ORIGINAL ARTICLE

Genomic Profile of Pancreatic Cancer by Next Generation Sequencing-A Single Center Experience

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ABSTRACT

Context Pancreatic cancer is the fourth most common cause of cancer death in Europe characterized by disappointing tumor response rates and high levels of resistance to standard treatments. Understanding genomic variations in pancreatic cancer is crucial, as they are known to contribute to pancreatic carcinogenesis and may provide fundamental knowledge for new and effective treatment strategies. **Objective** The main purpose of this exploratory study was to try to characterize the genomic profile of nine pancreatic tumors of patients treated at our institution. **Methods** Primary or metastatic tumor samples from patients with pancreatic cancer were analysed by FoundationOne CDx (F1CDx) Next Generation Sequencing (NGS) diagnostic test and clinical data were retrieved from the patients electronic medical record. **Results** Nine patients with cytological or histological documentation of pancreatic cancer treated at the Medical Oncology Department of Hospital da Luz Lisboa between October and December of 2017 were included. F1CDx NGS identified genomic variants with clinical significance in all 9 samples in the following genes: *KRAS* (7/9), *TP53* (5/9), *SMAD4* (3/9), *CTNNB1* (1/9), *CDKN2A* (1/9), *MDM4* (1/9), *ARID1A* (1/9), *ARID2* (1/9), *PIK3C2B* (1/9) and *FANCA* (1/9). No tumor sample had microsatellite instability or high mutational burden. Actionable genomic alterations were identified in 7 tumors. However, no patient underwent targeted therapy. **Conclusion** This exploratory cohort, although small in size, documents the genetic heterogeneity of pancreatic carcinoma and confirms *RAS*, *TP53* and *SMAD4* as the most common genetic alteration in this tumor type. However the utility of this test to foster inclusion in clinical trials is also conditioned by their accessibility (at the time no such trials were open in Portugal). Further studies are needed to validate the clinical utility of F1CDx in clinical practice.

INTRODUCTION

Pancreatic cancer is the fourth most common cause of cancer death in Europe, with stable or slightly increasing mortality rates [1, 2]. Despite decades of research and therapeutic development, five-year survival rate remains below 5%. The disappointing response rates and high levels of resistance to standard treatments highlights the urgent need need for novel treatments for patients with pancreatic cancer. The study of genomic alterations of pancreatic cancer may provide insight into targets for treatment [3]. Precision medicine clinical trials such as IMPACT [4], SAFIR 01 [5], MOSCATO [6] and SHIVA [7], suggest that this strategy is challenging but feasible. The recently published POLO clinical trial, brought new hope for target therapy in pancreatic cancer. It documented the benefit of olaparib, a poly adenosine diphosphate ribose polymerase (*PARP*) inhibitor, as maintenance therapy, in patients with germline *BRCA* mutation and metastatic pancreatic carcinoma without progression after at least 16 weeks of platinum-based chemotherapy metastatic pancreatic carcinoma with germline BRCA mutation without progression after at least 16 weeks of platinum-based chemotherapy. The median progression-free survival was significantly longer in the olaparib group than in the placebo group (7.4 months *vs.* 3.8 months; hazard ratio for disease progression or death, 0.53; 95% confidence interval [CI], 0.35 to 0.82; P=0.004) [8].

Also, patients with MSI-H and NTRK fusion-positive tumours presented meaningful clinical benefit with matched therapies in multi-histology studies [9, 10]. Currently, it is not recommended to perform tumour multigene Next-generation sequencing (NGS) in patients with pancreatic cancer in routine clinical practice unless in the context of molecular screening programmes, to allow access to innovative drugs [11]. The main purpose of this study was to characterize the genomic profile of nine pancreatic tumors of patients treated at our institution.

Methods

We applied FoundationOne®CDx (F1CDx) NGS test (provided by Roche Foundation Medicine®) to primary or metastatic lesions obtained from patients with pancreatic

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cancer. Eligible patients had to be 18 years or older and had to have a histologically/cytologically confirmed pancreatic cancer, American Joint Committee on Cancer (AJCC) stage I-IV, submitted to surgery, chemotherapy or/and radiotherapy and followed up in the Oncology Outpatient Clinic between October and December of 2017. Patients were excluded if they had documented hereditary cancer susceptibility syndromes, other neoplasms in the previous 5 years, except for non-melanoma skin cancer or life expectancy less than 3 months.

