ESMO Open
Cancer Horizons

Genetic mapping of pancreatic cancer by targeted next-generation sequencing in a cohort of patients managed with nab-paclitaxel-based chemotherapy or agents targeting the EGFR axis: a retrospective analysis of the Hellenic Cooperative Oncology Group (HeCOG)

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► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136esmoopen-2019-000525)

To cite: Zarkavelis G, Kotoula V, Kolliou G-A, *et al*. Genetic mapping of pancreatic cancer by targeted next-generation sequencing in a cohort of patients managed with nab-paclitaxel-based chemotherapy or agents targeting the EGFR axis: a retrospective analysis of the Hellenic Cooperative Oncology Group (HeCOG). *ESMO Open* 2019;**4**:e000525. doi:10.1136/esmoopen-2019-000525

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Received 4 April 2019
Revised 24 May 2019
Accepted 25 May 2019

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ABSTRACT

Pancreatic cancer is one of the most fatal malignancies ranking fourth among the leading causes of cancer death with diagnosis at late stages carrying a dismal prognosis. The aim of our retrospective study was to describe the nature and the incidence of gene mutations and genomic instability in advanced pancreatic adenocarcinomas of a Greek patient population fully annotated with clinicopathological data. We used a targeted next-generation sequencing (NGS) panel encompassing genes commonly mutated in pancreatic tumours in a patient population managed with either nab-paclitaxel regimens or targeted compounds modulating the epidermal growth factor receptor (EGFR)/AKT/mTOR axis. We identified *KRAS, TP53, SMAD4* and *CDKN2A* as being the most prevalent mutations in the study population with the exception of an intriguingly lower incidence regarding KRAS mutants. Homologous recombination gene mutations were found to be mutually exclusive with *CDKN2A* mutations. The coexistence of both *KRAS* and *TP53* mutation seems to adversely affect the outcome of the patients whether treated with targeted therapy against EGFR/Akt/mTOR axis or cytotoxic drugs. The poor prognosis observed, correlated to late presentation, specific molecular mutations and to high mutational load warrant prospective validating studies and research into the mechanistic pathophysiology of pancreatic tumours for more effective therapeutic targeting.

SUMMARY

The mutational landscape of pancreatic adenocarcinomas (PACs) has already been investigated with *KRAS, TP53, SMAD4* and *CDKN2A* being the most commonly mutated genes according to Cancer Genome Atlas. Patients with advanced PACs are usually treated with the use of monotherapy or combination regimens with modest results.

In the context of a retrospective translational study, clinical data and formalin-fixed paraffin-embedded (FFPE) tumour tissue were collected from patients with resected or inoperable, advanced PAC. The aim of our retrospective study was to describe the nature and the incidence of gene mutations and genomic instability in PACs of a Greek patient population fully annotated with clinicopathological data using next-generation sequencing (NGS) technique. In addition, as a hypothesis-generating experiment, we sought to examine prognostic/predictive impact of mutations separately in 89 patients who received first-line anti-epidermal growth factor receptor (EGFR)/AKT/mTOR therapy versus 49 patients who received nab-paclitaxel-based cytotoxic chemotherapy only.

We identified *KRAS, TP53, SMAD4* and *CDKN2A* as being the most prevalent mutations with the exception of an intriguingly lower incidence regarding KRAS mutants. The unexpected lower incidence of KRAS mutations in our study population compared with the reported incidence reaching up to 90% of PACs could imply a possible association of the incidence with environmental, ethic or geographical factors to be further

key questions

What is already known about the subject?

- The mutational landscape of pancreatic adenocarcinomas has already been investigated with KRAS, TP53, SMAD4 and CDKN2A being the most commonly mutated genes according to Cancer Genome Atlas.

What does this study add?

- The nature and the incidence of gene mutations and genomic instability in pancreatic adenocarcinomas of a Greek patient population fully annotated with clinicopathological data using NGS technique and investigation of the prognostic/predictive impact of mutations separately in 89 patients who received first line anti- EGFR/AKT/mTOR therapy versus 49 patients who received nab-paclitaxel based cytotoxic chemotherapy only.
- KRAS, TP53, SMAD4 and CDKN2A were the most prevalent mutations with the exception of an intriguingly lower incidence regarding KRAS mutants implying a possible association of KRAS incidence with environmental, ethic or geographic factors to be further investigated.

How might this impact on clinical practice?

- The coexistence of both KRAS and TP53 could possibly have a pathogenetic role in pancreatic adenocarcinomas and it seems to adversely affect the outcome of patients whether treated with targeted therapy against EGFR/Akt/mTOR axis or cytotoxic drugs.
- Of note, in the subgroup of patients who underwent EGFR/AKT/mTOR axis therapeutic modulation a statistically significant association could be observed between the presence of SMAD4 mutations and an increased probability of death.
- The high mutational load warrants prospective validating studies and research into the mechanistic pathophysiology of pancreatic tumors for more effective therapeutic targeting.

investigated. Homologous recombination gene mutations were found to be mutually exclusive with *CDKN2A* mutations. The coexistence of both *KRAS* and *TP53* could possibly have a pathogenetic role in PACs and it seems to adversely affect the outcome of patients whether treated with targeted therapy against EGFR/Akt/mTOR axis or cytotoxic drugs.

