chemotherapies is necessary to consider.

It is also important to consider that although the tumor stroma may be a physical barrier hampering drug delivery, it may also have protective effects in restraining tumor growth and progression. Sonic hedge hog (SHH) deficient tumors have reduced stromal content but surprisingly such tumors were more aggressive, exhibited undifferentiated histology, increased vascularity, and heightened proliferation.[21] Another study investigating role of adjuvant gemcitabine compared to observation in resectable pancreatic cancer patients analyzed tissue samples of 162 patients. They found that dense stromal reaction was actually associated with improved disease free survival (DFS) and OS with median DFS and OS of 13.8 and 28 months in weak stroma (p=0.05) *versus* 10.1 and 20.2 months in strong stroma (p=0.047).[22] Therefore, reversal of hypovascular stroma with stromal depleting and penetrating agents such as PEGPH20 may simply unleash the strain of aggressive cancer clones potentiating their metastatic capacity.

In summary, the phylogenesis of pancreatic cancer is much more complex than originally thought. It may indeed be a combination of stromal modifying agents as well as other strategies to overcome chemoresistance to better fight pancreatic cancer. Further studies in molecular biology to better characterize the complex interaction between the microenvironment and cancer cells are warranted.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

1. American Cancer Society. Cancer Facts & Statistics. Web site. https://www.cancerstatisticscenter.cancer.org/. Accessed November 5, 2019.

2. Noonan AM, Howlader N, Krapcho M. SEER Cancer Statistics Review, 1975-2015. Web site. https://seer.cancer.gov/csr/1975_2015/. Accessed November 5, 2019.

3. Conroy T, Desseigne F, Ychou M, et al. FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. *N Engl J Med.* 2011; 364(19): 1817-1825. doi: 10.1056/NEJMoa1011923

4. Von Hoff DD, Ervin T, Arena FP, et al. Increased survival in pancreatic cancer with nab-paclitaxel plus gemcitabine. *N Engl J*

Med. 2013; 369(18): 1691-1703. doi: 10.1056/NEJMoa1304369

5. Moore MJ, Goldstein D, Hamm J, et al. Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: A phase III trial of the National Cancer Institute of Canada Clinical Trials Group. *J Clin Oncol.* 2007; 25(15): 1960-1966. doi: 10.1200/JCO.2006.07.9525

6. Golan T, Hammel P, Reni M, et al. Maintenance olaparib for germline BRCA-mutated metastatic pancreatic cancer. *N Engl J Med.* 2019; 381(4): 317-327. doi: 10.1056/NEJMoa1903387

7. Merika EE, Syrigos KN, and M.W. Saif, Desmoplasia in pancreatic cancer. Can we fight it? *Gastroenterol Res Pract.* 2012; 2012: 781765. doi: 10.1155/2012/781765

8. Cheng XB, Sato N, Kohi S, Yamaguchi K. Prognostic impact of hyaluronan and its regulators in pancreatic ductal adenocarcinoma. *PLoS One.* 2013; 8(11): e80765. doi: 10.1371/journal.pone.0080765

9. Hingorani SR, Harris WP, Beck JT, et al. Phase Ib study of PEGylated recombinant human hyaluronidase and gemcitabine in patients with advanced pancreatic cancer. *Clin Cancer Res.* 2016; 22(12): 2848-2854. doi: 10.1158/1078-0432.CCR-15-2010

10. Hingorani SR, Zheng L, Bullock AJ, et al. HALO 202: Randomized Phase II study of PEGPH20 plus nab-paclitaxel/gemcitabine versus nab-paclitaxel/gemcitabine in patients with untreated, metastatic pancreatic ductal adenocarcinoma. *J Clin Oncol.* 2018; 36(4): 359-366. doi: 10.1200/JCO.2017.74.9564

11. Doherty GJ, Tempero M, Corrie PG. HALO-109-301: A Phase III trial of PEGPH20 (with gemcitabine and nab-paclitaxel) in hyaluronic acid-high stage IV pancreatic cancer. *Future Oncol.* 2018; 14(1): 13-22. doi: 10.2217/fon-2017-0338

12. Diego S. Halozyme Announces HALO-301 Phase 3 Study Fails to Meet Primary Endpoint. Halozyme Therapeutics. Web site. https://www.halozyme.com/investors/news-releases/news-release-details/2019/Halozyme-Announces-HALO-301-Phase-3-Study-Fails-To-Meet-Primary-Endpoint/default.aspx. Accessed November 4, 2019.