F1CDx uses DNA isolated from formalin-fixed paraffin embedded (FFPE) tumor tissue specimens and applies NGS to the detection of point mutations (substitutions, insertions and deletions), copy number alterations (CNAs) and selected gene rearrangements, in 324 genes. F1CDx also enables the evaluation of genomic signatures including microsatellite instability (MSI) and tumor mutational burden (TMB) [12]. The test report describes the clinically relevant alterations found in each patient´s tumor and identifies target therapies and clinical trials are indicated for those genomic events. The cut-off date for the data included in this report was 31 March 2021.

RESULTS

An observational descriptive cross-sectional study of 9 patients with pancreatic cancer with tumor samples analysed by F1CDx NGS was done. Clinical data were retrieved from the electronic medical record (EMR) and NGS results from the F1CDx report. The median age of patients was 65 [range 25; 85]; 6 were females and 3 males. Eastern Cooperative Oncology Group Performance Status (ECOG PS) of 0 in 6 patients, of 1 in 2 patients, and of 2 in 1 patient **(Table 1).** The diagnoses were made between October 2013 and December 2017 (median of 9.9 months between cancer diagnosis and test request).

There were seven ductal adenocarcinomas, 1 a solid pseudopapillary tumor of pancreas (SPTP) and 1 a carcinoma not otherwise specified (NOE). The study samples were obtained from the primary tumor in all but in 2 cases, both with samples obtained from peritoneal tumor metastases **(Table 2).** Stage at diagnosis was (8th edition of the American Joint Committee on Cancer (AJCC)): stage IIA (n = 1), stage IIB (n = 3) and stage IV (n = 4) (the other patients had an SPTP). Among the patients

Table 1: Baseline Characteristics and Survival.

Samples	Age	Stage	Metastatic disease at sample collection	ECOG PS	Survival from diagnosis (months)
Patient 1	27	NA	Yes	0	39,4 (alive)
Patient 2	64	IIA	No	0	48,3 (alive)
Patient 3	85	IV	Yes	1	3
Patient 4	68	IV	Yes	1	19,1
Patient 5	69	IV	Yes	0	24,7
Patient 6	61	IV	No	2	32,3
Patient 7	66	IIIB	No	0	63,3 (alive)
Patient 8	60	IIIB	No	0	29,8
Patient 9	48	IV	Yes	0	13,4

Table 2. Genomic alterations identified per patient.

Samples	Tissue of origin	Gene	Mutation
Patient 1	Peritoneal implants	*CTNNB1*	S37F
Patient 2	Pancreatic tumor	*MDM4* *ARID1A* *PIK3C2B*	Amplification Q176* Amplification
Patient 3	Pancreatic tumor	*KRAS* *FANCA* *TP53*	G12D Loss exons 1-28 Y220C
Patient 4	Pancreatic tumor	*KRAS* *TP53*	G12V H193R
Patient 5	Peritoneal implants	*KRAS* *ARID2*	G12R R601Q
Patient 6	Pancreatic tumor	*KRAS*	G12R
Patient 7	Pancreatic tumor	*KRAS* *SMAD4* *TP53*	G12D Y412* R282G splice site 559+2T>G
Patient 8	Pancreatic tumor	*KRAS* *MYST3* *SMAD4* *TP53*	G12V Amplification C25fs*4 R175H
Patient 9	Pancreatic tumor	*KRAS* *CDKN2A* *SMAD4* *TP53*	G12V p16INK4a V82fs*39 p14ARF R96fs*66 Loss exon 12 R156H R273C

with advanced disease (n=4), three had received 2 or more lines of systemic treatment. At the time of this analysis, 6 patients have died, and 3 patients are alive and disease free. The overall survival for each patient is described in Table 1.