INTRODUCTION

Pancreatic cancer is one of the most fatal malignancies ranking fourth among the leading causes of cancer death with patients being diagnosed at late stages carrying a dismal prognosis. The median overall survival (OS) is estimated at 6–11 months while the 5-year survival rate is below 10%. Although much progress has been made on unravelling the biology of cancer and consequently the identification of targetable molecular triggering mechanisms, this is merely not the case for patients with pancreatic cancer. Targeted therapies and immunotherapies failed to establish clinically meaningful efficacy. Combination chemotherapy regimens including gemcitabine, 5-fluorouracil, oxaliplatin and irinotecan among others are commonly administered with modest results. The FOLFIRINOX combination has provided better therapeutic results, whereas albumin-bound paclitaxel regimens also provide marginally superior efficacy and tolerance and are nowadays gemcitabine in combination with nab-paclitaxel as well as FOLFIRINOX are the standards of care, still arguing for an imperative need for novel therapeutic agents.[1]

The aim of our retrospective study was to describe the nature and the incidence of gene mutations and genomic instability in pancreatic adenocarcinomas (PACs) of a Greek patient population fully annotated with clinicopathological data. We used a targeted next-generation sequencing (NGS) panel interrogating genes commonly mutated in pancreatic tumours in a patient population managed with either nab-paclitaxel regimens or targeted compounds modulating the EGFR-AKT-mTOR axis. Our efforts resulted in the development of a pancreatic cancer genetic map from the Hellenic area and further comparison of its characteristics with those reported for American, Asian and European populations. In addition, we assessed the prognostic and predictive significance of the genetic abnormalities under study and evaluated the presence of aberrations in biomolecules that are potentially targetable.

Patients and methods

In the context of a retrospective translational study, clinical data and formalin-fixed paraffin-embedded (FFPE) tumour tissue were collected from patients with resected or inoperable, advanced PAC. The majority of the patients had been prospectively treated according to first-line treatment protocols or with regimens in the context of a clinical trial, while a small percentage of them had only received adjuvant treatment. Specifically, patients had been treated with:

1. gemcitabine and EGFR tyrosine kinase inhibitor (TKI, erlotinib or gemcitabine)
2. the combination of gemcitabine +temsirolimus in the context of a clinical trial
3. nab-paclitaxel-based chemotherapy (nab-paclitaxel (Abraxane) in combination with gemcitabine

In view of the recent MPACT (A Randomized Phase III Study of Weekly nab-paclitaxel plus Gemcitabine versus Gemcitabine Alone in Patients with Metastatic Adenocarcinoma of the Pancreas) trial data, every effort was made to further enrich the patient population with FFPE blocks from nab-paclitaxel treated patients, identified retrospectively. All patients provided written informed consent for the research use of their biological material, which included FFPE tumour tissues and peripheral blood samples. The translational research protocol was approved by the Institutional Review Board of the Papageorgiou Hospital (#982/20.3.14). Overall, a total of 289 tumour blocks were available for the study. All tissues, primary or metastatic, had been collected before treatment start. For 111 patients, matched tumour blocks and peripheral blood samples were available.

Tumour tissue processing

Histology review of FFPE tumours, tissue evaluation and processing, NGS genotyping and bioinformatics analysis were performed in the Laboratory of Molecular Oncology (MOL by Hellenic Foundation for Cancer Research]/ HeCOG (Aristotle University of Thessaloniki), Thessaloniki, Greece).

Haematoxylin and eosin sections were reviewed for tumour presence and histologic characteristics; for marking areas for macrodissection and for tumour cell content (TCC%) evaluation in the molecular template. Because pancreatic cancer stroma is implicated in tumour behaviour, the aim was to mark tissue areas containing ideally 50% tumour and 50% stromal cells for genotyping. Following macrodissection, DNA was extracted from marked areas on 10 µm thick whole unstained sections with the QIAamp DNA Mini Kit (Qiagen GmbH, Hilden, Germany). For comparison with germline status, peripheral blood DNA was extracted with a standard desalting method. DNA quantity was measured with the Qubit fluorometer (Life Technologies, Paisley, UK). Samples were ineligible for NGS if DNA quantity was <2 ng/µL. Where possible, DNA samples from normal pancreatic ductal epithelium were also prepared.

In total, 205 FFPE samples with adequate DNA from 193 patients were submitted for NGS. Mean TCC% for tumour samples was 50.3% (median 45%; range 10%–90%; 90% of the samples contained >30% tumour cell DNA). For 75 patients, matched germline DNA was available (27 among the patients treated with nab-paclitaxel plus gemcitabine; 46 among the patients who received gemcitabine plus temsirolimus; and, from two patients who received erlotinib).

Targeted NGS and genotype analysis

We performed targeted NGS with a custom Ampliseq panel (Applied Biosystmes/Ion Torrent/Thermo-Fisher Scientific, Paisley, UK). The panel, provided in full in online supplementary table S1, targeted (a) coding regions with previously identified mutations in PAC[2] and (b) germline mutations previously identified and recorded for the Greek population, based on recent reports on patients carrying germline mutations in cancer susceptibility genes, including *BRCA1/2*-related ones (data kindly provided by the Molecular Diagnostics Laboratory of NCSR Demokritos). Details on the NGS method and on the criteria for variant, sample and case eligibility are provided in **Supplementary Methods**. Based on these criteria, we accepted for analysis: (i) 5703 out of the originally returned 6791 variants by Ion Reporter (84%); (ii) 186 out of 205 FFPE (90.7%) and 70 out 75 germline DNA (93.3%) sequenced samples; (iii) 172 out of 193 patients (89.1%) for whom biological material was submitted for NGS genotyping. The median value of mean reading depth for the 185 informative FFPE samples was 3193x (mean value >4500x; >484 in 90% of the samples) and for uniformity of reads 92.6% (mean 91.8%; >89% in 90% of the samples) (online supplementary figure S1). Variants