13. Ramanathan RK, McDonough SL, Philip PA, et al. Phase IB/II randomized study of FOLFIRINOX plus pegylated recombinant human hyaluronidase versus FOLFIRINOX alone in patients with metastatic pancreatic adenocarcinoma: SWOG S1313. *J Clin Oncol.* 2019; 37(13): 1062-1069. doi: 10.1200/JCO.18.01295

14. Uzunparmak B, Sahin IH. Pancreatic cancer microenvironment: A current dilemma. *Clin Transl Med.* 2019; 8(1): 2. doi: 10.1186/s40169-019-0221-1

15. Beatty GL, Eghbali S, Kim R. Deploying immunotherapy in pancreatic cancer: Defining mechanisms of response and resistance. *Am Soc Clin Oncol Educ Book.* 2017; 37: 267-278. doi: 10.14694/EDBK_175232

16. Le DT, Ko AK, Wainberg ZA, et al. Results from a phase 2b, randomized, multicenter study of GVAX pancreas and CRS-207 compared to chemotherapy in adults with previously-treated metastatic pancreatic adenocarcinoma (ECLIPSE Study). *Journal of Clinical Oncology.* 2017; 35(4_suppl): 345-345. doi: 10.1200/JCO.2017.35.4_suppl.345

17. Burris HA, 3rd, Moore MJ, Andersen J, et al. Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: A randomized trial. *J Clin Oncol.* 1997; 15(6): 2403-2413. doi: 10.1200/JCO.1997.15.6.2403

18. Ueno H, Ioka T, Ikeda M, et al. Randomized phase III study of gemcitabine plus S-1, S-1 alone, or gemcitabine alone in patients with locally advanced and metastatic pancreatic cancer in Japan and Taiwan: GEST study. *J Clin Oncol*, 2013; 31(13): 1640-1648. doi: 10.1200/JCO.2012.43.3680

19. O'Reilly EM, Oh DY, Dhani N, et al. Durvalumab with or without tremelimumab for patients with metastatic pancreatic ductal adenocarcinoma: A Phase 2 randomized clinical trial. *JAMA Oncol.* 2019. doi: 10.1001/jamaoncol.2019.1588

20. Zheng X, Carstens JL, Kim J, et al. Epithelial-to-mesenchymal transition is dispensable for metastasis but induces chemoresistance in pancreatic cancer. *Nature.* 2015; 527(7579): 525-530. doi: 10.1038/nature16064

21. Rhim AD, Oberstein PE, Thomas DH, et al. Stromal elements act to restrain, rather than support, pancreatic ductal adenocarcinoma. *Cancer Cell.* 2014; 25(6): 735-47. doi: 10.1016/j.ccr.2014.04.021

22. Sinn M, Denkert C, Striefler JK, et al. α-Smooth muscle actin expression and desmoplastic stromal reaction in pancreatic cancer: results from the CONKO-001 study. *Br J Cancer.* 2014; 111(10): 1917-1923. doi: 10.1038/bjc.2014.495

ISSN 2471-142X

PANCREAS
Open Journal

Openventio
PUBLISHERS

Editorial

PARP Inhibitors in Pancreatic Cancer: From Phase I to Plenary Session

Rajvi Patel, MD[1]; Daniel Fein, MD[2]; Carolina B. Ramirez, MD[1]; Kevin Do, MD[2]; Muhammad W. Saif, MD[1*]

[1]*Northwell Health Cancer Institute and Donald and Barbara Zucker School of Medicine, Hofstra/Northwell, NY, USA*
[2]*Tufts University, School of Medicine, Tufts Cancer Center, Boston, MA, USA*