All F1CDx NGS were performed on tumor samples obtained prior to systemic therapy administration. The average time between the date of test request and receipt of the report was 14 days [10; 20], and between the receipt of the report and the date of death was 180 days [15; 440]. Genomic variants with known clinical significance were found in all tumor samples involving the following genes: *KRAS* (7/9), *TP53* (5/9), *SMAD4* (3/9), *CTNNB1* (1/9), *CDKN2A* (1/9), *MDM4* (1/9), *ARID1A* (1/9), *ARID2* (1/9), *PIK3C2B* (1/9) and *FANCA* (1/9) (Table 2). Breakdown of mutations according to the histological subtype are listed in **Table 3**. Major pathways targeted by genomic alterations are shown in **Table 4**. Ras-ERK and DNA damage response pathways were the intracellular pathways more commonly involved (8 and 6 samples respectively) (Table 4). Variants of unknown significance (VUS) are listed on **Table 5.** No tumor sample had microsatellite instability or high mutational burden (but in 2 cases the result was undetermined due to low tumor purity). Druggable genomic alterations were identified in 6 mutated RAS tumors (cobimetinib and trametinib). However, no clinical trials were available in Portugal. No patient received targeted therapy.

Table 3: Genomic Alterations by histological subtype.

n	Histological Subtype	Genomic Alterations Identified
7	Ductal Adenocarcinoma	*KRAS (n=6), TP53 (n=4), SMAD4 (n=2), MDM4, ARID1A, ARID2, PIK3C2B, FANCA, MYST3*
1	Solid Pseudo papillary Tumor of Pancreas	*CTNNB1*
1	Carcinoma NOE	*KRAS, CDKN2A, SMAD4, TP53*

Table 4: Major Pathways targeted by genomic alterations.

Pathway	n	Genomic Alterations Identified
Ras-ERK	8	*KRAS, ARAF*
DNA damage response	6	*TP53* *MDM4*
TGF-β/SMAD4	3	*SMAD4*
FA/BRCA	1	*FANCA*
SWI/SNF	2	*ARID1A, ARID2*
CDK4/6-cyclin-Rb	1	*CDKN2A*
Wnt/beta-catenin	1	*CTTNB1*
PI3K	1	*PIK3C2B*
KAT6A	1	*MYST*

Table 5. Variants of Unknown Significance.

Samples	Variants of Unknown Significance (Reports date between December of 2017 and January of 2018)
Patient 1	*ASXL1 H633R* *MLL2 F2369S* *BRCA1 M1783T* *PMS2 G29A* *GATA2 P161A* *ZNF217 E349K* *HSD3B1 G90S* *ZNF703 A401_H402insPTH; LGGSSCSTCSA* *KDM6A R559H* *MLL T2230I*
Patient 2	*HGF S433G* *HSP90AA1 Q130 R* *RICTOR A713G* *IRS2 P780L* *MED12 Q2119_Q2120insH; QQQ* *NOTCH1* *R1598H* *RET G7D*
Patient 3	*BRIP1 amplification* *CDKN2A G135fs*22* *IRS2 A701_V702insA* *MYST3 R1926G* *SMAD4 E330G*

Patient 4	*ATM 274C* *SUFU G19S* *GPR124 V1109M* *MYCL1 S26R* *MYD88 A6fs*39* *ROS1 splice site 1165-1 G>A* *SDHB S152F*
Patient 5	*AKT2 T213I* *RANBP2 N1403S* *CARD11 S442R* *SOX9 S216del* *CHD2 P1749S* *WT1 R34W* *LRP1XB M131l* *MLL G909D* *NTRK1 G18E*
Patient 6	*BCORL1 D94N* *CDK6 Q318E* *NTRK1 G20D*
Patient 7	*EPHB1 R190C* *FGF3 T140M* *KEL D378E* *MLL3 L804V* *PRKDC R2731W* *ZNF217 F560C*
Patient 8	*BCORL1 T1490M* *MET 156L* *PIK3C2B Y653fs*44* *BRCA1 C675**
Patient 9	*C11orf30 P291S* *MLL2T429I* *NOTCH1 E515K* *RAF1 P332S*