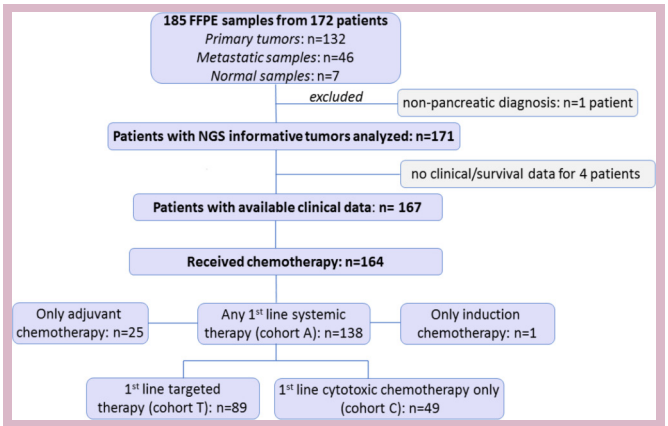

Figure 1 Remark diagram.

were classified as mutations if resulting in amino acid or splice site change, with population frequencies (Mutant Allele Frequency) <0.1%, based on the NCBI database of genetic variation (dbSNP), the Exome Aggregation Consortium (ExAC) (ANNOVAR) and 1000 Genomes. Mutations were further characterised as clonal for variant allelic frequencies >25% in the sample[3] and as pathogenic based on ClinVar, catalogue of somatic mutations in cancer (COSMIC) and on deleterious functional analysis through hidden markov models (FATHMM) scores. In cases with matched samples, variants were categorised as shared and private by taking into account informative position reads for both samples under comparison.

Statistical analysis

On inspection of the clinical data, we further excluded one patient with adenocarcinoma of non-pancreatic origin. Thus, the final number of patients with informative NGS data in the present analysis was 171 (figure 1).

Percentages were used to describe categorical variables, whereas medians, means, SD and range were used to provide descriptive statistics of continuous variables. χ^2 or Fisher's exact test (where appropriate) were performed to evaluate differences between clinicopathological parameters and the mutational status of several genes, whereas the non-parametric Wilcoxon rank-sum or Kruskal-Wallis tests were applied for continuous variables.

The primary endpoint of interest was OS, defined as the time (in months) from diagnosis of pancreatic cancer to death from any cause or last contact, whichever occurred first. Survival distributions were estimated using the Kaplan-Meier method and compared across groups with the Log-rank test. All parameters were tested for proportionality using time-dependent covariates. Univariate Cox proportional hazard regression models were applied to analyse the association of several variables of interest (clinicopathological parameters and genes' mutational status) with death rates in the Entire Cohort of patients with available follow-up data (n=167) as well as among:

a) patients with advanced PAC treated with any first-line systemic therapy either targeted therapy (cohort T)

or cytotoxic only chemotherapy (cohort C), cohort A (n=138): ADVANCED DISEASE PATIENTS,

b) patients treated with first-line targeted therapy either an EGFR TKI (erlotinib) or a PI3K/Akt/mTOR inhibitor (temsirolimus), cohort T (n=89): TARGETED THERAPY ADVANCED DISEASE PATIENTS and

c) patients treated with first-line cytotoxic chemotherapeutic drugs only, cohort C (n=49): CYTOTOXIC CHEMOTHERAPY ONLY ADVANCED DISEASE PATIENTS.

Multivariate models were also applied in the Entire Cohort, in patients in cohorts A and T, as above. Multivariate models were not examined in the subgroup of patients treated with first-line cytotoxic chemotherapeutic drugs only (cohort C) due to the limited sample size. The final models included variables remaining from a backwards selection procedure with a removal criterion of p>0.10.

Details on model construction are provided in Supplementary Methods and Figures. All tests were two-sided at an alpha 5% level of significance. No adjustment for multiple comparisons was performed since this study was exploratory and mainly hypothesis generating with predefined parameters. The SAS V.9.3 (SAS Institute) was used for data manipulation and statistical analysis. The R studio V.3.5.0 was used to produce maps with mutated gene prevalence and profiles in the examined tumours.

RESULTS

Patient characteristics

Clinical and follow-up data were available for 167 of the 171 patients (97.7%) with NGS informative tumours. Basic patient and tumour characteristics are presented in table 1. The median age at diagnosis was 64 years while most patients were men (59.9%) and were initially diagnosed with stage IV pancreatic cancer. Three patients (1.8%) did not receive any systemic treatment while 138 patients had received first-line systemic therapy (cohort A). Of them, 89 patients received treatment against the EGFR/AKT/mTOR axis (cohort T), while the rest of the patients were treated with cytotoxic chemotherapeutic drugs only (cohort C).

At a median follow-up of 71.4 months (95% CI 41.0 to 110.0), 143 deaths were recorded. The median OS was 10.8 months (95% CI 9.3 to 13.0) while 42 patients (25.1%) died within 6 months and 86 patients (51.5%) within 1 year since diagnosis.

Table 1 Basic patient and tumour characteristics

(n=167)	
Age (years)	
Median, min-max	**63.7 (34.8,80.8)**
	N (%)
Sex	
Female	67 (40.1)
Male	100 (59.9)
Histological grade	
G1	17 (10.2)
G2	73 (43.7)
G3-G4	59 (35.3)
Unknown	18 (10.8)
Initial stage	
I-III	67 (40.1)
IV	99 (59.3)
Unknown	1 (0.6)
Radical operation	
Yes	56 (33.5)
No	111 (66.5)
Chemotherapy	
Yes	164 (98.2)
No	3 (1.8)
Type of chemotherapy	
Only adjuvant chemotherapy	25 (15.0)
Only induction chemotherapy	1 (0.6)
First-line chemotherapy*	138 (82.6)
Follow-up (months)	
Median, 95% CI	71.4 (41.0 to 110.0)
Overall survival (months)	
Median, 95% CI	10.8 (9.3 to 13.0)

*With or without prior chemotherapy/radiotherapy.
N, Number.