[*]***Corresponding author***
Muhammad W. Saif, MD
Professor, Deputy Physician-in-Chief, Northwell Health Cancer Institute and Donald and Barbara Zucker School of Medicine, Hofstra/Northwell, NY, USA;
Tel. (516) 321-2238; E-mail: wsaif@northwell.edu

Article information
Received: December 3rd, 2019; **Revised:** December 14th, 2019; **Accepted:** December 16th, 2019; **Published:** December 20th, 2019

Cite this article
Patel R, Fein D, Ramirez CB, Do K, Saif MW. PARP inhibitors in pancreatic cancer: From phase I to plenary session. *Pancreas Open J.* 2019; 3(1): e5-e8. doi: 10.17140/POJ-3-e011

ABSTRACT

Survival rates for pancreatic cancer remain dismal. Current standard of care treatment regimens provide transient clinical benefit but eventually chemoresistance develops. Tumors deficient in deoxyribonucleic acid (DNA) damage repair mechanisms such as *BRCA* mutants show better responses to platinum based agents, however, such tumors can utilize the poly(adenosine diphosphate [ADP]–ribose) polymerase (PARP) pathway as a salvage mechanism. Therefore, inhibition of PARP pathway could lead to tumor destruction and synthetic lethality in presence of *BRCA* mutation. Various PARP inhibitors have been approved for treatment of patients with germline or somatic *BRCA* mutant breast and ovarian cancer. This provides basis of using PARP inhibitors in patients with pancreatic cancer that harbor *BRCA* mutation. A recent phase III Pancreas Cancer Olaparib Ongoing (POLO) study showed impressive results with near doubling of progression free survival compared to placebo (7.4 *vs* 3.8 months). These results highlight the importance of germline testing for all patients with pancreatic cancer and inclusion of additional deficiencies in homologous recombination repair *(ATM* and *PALB2*) including *BRCA* variants of uncertain significance should be further explored.

Keywords
Pancreatic cancer; Chemoresistance; DNA damage repair; Synthetic lethality; *BRCA1/2*; Germline mutations; Genomics;

The fatality of pancreatic ductal adenocarcinoma (PDAC) continues to rise regardless of current efforts to improve survival. Given the subtle clinical presentation, most patients have advanced disease at the time of diagnosis. Hence, the 5-year-survival rate is 3% and the median overall survival (OS) is around 6-months for patients with metastatic disease.[1] Currently, there are limited treatment options that include combination regimens with oxaliplatin, irinotecan, fluorouracil and leucovorin (FOLFIRINOX) or gemcitabine plus nab-paclitaxel.[2,3] However, chemotherapy provides a transient clinical benefit and eventually PDAC becomes resistant to conventional therapies.

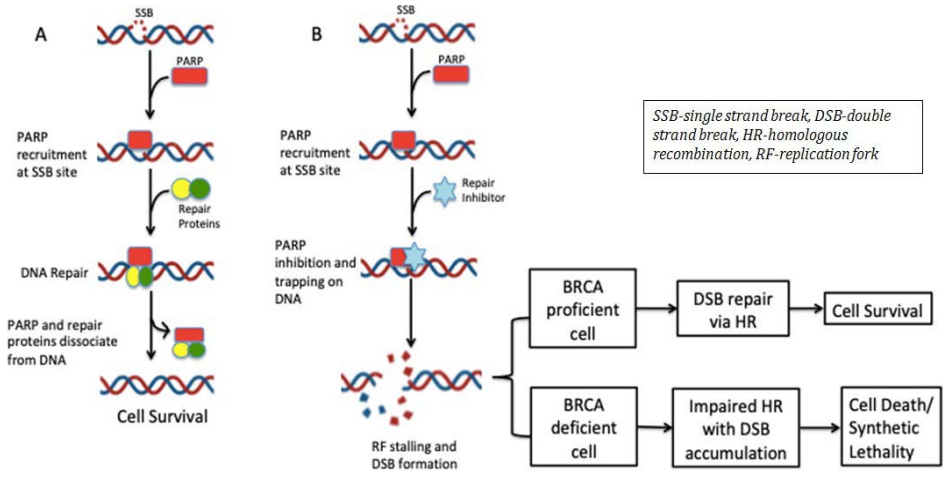
Mechanisms for chemotherapy resistance may be related to tumor microenvironment or intrinsic genetic alterations. A proposed mechanism for development of PDAC includes multiple steps where premalignant lesions called pancreatic intraepithelial neoplasia (PanIN), intraductal papillary mucinous neoplasm (IPMN), and mucinous cystic neoplasm (MCN) develop into carcinoma. *KRAS* mutation is crucial for pancreatic carcinogenesis and more than 90% of pancreatic tumors express KRAS mutated protein.[4-7] Inactivation of cyclin-dependent kinase inhibitor 2A (*CDKN2A*), *SMAD4*, *TP53*, and other tumor suppressor genes are also key elements implicated in this progression model.