DISCUSSION

Pancreatic cancer treatment is an unmet medical need. Understanding genomic alterations in pancreatic cancer is crucial, as they are known to contribute to pancreatic carcinogenesis and may provide fundamental knowledge for new and effective treatment strategies [3]. In these nine patients evaluated by F1CDx, the most prevalent genomic alteration was in the *KRAS* gene [G12V (n=3), G12D (n=2), G12R (n=2)]. The proto-oncogene *KRAS* is known to be mutated in almost 95% of pancreatic ductal adenocarcinoma (PDAC) and unfortunately, there are no therapeutic options that successfully target mutant *KRAS* [13]. Approximately 90% of patients with PDAC harbour the G12 mutation in KRAS [14]. The presence of a *KRAS* mutation seems to negatively influence the prognosis, although large-scale studies are certainly required. Targeting of *KRAS* to treat PDAC has been applied at different stages of the *RAS* molecular pathway [15]. Proteins downstream of KRAS, such as the RAF/MEK/ERK pathway have also attracted increasing interest [14]. The clinical trials proposed to the patients with *KRAS* mutations used *MEK* inhibitors as therapies: cobimetinib and trametinib.

TP53 is somatically mutated in up to 85% of pancreatic cancers [16] and it was the second most frequent genomic alteration in our cohort. *TP53* has an effect on DNA repair and responds to diverse cellular stresses to regulate target genes that induce cell cycle arrest, thus inducing growth arrest or apoptosis [17], and is the most commonly inactivated tumour suppressor in PDAC [14]. However, there were no clinical trials targeting *TP53* at the time in Portugal. *SMAD4* is inactivated in approximately 55% of pancreatic cancers, either by homozygous deletion or by an intragenic mutation in association with loss of the second copy [16]. *SMAD4* was the third most frequently identified genomic alteration. *SMAD4* mediates the pleiotropic signalling network downstream of the transforming growth factor-β (TGF-β) pathway and exerts paradoxical effects on tumorigenesis. In PDAC, *SMAD4* mutations interfere with the trimeric assembly of its C-terminal domain, thus therefore preventing the normal transduction of TGF-β signals [14]. Clinical studies have suggested that *SMAD4* inactivation is associated with a poor prognosis [18, 19].

In contrast to ductal adenocarcinomas, the genomic alteration found in SPTP was in the somatic β-catenin coding gene (*CTNNB1* S37F) (Table 2). Genomic alterations of *KRAS, SMAD4, TP53* and *CDKN2A* have never been detected in SPTP, differing from the molecular changes seen in adenocarcinoma of the pancreas. Almost all patients with SPTP have mutations of the *CTNNB1*, and multiple proteins associated with β-catenin have been detected as dysfunctional [20, 21]. Our cohort, although small in size, illustrates the genetic heterogeneity of pancreatic carcinoma. We stress the short time between sample dispatchment and test results, a logistic variable that may be relevant in clinical practice. Furthermore, the report of tests results is easy to interpret and the information on available clinical trials is helpful.

This study has limitations. First, given the small sample size it is not possible to make a correlation between the identified mutations and risk of recurrence or survival. Second, the tumor samples used for analysis were collected at diagnosis, prior to therapy. While this represents the true genetic changes of pancreatic cancer, it will miss treatment-induced changes that may be druggable. According to the European Society for Medical Oncology recommendations, tumor multigene NGS may be offered to patients with advanced PDAC in the context of molecular screening programs. To screen for access to innovative drugs [11]. However the application of this diagnostic test to foster clinical trials enrolment is also conditioned by their availability.

CONCLUSIONS

F1CDx, a NGS diagnostic test allows the rapid characterization of tumor genomic alterations and is a helpful tool to identify potential molecular targets for cancer treatment. This exploratory study of patients with pancreatic cancer studied by F1CDx validates *RAS, TP53* and *SMA*D4 as the most common genomic alteration in this tumor type. The genomic profiling of pancreatic cancer, a disease with limited systemic therapeutic options, may lead to the identification of druggable intracellular pathway targets, tested in early phase clinical trials. However, further studies are needed to validate its application in clinical practice.

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CONFLICTS OF INTEREST

The author Patrícia Machado has declared associations with Roche Farmacêutica Química as an employee for the last 2 years. The author Catarina Pulido has declared receiving speaker fees from Roche.

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