Genomic mapping and molecular data

The 185 informative FFPE samples corresponded to 132 primary tumours; 46 metastatic samples, 39 out of which constituted the only available sample per patient; and, 7 normal samples. Out of 5703 informative variants 799 were mutations; these positions were read at a median coverage of 1704x. Mutations were distributed in tumour tissues from 150 out of the 171 (87.7%) examined patients at a median value of 2 per tumour (range 0–116). In most cases, the number of tissue mutations per patient was 1–3, but it was surprisingly high (>8 mutations/sample) in nine cases. Mutated and non-mutated samples did not differ in technical performance (online supplementary file 2).

Out of all mutations, 374 were pathogenic distributed in the tissues of 141 patients (82.5% of all patients; median: two pathogenic tissue mutations per patient; range 0–35); 128/374 pathogenic mutations were clonal and were observed in tumours from 70 patients.

KRAS, TP53, SMAD4 and *CDKN2A* were the most frequently mutated genes. Median position coverage was 1993x, 1961x, 1998x and 1448x, respectively; median variant allele frequency was 18%, 17%, 17% and 24%, respectively. In total, 90 patients (52.6%) carried mutations in *TP53* and 83 of them presented with *TP53* pathogenic mutations, while all *KRAS* mutations detected in

Figure 2 Map showing the distribution of pathogenic mutations per gene per tumour.

112 patients (65.5% of all tumours; 74.5% of mutated tumours) were pathogenic (online supplementary table S2). The distribution of all mutations and pathogenic mutations per gene is presented in online supplementary figure S2 and figure 2, respectively.

Co-occurrence of tumour pathogenic mutations in *KRAS* and *TP53* was observed in 67 out of 171 patients (39.2%). In all, 23 patients (13.5%) carried pathogenic mutations in any of the examined homologous recombination (HR) genes (*BRCA1, BRCA2, CHEK2, PALB2, NBN, BAP1*) while 15 patients (8.8%) presented with pathogenic mutations in *BRCA1* and/or *BRCA2*. Pathogenic mutations in HR genes and/or *CDKN2A* were detected in 47 patients (27.5%). Pathogenic mutations in HR genes were mutually exclusive with pathogenic mutations in the *CDKN2A* gene with only three patients presenting with pathogenic mutations in HR genes as well as in *CDKN2A* (Fisher's p=0.010). It is of note that two of these patients also carried pathogenic mutations in *BRCA1/BRCA2* in their tumours.

No pathogenic mutations were detected in the 55 germline DNA samples matched to the patients in the present study. However, variants of unknown significance or benign variants in *BRCA1, MSH2, PMS2, CHEK2* and one *TP53* variant of conflicting pathogenic significance were observed in the germline samples of five patients. Furthermore, mutations were observed in three out of seven normal samples, one of them harbouring pathogenic *KRAS* and *TP53* mutations. All germline and normal tissue variants were shared in matched tumours (online supplementary table S3).

Associations and prognostic/predictive analyses

The associations of clinicopathological parameters with the mutational status of several genes are presented in online supplementary table S4. Patients with clonal pathogenic mutations had less frequently undergone radical operation compared with those without (23.2% vs 40.8%, χ^2 p=0.018) while the number of mutations was lower in stage I-III disease and that of pathogenic mutations was lower in patients who underwent radical operation (online supplementary table S5).

In the Entire Cohort, the presence of clonal pathogenic mutations, *KRAS* mutations, pathogenic mutations in HR genes/*CDKN2A* and in *BRCA1/2* were associated with worse survival while patients carrying pathogenic mutations in both *KRAS* and *TP53* were at significantly higher risk of death as well (table 2).

Among patients treated with first-line systemic therapy (cohort A), the presence of pathogenic mutations in *KRAS* and *SMAD4* increased the risk of death (HR=2.16, Wald's p<0.001 and HR=1.81, p=0.011, respectively). In addition, patients with pathogenic mutations in *BRCA1/BRCA2*, both *KRAS* and *TP53* and only in *KRAS* were at higher risk of death compared with those without mutations in any of these genes (HR=2.15; p=0.011, HR=2.12; p=0.003 and HR=3.17; p<0.001, respectively) (table 2).

Table 2 HRs (95% CIs) estimated by univariate COX regression with respect to OS in the entire cohort, cohort A and cohort T