Additionally, genes involved in deoxyribonucleic acid (DNA) damage repair (DDR) may also contribute to the pathogenesis of PDAC and deficiency in DDR mechanism leads to an increased risk of cancer. It is known that poly(adenosine diphosphate [ADP]–ribose) polymerase (PARP) 1/2 detect DNA damage and promote its repair.[8] Importantly, studies have demonstrated the clinical benefit of PARP inhibition in carriers of *BRCA1* or *BRCA2* mutation.[9]

Tumors that harbor mutations in genes related to double strand DNA repair such as *BRCA1*, *BRCA2*, *PALB2*, or *ATM* have been associated to have better responses to platinum based

Pancreas Open J. 2019; 3(1): e5-e8. doi: 10.17140/POJ-3-e011

Figure 1. *Mechanism of PARP Inhibitors Leading to Synthetic Lethality*



A) Normal DNA repair mechanism with functional PARP protein and DNA repair proteins B) DNA repair of SSB in the presence of PARP inhibitor resulting in DSB formation. BRCA-proficient cells have the ability to repair the DSB maintaining cell survival. BRCA deficient cells are unable to repair the accumulating double stranded breaks resulting in cell death.

Table 1. *Current PARP Inhibitors and Food and Drug Administration (FDA) Indications*

Drug	FDA Indications	Key Trials
Olaparib	Ovarian Cancer • Maintenance in patients with germline (gBRCAm) or somatic (sBRCAm) *BRCA* mutation with response to platinum based chemotherapy • Treatment in advanced ovarian cancer with *gBRCAm* with 3 or more prior lines of chemotherapy breast cancer • gBRCAm, human epidermal growth factor receptor 2 (HER2)-negative metastatic breast cancer who have previously been treated with chemotherapy in the neoadjuvant, adjuvant, or metastatic setting	SOLO-1 SOLO-2 OlympiAD
Niraparib	Ovarian Cancer • Maintenance treatment in patients with recurrent epithelial ovarian, fallopian tube, or primary peritoneal cancer who are in a complete or partial response to platinum-based chemotherapy	NOVA
Rucaparib	Ovarian Cancer • Monotherapy in patients with advanced ovarian cancer with gBRCAm or sBRCAm who have been treated with two or more lines of chemotherapy	ARIEL2

chemotherapy agents. This is explained by the fact that platinum compounds generate double-strand DNA breaks that cannot be repaired due to mutation in double-strand DNA repair genes. However, tumors that harbor mutations in homologous recombination genes, can utilize the Poly (ADP-ribose) polymerase (PARP) pathway that is involved in single strand DNA break repair as a salvage mechanism to repair DNA damage. Therefore, inhibition of PARP mediated pathway could lead to tumor destruction and synthetic lethality in presence of *BRCA* mutations (Figure 1). Based on this, various PARP inhibitors have been approved for treatment of patients with germline or somatic *BRCA* mutant breast and ovarian cancer (Table 1).

A recent study of whole genome sequencing of 638 patients with familial pancreatic cancer showed mutations in the *BRCA2* gene accounted for the largest fraction of known familial pancreatic cancer genes and was found in 5-10% of the families.[10] Among patients with no family history of PDAC, *BRCA2* mutation is found in 2% and *BRCA1* mutation is found in 1% of the patients or less.[11] In the Ashkenazi Jewish population with PDAC, a much higher incidence of *BRCA* mutations are found and seen in up to 13.7% of unselected cases.[12] Therefore, PARP inhibitors may play an important role in treatment of pancreatic cancer with germline or somatic *BRCA1/BRCA2* mutations as well.

PARP INHIBITORS PHASE I STUDIES

Phase I studies were conducted after having demonstrated synthetic lethality with PARP inhibitors using *in vitro* models. In a study of 60 patients, 22(37%) of whom were known carriers of a *BRCA 1* or *BRCA 2* mutation, received olaparib at escalating doses. This study recruited patients 18-years of age or older with treatment refractory solid tumors. Dosing of olaparib was started at 10 mg daily for two of every three weeks, and was doubled every cycle of treatment, if tolerated. This trial demonstrated that the maximum tolerated dose of olaparib to be 400 mg twice daily. The most common side effects included grade 1-2 nausea, and fatigue in one third of patients. The most common grade 3-4 toxicities were lymphopenia and anemia, which occurred in up to 5% of the study population. Overall, 23 patients with *BRCA* mutation were treated

Pancreas Open J. 2019; 3(1): e5-e8. doi: 10.17140/POJ-3-e011

and 19 patients were evaluated for response. 12 of 19 (63%) patients had clinical benefit defined as radiologic response (complete or partial response) based on response evaluation criteria in solid tumors (RECIST), tumor-marker responses defined as a decline in the tumor-marker level of more than 50% that was sustained for at least 4-weeks, or stable disease for a period of 4-months or more.[9]

PARP INHIBITORS PHASE II STUDIES

Following promising phase I trial results, several phase II trials were conducted looking at PARP inhibitors in patients with germline *BRCA1* or *BRCA2* mutations. In a study of 298 patients with pretreated solid tumors, 23 of whom had pancreatic cancer, patients received olaparib at 400 mg twice daily. The response rate was found to be 26% in all patients and 22% in patients with pancreatic specifically.[13] Another multicenter phase II study, RUCAPANC, enrolled patients with *BRCA1* or *BRCA2* mutations and pancreatic cancer. In this study, 19 patients were enrolled and treated with the PARP inhibitor Rucaparib at 600 mg twice a day. Clinical responses were seen in 3/19 (16%) of the patients.[14] Currently there are no head to head trials comparing various PARP inhibitors comparing efficacy. A meta-analysis presented at the Society of Gynecologic Oncology (SGO) meeting 2018 showed no difference in efficacy among the three PARP inhibitors but demonstrated a more favorable safety profile for olaparib, associated with a reduced odds ratio of grade 3 or greater adverse events and treatment interruption.[15]

PARP INHIBITORS PHASE III STUDIES

The results of a recently published phase III Pancreas Cancer Olaparib Ongoing (POLO) trial were chosen for presentation at the plenary session of the American Society of Clinical Oncology (ASCO) 2019 meeting, highlighting the great excitement for the emerging roles of PARP inhibitor therapy in numerous solid tumors.[16] Patients with metastatic pancreatic cancer enrolled for this randomized, double-blind, placebo-controlled phase III trial must have harbored a deleterious germline mutation of *BRCA1* or *BRCA2* (confirmed by central testing with the BRCAnalysis CDx test) and received at least 16-weeks of continuous first-line platinum-based chemotherapy for eligibility. Patients were randomly assigned in a 3:2 ratio to receive olaparib 300 mg twice daily or placebo (PBO) as a maintenance therapy started within 4-8 weeks after the final dose of first-line chemotherapy. Of 3315 patients screened for eligibility, 154 underwent randomization with 92 assigned to receive olaparib and 62 to receive placebo. The majority (>80% in each group) received a variant of FOLFIRINOX with an option to hold the platinum component after 16-weeks if toxicities arised. Although the duration of first-line chemotherapy was not limited, the majority in each group received therapy ranging from 16-weeks to 6-months (66% in olaparib, 65% in PBO).