		Entire cohort (n=167)					Cohort A (n=138)					Cohort T (n=89)				
Parameter	**Categories**	**N pts**	**N evts**	**HR**	**95% CI**	**P value**	**N pts**	**N evts**	**HR**	**95% CI**	**P value**	**N pts**	**N evts**	**HR**	**95% CI**	**P value**
Age				1.01	1.00 to 1.03	0.14			1.01	1.00 to 1.03	0.37			0.99	0.97 to 1.02	0.48
Gender	Male vs female	100 vs 67	86 vs 57	1.01	0.72 to 1.41	0.96	81 vs 57	73 vs 50	1.11	0.77 to 1.59	0.58	51 vs 38	48 vs 36	1.26	0.81 to 1.95	0.31
Histological grade						0.30					0.34					0.51
	G2 vs G1	73 vs 17	60 vs 14	1.23	0.69 to 2.20	0.49	61 vs 14	51 vs 12	1.30	0.69 to 2.45	0.41	38 vs 7	34 vs 7	1.07	0.47 to 2.41	0.88
	G3-G4 vs G1	59 vs 17	51 vs 14	1.52	0.84 to 2.76	0.16	45 vs 14	42 vs 12	1.58	0.83 to 3.02	0.17	29 vs 7	28 vs 7	1.40	0.61 to 3.22	0.43
Stage	Stage IV vs Stage I-III	99 vs 67	93 vs 49	2.40	1.69 to 3.43	*<0.001*	97 vs 40	91 vs 31	2.27	1.49 to 3.44	*<0.001*	69 vs 19	67 vs 16	2.07	1.18 to 3.62	*0.011*
Radical operation	Yes vs no	56 vs 111	41 vs 102	0.41	0.28 to 0.59	*<0.001*	31 vs 107	24 vs 99	0.41	0.26 to 0.65	*<0.001*	13 vs 76	ten vs 74	0.41	0.21 to 0.80	*0.009*
Number of mutations per tumour				1.01	1.00 to 1.02	*0.037*			1.01	1.00 to 1.02	0.082			1.01	1.00 to 1.02	0.27
Number of pathogenic mutations per tumour				1.06	1.02 to 1.10	*0.001*			1.05	1.02 to 1.10	*0.006*			1.04	1.00 to 1.09	0.059
Presence of mutations	Yes vs no	148 vs 19	130 vs 13	1.81	1.02 to 3.20	*0.043*	125 vs 13	113 vs 10	2.10	1.10 to 4.03	*0.026*	78 vs 11	76 vs 8	3.45	1.57 to 7.62	*0.002*
Presence of pathogenic mutations	Yes vs no	139 vs 28	122 vs 21	2.05	1.28 to 3.28	*0.003*	117 vs 21	106 vs 17	2.52	1.46 to 4.40	*<0.001*	73 vs 16	71 vs 13	2.71	1.44 to 5.11	*0.002*
Presence of clonal pathogenic mutations	Yes vs no	69 vs 98	59 vs 84	1.48	1.05 to 2.07	*0.025*	59 vs 79	51 vs 72	1.42	0.98 to 2.06	0.064	39 vs 50	38 vs 46	1.51	0.97 to 2.35	0.070
KRAS	Mut vs no mut	111 vs 56	99 vs 44	2.13	1.46 to 3.10	*<0.001*	92 vs 46	84 vs 39	2.16	1.45 to 3.24	*<0.001*	58 vs 31	57 vs 27	2.16	1.33 to 3.52	*0.002*
TP53	Mut vs no mut	89 vs 78	77 vs 66	1.31	0.94 to 1.82	0.11	74 vs 64	67 vs 56	1.27	0.89 to 1.82	0.19	47 vs 42	46 vs 38	1.33	0.86 to 2.06	0.20
CDKN2A	Mut vs no mut	30 vs 137	29 vs 114	1.50	0.99 to 2.26	0.056	27 vs 111	26 vs 97	1.38	0.89 to 2.14	0.15	14 vs 75	14 vs 70	1.39	0.78 to 2.49	0.27
SMAD4	Mut vs no mut	34 vs 133	32 vs 111	1.40	0.95 to 2.09	0.093	29 vs 109	28 vs 95	1.57	1.03 to 2.41	*0.039*	23 vs 66	23 vs 61	1.73	1.05 to 2.84	*0.032*
Pathogenic mutations in TP53	Yes vs no	82 vs 85	70 vs 73	1.21	0.87 to 1.68	0.26	67 vs 71	60 vs 63	1.18	0.83 to 1.69	0.37	41 vs 48	40 vs 44	1.22	0.79 to 1.88	0.36
Pathogenic mutations in CDKN2A	Yes vs no	27 vs 140	26 vs 117	1.46	0.95 to 2.25	0.084	25 vs 113	24 vs 99	1.31	0.84 to 2.06	0.23	13 vs 76	13 vs 71	1.34	0.73 to 2.43	0.35
Pathogenic mutations in SMAD4	Yes vs no	29 vs 138	28 vs 115	1.51	1.00 to 2.29	0.052	24 vs 114	24 vs 99	1.81	1.15 to 2.84	*0.011*	19 vs 70	19 vs 65	1.85	1.09 to 3.14	*0.022*
KRAS/TP53 pathogenic mutations						*<0.001*					*<0.001*					*0.002*
	Both KRAS&TP53 vs none	67 vs 41	57 vs 31	2.10	1.34 to 3.30	*0.001*	54 vs 33	48 vs 27	2.12	1.30 to 3.47	*0.003*	32 vs 22	31 vs 18	2.21	1.21 to 4.05	*0.010*
	Only KRAS vs none	44 vs 41	42 vs 31	2.66	1.65 to 4.29	*<0.001*	38 vs 33	36 vs 27	3.17	1.88 to 5.37	*<0.001*	26 vs 22	26 vs 18	3.60	1.89 to 6.83	*<0.001*
	Only TP53 vs none	15 vs 41	13 vs 31	1.39	0.72 to 2.66	0.32	13 vs 33	12 vs 27	1.61	0.81 to 3.20	0.17	nine vs 22	nine vs 18	2.16	0.96 to 4.87	0.063

Continued

Table 2 Continued

Parameter	Categories	Entire cohort (n=167)					Cohort A (n=138)					Cohort T (n=89)				
		N pts	N evts	HR	95% CI	P value	N pts	N evts	HR	95% CI	P value	N pts	N evts	HR	95% CI	P value
Pathogenic mutations in HR genes	Yes vs no	22 vs 145	21 vs 122	1.40	0.88 to 2.23	0.16	21 vs 117	20 vs 103	1.25	0.77 to 2.02	0.37	16 vs 73	16 vs 68	1.20	0.69 to 2.08	0.52
Pathogenic mutations in HR genes/CDKN2A	Yes vs no	46 vs 121	44 vs 99	1.50	1.05 to 2.16	*0.028*	43 vs 95	41 vs 82	1.32	0.90 to 1.93	0.16	27 vs 62	27 vs 57	1.37	0.85 to 2.19	0.20
BRCA1/2 pathogenic mutations	Yes vs no	14 vs 153	14 vs 129	2.42	1.38 to 4.26	*0.002*	13 vs 125	13 vs 110	2.15	1.19 to 3.86	*0.011*	12 vs 77	12 vs 72	1.56	0.84 to 2.90	0.16

evts, events; pts, patients.

Similar results were observed among patients treated with targeted therapy containing either an EGFR TKI or a PI3K/Akt/mTOR inhibitor (cohort T), with patients with pathogenic mutations in *KRAS* and *SMAD4* presenting with increased risk of death (HR=2.16; p=0.002 and HR=1.85; p=0.022, respectively). The presence of pathogenic mutations in both *KRAS* and *TP53* was also associated with increased risk of death (HR=2.21; p=0.010) while the existence of pathogenic mutations in *BRCA1/BRCA2* was not found to affect the risk of death in this subgroup (p=0.16) (table 2).

In Cohort C of patients treated with first-line cytotoxic chemotherapy only, pathogenic mutations in *KRAS* increased the risk of death (HR=2.38, p=0.022) (online supplementary table S6). It is of note that radical operation was associated with longer survival in the entire cohort as well as in all examined population subgroups. In addition, the number of pathogenic mutations per tumour conferred higher risk of death in the entire cohort and in cohort A in cohort (HR=1.06; p=0.001 and HR=1.05; p=0.006, respectively) while marginal significance was reached in Cohort T (HR=1.04; p=0.059).

On multivariate analyses, in the Entire Cohort, the presence of pathogenic mutations in both *KRAS* and *TP53* remained an unfavourable prognostic factor of OS (HR=2.22; p<0.001), along with late stage (HR=2.48; p<0.001). Similarly, in the Cohort A of patients with advanced disease treated with first-line systemic therapy as well as among those in Cohort T, the presence of pathogenic mutations in both *KRAS* and *TP53* was an independent prognostic factor associated with worse survival (HR=2.12; p=0.003 and HR=2.21; p=0.010, respectively) (table 3).

DISCUSSION

According to published data regarding the molecular landscape of PACs, *KRAS, TP53, SMAD4* and *CDKN2A* are the most commonly mutated genes among both oncogenes and tumour suppressors.[4] Wadell *et al* have proposed a classification model for PACs according to structural rearrangements observed describing stable, locally rearranged, scattered and unstable subtypes. The unstable subtype correlates to mutations affecting *BRCA1* and *BRCA2* genes along with cases where *PALB2* and *ATM* mutations are identified.[5] PACs are usually characterised by low amounts of cellularity in the setting of advanced desmoplastic reaction, thus confounding the actual impact of neoplastic cell mutational burden. Mutations in other genes including *ARID1A, ERBB2, MET, FGFR1, CDK6, PIK3R3* and *PIK3CA* have also been reported but at a lower prevalence.[4 6]

The results of our study confirm the described mutational landscape of PACs with pathogenic mutations in *KRAS, TP53, SMAD4* and *CDKN2A* genes being the most prevalent. Additionally, mutations were identified in *BRCA1, BRCA2, KMT2C, PALB2* (up to 7% prevalence). What is however intriguing is the fact that KRAS mutations

Table 3 HRs (95% CIs) estimated by multivariate COX regression with respect to OS in (a) the entire cohort, (B) cohort A and (C) cohort T; results of the backwards selection models

Parameter	Category	N events/ N patients	HR	95% CI	P value
		A) Entire cohort			
n=166 patients					
Stage	Stage I-III	49/67	1 (Reference)		
	Stage IV	93/99	2.48	1.73 to 3.56	*<0.001*
KRAS/TP53 pathogenic mutations					*<0.001*
	None	30/40	1 (Reference)		
	Both KRAS&TP53	57/67	2.22	1.39 to 3.53	*<0.001*
	Only KRAS	42/44	2.84	1.74 to 4.66	*<0.001*
	Only TP53	13/15	1.67	0.87 to 3.23	0.13
		B) Cohort A			
n=138patients					
KRAS/TP53 pathogenic mutations					*<0.001*
	None	27/33	1(Reference)		
	Both KRAS&TP53	48/54	2.12	1.30 to 3.47	*0.003*
	Only KRAS	36/38	3.17	1.88 to 5.37	*<0.001*
	Only TP53	12/13	1.61	0.81 to 3.20	0.17
		C) Cohort T			
n=89 patients					
KRAS/TP53 pathogenic mutations					*0.002*
	None	18/22	1 (Reference)		
	Both KRAS and TP53	31/32	2.21	1.21 to 4.05	*0.010*
	Only KRAS	26/26	3.60	1.89 to 6.83	*<0.001*
	Only TP53	9/9	2.16	0.96 to 4.87	0.063

N, number.

could be identified in only 65.5% of our patient population. The Kirsten rat sarcoma viral oncogene homolog is found to be mutated in 80%–93% of PACs being the most frequent and the most early mutation during the oncogenesis procedure affecting multiple downstream signalling pathways, mainly RAF/MAPK/Mek/Erk, PI3K/Pdk1/AKT/mTOR and RalGDS/p38MAPK among others which can partially explain the futility in using an anti-EGFR targeted therapy.[7 8] Although technical issues can never be excluded when dealing with FFPE samples, it seems unlikely that the observed 65.5% of *KRAS* mutations among all tumours and 74.5% among mutated tumours is due to low assay sensitivity; for example, the 93% incidence of *KRAS* mutations in the TCGA data was obtained with lower sample coverage than we had here, while enriched mutation targeting with other methods concerned *KRAS* exons 2 and 3, as in our case. Furthermore, our FFPE samples were enriched in tumour cell DNA; hence, the general statement that PAC samples are often poor in tumour DNA[9] does not apply in our case. The observed lower *KRAS* mutation rates may be due to study sample bias, different genetic or ethnic characteristics or geography-related environmental factors, particularly dietary, according to a Spanish study[10] and possibly air-pollution parameters.[11] Still, it definitely raises a question regarding the most common pathogenic mutation of pancreatic cancer in Greek or southeast Mediterranean natives, similarly to reports from further geographical areas.[12 13]