The study met its primary endpoint impressively with near doubling of progression free survival (PFS) in the olaparib group compared to placebo (mPFS 7.4 *vs.* 3.8 mo, HR 0.53, 95% CI 0.35-0.82, p=0.004), generating an enthusiasm shown similar to the practice-changing SOLO1 olaparib maintenance trial for newly diagnosed advanced ovarian cancer (Table 2).[17] Significant responses were seen in 20 patients in the olaparib group (20%) compared to 6 in PBO (10%), with 2 complete responses seen with olaparib alone. Olaparib was very well tolerated with only 5% rate of discontinuation for toxicity with no changes in quality of life compared to PBO as measured by the EORTC QLQ-C30 score. At this interim analysis with data maturity of only 46%, there was no OS benefit yet seen (mOS 18.9 *vs.* 18.1, HR 0.91 95% CI, 0.56-1.46, p=0.68). In addition to the immaturity of the data for this secondary endpoint, PARP inhibitor use in the PBO group after discontinuation of study drug and increased use of chemotherapy upon progression (49% in olaparib group *vs.* 74% in PBO group) may have contributed to the lack of OS benefit.

Table 2. *POLO Trial PFS and OS*

PFS (mos)	Olaparib Group	Placebo Group	Hazard Ratio	p-value
6	53.0%	23.0%		
12	33.7%	14.5%		
18	27.6%	9.6%		
24	22.1%	9.6%		
Median PFS	7.4-months	3.8-months	0.53	0.004
Median OS	18.9-months	18.1-months	0.91	0.68

As shown repeatedly in both ovarian and breast cancer, the commended POLO trial has strengthened the encouragement for PARP inhibition in solid tumors, now likely setting a new standard of care in pancreatic cancer for those with germline *BRCA1* or *BRCA2* mutations. These impressive results continue to support the use of PARP inhibitors as an adjunct to DNA-damaging agents for those with homologous recombination repair deficiencies, even after prolonged exposure to platinum agents.

The growing success in this space calls for further inclusion of those with additional deficiencies of homologous recombination repair, particularly *ATM* and *PALB2* which are also of interest given their prevalence in pancreatic cancer.[18] A phase II trial including patients with metastatic castrate resistant prostate cancer (mCRPC) treated with olaparib 400 mg twice daily identified mutations in DNA repair related genes including *BRCA 1/2*, *ATM*, and *PALB2* in 16 out of 49 (33%).[19] Subgroup analysis per altered gene identified response rates defined as radiologic response per RECIST criteria or greater than 50% reduction in PSA. Response rates of 80%, 57%, and 37% were seen in patients with *BRCA 1/2*, *PALB2*, and *ATM* mutations respectively.[19] The highest response rate in PSA reduction was noted in patients with *BRCA 1/2* and *PALB2* subgroups. This study corroborates the rationale in developing PARP inhibition in DDR-defective patients beyond *BRCA* mutations and has implications in treatment of PDAC as well.[19] Periodic re-evaluation of those found with *BRCA* variants of uncertain significance should also be warranted for appropriate grouping of this unclear alterations to "likely benign" or "likely pathogenic" classification. The benefits of PARP inhibitor-mediated synthetic lethality for those with germline *BRCA1* or *BRCA2* mutations are now undeniable and highlights the importance of germline testing for all patients with pancreatic cancer.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

1. American Cancer Society. Cancer Facts & Statistics. American Cancer Society. Web site. https://www.cancerstatisticscenter.cancer.org/. Accessed July 9, 2019.

2. Conroy T, Desseigne F, Ychou M, et al. FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. *N Engl J Med.* 2011; 364(19): 1817-1825. doi: 10.1056/NEJMoa1011923

3. Von Hoff DD, Ramanathan RK, Borad MJ, et al. Gemcitabine plus nab-paclitaxel is an active regimen in patients with advanced pancreatic cancer: a phase I/II trial. *J Clin Oncol.* 2011; 29(34): 4548-4554. doi: 10.1200/JCO.2011.36.5742

4. Almoguera C, Shibata D, Forrester K, Martin J, Arnheim N, Perucho M. Most human carcinomas of the exocrine pancreas contain mutant c-K-ras genes. *Cell.* 1988; 53(4): 549-554. doi: 10.1016/0092-8674(88)90571-5

5. Pellegata NS, Sessa F, Renault B, et al. K-ras and p53 gene mutations in pancreatic cancer: Ductal and nonductal tumors progress through different genetic lesions. *Cancer Res.* 1994; 54(6): 1556-1560.