As expected, patients carrying *KRAS* mutations were found to have worse OS and higher probability of death. The knowledge of *KRAS* mutational status in lung and colorectal cancer and its implications in the prognosis and therapy of these two neoplasms along with the high incidence of the particular mutations in pancreatic cancer has triggered a number of investigational efforts in PAC investigating the potential of *KRAS* either in the diagnostic algorithm or as a possible biomarker of prognostic and predictive significance.[14 15] In accordance with the published results, the presence of a detectable *KRAS* mutation is a strong factor correlating with dismal prognosis leading to shortened survival not being influenced by the administration of gemcitabine-based chemotherapy.[8] More recently, the hypothesis of detecting *KRAS* mutation in the peripheral blood of patients with PAC, as this could provide prognostic information with patients harbouring *KRAS* mutated clones having shortened OS is being investigated. The question that arises is whether the serial monitoring of *KRAS* mutational load with liquid biopsy techniques during therapy can provide real-time prognostic and predictive information. This remains to be answered in large scale trials.[8 16]

TP53 mutations were detected in 52.6% of the population ranking second among the most prevalent pathogenic mutations. When investigating the occurrence of both *KRAS* and *TP53* mutations, we observed them in 67 out 171 patients (39.2%). *TP53* is a known tumour suppressor gene activating response to cellular stress and DNA damage while stopping the cellular cycle process. Mutations in *TP53* are found in about 60%–70% of pancreatic cancers where it usually appears in the latest stages of pancreatic dysplasia.[17] In our study, patients harbouring both *KRAS* and *TP53* mutations had a higher probability of death. However, the adverse prognostic significance of *TP53* mutations has not been confirmed in pancreatic cancer. No significant association between *TP53* and patient OS could be established in a number of studies. Moreover, the co-expression of these mutations has been investigated and data support that they can co-occur in pancreatic cancer but are found in different neoplasia pathways. More recent research in animal models suggests that alterations in both *KRAS* and *TP53* can induce the onset of PACs indicating a possible pathogenetic role of this co-occurrence.[18]

BRCA1 and *BRCA2* genes are associated with both familial and sporadic pancreatic cancers. *BRCA1* mutations are identified in approximately 6% of pancreatic cancer cases indicative of an association with familial pancreatic cancer risk, whereas *BRCA2*, responsible for DNA double strand break repair, is found to be inactivated in about 7% of familial pancreatic cancers. Sporadic *BRCA* mutations are rather rare, although more research is needed as the association with familial cancer risk did not follow standardised criteria in some series.[19] Unfortunately, informative germline DNA data were available in only 70 patients in our series; thus, we were not able to assign an incidence of sporadic *BRCA* mutations with confidence. In our study, 13.5% of the patients harboured pathogenic mutation in one of the HR genes (*BRCA1, BRCA2, CHEK, PALB2, NBN* and *BAP10*) in their tumours, while in 8.8% mutations could be identified in *BRCA1* or *BRCA2* genes. In any case, alterations in HR component genes result in HR deficiency and there are data pointing towards improved outcomes for the carriers when cisplatin-based chemotherapy is administered or with the use of DNA intercalating agents.[19 20] Ongoing research is currently investigating the role of poly ADP ribose polymerase (PARP) inhibitors alone or in combination with cytotoxic regimens for patients with pancreatic cancer found to have HR deficiency.[21] *CDKN2A* mutations are associated with Familial Atypical Multiple Mole Melanoma Syndrome, an autosomal dominant inherited disorder which in some families correlates with significantly increased risk of PAC.[22] When investigating the relationship between HR genes and *CDKN2A*, only three patients were found to carry both HR and *CDKN2A* mutation with two patients carrying *BRCA* mutation as well. It seems that the incidence of both HR gene and *CDKN2A* mutations is mutually exclusive. Patients in whom HR, *CDKN2A* or only *BRCA1/2* mutations were identified fared worse outlining the adverse prognostic role of these mutations in OS as has already been described.

The median OS of the study population was 10.8 months during a 71.4-month follow-up. According to our results, patients who underwent a radical surgery on presentation had a significantly lower number of mutations. It is

probable that during time lapse and disease progression accumulating mutations may result in the aggressiveness seen when the disease is disseminated. Patients with higher numbers of pathogenic mutations had a higher risk of dying irrespective of therapy administered, reflecting the impact of genetic alterations on patient survival. Pancreatic cancer, according to latest published data, seems to be a malignancy where a sufficient amount of mutations can be identified.[23 24] Recently, with the advent of immunotherapy, tumour mutational load has been identified as a possible predictive biomarker irrespective of programmed death ligand 1 (PD-L1) expression.[25 26] Tumours with more neoantigens and high numbers of infiltrating lymphocytes may respond better to immunotherapy administration. In the case of pancreatic cancer, mutations can be identified, neoepitopes are present but there seems to be suppression of lymphocyte activation and neoantigen immunoediting thus rendering pancreatic cancer a neoplasm where immunotherapy cannot be applied for the time being.[27]

As a hypothesis-generating experiment, we sought to examine prognostic/predictive impact of mutations separately in 89 patients who received first-line targeted therapy with either an EGFR TKI or a PI3K/Akt/mTOR inhibitor versus 49 patients who received nab-paclitaxel-based cytotoxic chemotherapy only. In both subgroups of patients, the presence of pathogenic mutations and in particular *KRAS*, and combination of *KRAS* and *TP53* mutations were associated with higher probability of death. Of note, in the subgroup of patients who underwent EGFR/AKT/mTOR axis therapeutic modulation, a statistically significant association could be observed between the presence of *SMAD4* mutations and an increased probability of death. It has been proposed that *SMAD4* mutations are associated with poor prognosis and that protein (SMAD) deficiency may result in EGFR-enhanced expression and axis activation in PAC cell lines, probably unsuccessfully abrogated by anti-EGFR-targeted compounds in our study.[6 28] Regarding the cohort of patients who underwent cytotoxic chemotherapy consisting of gemcitabine plus nab-paclitaxel no specific correlation with any parameter could be established apart from the adverse impact of *KRAS* mutation and non-radical surgical intervention. According to results from the MPACT trial, nab-paclitaxel is an effective choice for patients with locally advanced or metastatic pancreatic cancer. To date, there have been no associations of nab-paclitaxel activity with specific mutations although emerging data suggest possible improved efficacy with the use of RAF-MEK-ERK inhibitors in combination with nab paclitaxel, to be further validated.[29 30]