6. Hezel AF, Kimmelman AC, Stanger BZ, Bardeesy N, Depinho RA. Genetics and biology of pancreatic ductal adenocarcinoma. *Genes Dev.* 2006; 20(10): 1218-1249. doi: 10.1101/gad.1415606

7. Maitra A, Hruban RH. Pancreatic cancer. *Annu Rev Pathol.* 2008; 3: 157-188. doi: 10.1146/annurev.pathmechdis.3.121806.154305

8. Helleday T. The underlying mechanism for the PARP and BRCA synthetic lethality: Clearing up the misunderstandings. *Mol Oncol.* 2011; 5(4): 387-393. doi: 10.1016/j.molonc.2011.07.001

9. Fong PC, Boss DS, Yap TA, et al. Inhibition of poly (ADP-ribose) polymerase in tumors from BRCA mutation carriers. *N Engl J Med.* 2009; 361(2): 123-134. doi: 10.1056/NEJMoa0900212

10. Roberts NJ, Norris AL, Petersen GM, et al. Whole genome sequencing defines the genetic heterogeneity of familial pancreatic cancer. *Cancer Discov.* 2016; 6(2): 166-75. doi: 10.1158/2159-8290.CD-15-0402

11. Shindo K, Yu J, Suenaga M, et al. Deleterious germline mutations in patients with apparently sporadic pancreatic adenocarcinoma. *J Clin Oncol.* 2017; 35(30): 3382-3390. doi: 10.1200/JCO.2017.72.3502

12. Ferrone, C.R., Levine DA, Tang LH, et al. BRCA germline mutations in jewish patients with pancreatic adenocarcinoma. *J Clin Oncol.* 2009. 27(3): 433-438. doi: 10.1200/JCO.2008.18.5546

13. Kaufman B, Shapira-Frommer R, Schmutzler RK, et al. Olaparib monotherapy in patients with advanced cancer and a germline BRCA1/2 mutation. *J Clin Oncol.* 2015; 33(3): 244-250. doi: 10.1200/JCO.2014.56.2728

14. Shroff RT, Hendifar A, McWilliams RR, et al. Rucaparib monotherapy in patients with pancreatic cancer and a known deleterious BRCA mutation. *JCO Precis Oncol.* 2018; 2018. doi: 10.1200/PO.17.00316

15. Similar Efficacy Across PARP Inhibitors in Ovarian Cancer, But Safety Analysis Favors Olaparib. Paper presented at: SGO Annual Meeting; 2018; New Orleans, LA, USA.

16. Golan T, Hammel P, Reni M, et al. Maintenance olaparib for germline BRCA-mutated metastatic pancreatic cancer. *N Engl J Med.* 2019; 381(4): 317-327. doi: 10.1056/NEJMoa1903387

17. Moore K, Colombo N, Scambia G, et al. Maintenance olaparib in patients with newly diagnosed advanced ovarian cancer. *N Engl J Med.* 2018; 379(26): 2495-2505. doi: 10.1056/NEJMoa1810858

18. Heeke AL, Pishvaian MJ, Lynce F, et al. Prevalence of homologous recombination-related gene mutations across multiple cancer types. *JCO Precis Oncol.* 2018; 2018. doi: 10.1200/PO.17.00286

19. Mateo J, Porta N, McGovern UB, et al. TOPARP-B: A phase II randomized trial of the poly(ADP)-ribose polymerase (PARP) inhibitor olaparib for metastatic castration resistant prostate cancers (mCRPC) with DNA damage repair (DDR) alterations. *J Clin Oncol.* 2019; 37(15_suppl): 5005-5005. doi: 10.1200/JCO.2019.37.15_suppl.5005