In summary, we identified *KRAS, TP53, SMAD4* and *CDKN2A* as being the most prevalent mutations with the exception of an intriguingly lower incidence regarding *KRAS* mutants. HR gene mutations were found to be mutually exclusive with *CDKN2A* mutations. The coexistence of both *KRAS* and *TP53* mutation seems to adversely affect the outcome of patients whether treated with targeted therapy containing either an EGFR TKI or a PI3K/Akt/mTOR inhibitor or cytotoxic drugs. Limitations of our study are the small number of patients studied with even smaller subpopulations treated with different therapies as well as its retrospective nature. Still, the poor prognosis observed, correlated to late presentation, specific molecular mutations and high mutational load warrant prospective validating studies and research into the mechanistic pathophysiology of pancreatic tumours for more effective therapeutic targeting.

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Funding This study was supported by Celgene Corporation, Summit, NJ and Genesis Pharma S.A., Greece and by an internal Hellenic Cooperative Oncology Group (HeCOG) research grant (TR_3/19).

Competing interests VKa: Advisory Board of Amgen, Pfizer, Novartis, BI, Lilly, Astellas, Genesis-Pharma and Janssen. ES: Advisory Board of Merck, MSD, AstaZeneca, Roche, Amgen and Genesis. GF: Advisory Board of Pfizer, Sanofi and Roche. Honoraria from Astra-Zeneca. GP: Advisory Role: Roche, Honoraria: Roche, Speaker bureau: Roche, Grants: Amgen.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement All data relevant to the study are included in the article or uploaded as supplementary information.

REFERENCES

1. Ducreux M, Cuhna AS, Caramella C, *et al*. Cancer of the pancreas: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2015;26 Suppl 5(suppl 5):v56–68.

2. Cancer Genome Atlas Research Network. Electronic address aadhe, cancer genome atlas research N. integrated genomic characterization of pancreatic ductal adenocarcinoma. *Cancer Cell* 2017;32(2:185–203.
3. McGranahan N, Favero F, de Bruin EC, *et al*. Clonal status of actionable driver events and the timing of mutational processes in cancer evolution. *Sci Transl Med* 2015;7:283ra54.
4. Cicenas J, Kvederaviciute K, Meskinyte I, *et al*. Kras, TP53, CDKN2A, Smad4, BRCA1, and BRCA2 mutations in pancreatic cancer. *Cancers* 2017;9:42. 5.
5. Waddell N, Pajic M, Patch A-M, *et al*. Whole genomes redefine the mutational landscape of pancreatic cancer. *Nature* 2015;518:495–501.
6. Hayashi H, Kohno T, Ueno H, *et al*. Utility of assessing the number of mutated KRAS, CDKN2A, TP53, and Smad4 genes using a targeted deep sequencing assay as a prognostic biomarker for pancreatic cancer. *Pancreas* 2017;46:335–40.
7. Eser S, Schnieke A, Schneider G, *et al*. Oncogenic KRAS signalling in pancreatic cancer. *Br J Cancer* 2014;111:817–22.
8. Bournet B, Buscail C, Muscari F, *et al*. Targeting KRAS for diagnosis, prognosis, and treatment of pancreatic cancer: hopes and realities. *Eur J Cancer* 2016;54:75–83.
9. Dougan SK. The pancreatic cancer microenvironment. *Cancer J* 2017;23:321–5.
10. Morales E, Porta M, Vioque J, *et al*. Food and nutrient intakes and K-ras mutations in exocrine pancreatic cancer. *J Epidemiol Community Health* 2007;61:641–9.
11. Soliman AS, Lo A-C, Banerjee M, *et al*. Differences in K-Ras and p53 gene mutations among pancreatic adenocarcinomas associated with regional environmental pollution. *Carcinogenesis* 2007;28:1794–9.
12. Kwon MJ, Jeon JY, Park H-R, *et al*. Low frequency of KRAS mutation in pancreatic ductal adenocarcinomas in Korean patients and its prognostic value. *Pancreas* 2015;44:484–92.
13. Amaral NS, Resende V, Dos Santos JS, *et al*. Impact of ethnicity on somatic mutation rates of pancreatic adenocarcinoma. *In Vivo* 2018;32:1527–31.
14. Maus MKH, Grimminger PP, Mack PC, *et al*. Kras mutations in non-small-cell lung cancer and colorectal cancer: implications for EGFR-targeted therapies. *Lung Cancer* 2014;83:163–7.
15. Compagnone M, Chetaille B, Olschwang S. Kras mutation spectrum notably diverges between non-small cell lung and colorectal carcinomas. *J Thorac Oncol* 2012;7:773–4.
16. Allenson K, Castillo J, San Lucas FA, *et al*. High prevalence of mutant KRAS in circulating exosome-derived DNA from early-stage pancreatic cancer patients. *Ann Oncol* 2017;28:mdx004–7.
17. Olivier M, Hollstein M, Hainaut P. Tp53 mutations in human cancers: origins, consequences, and clinical use. *Cold Spring Harb Perspect Biol* 2010;2:a001008.
18. Lu L, Zeng J. Evaluation of K-Ras and p53 expression in pancreatic adenocarcinoma using the cancer genome atlas. *PLoS One* 2017;12:e0181532.
19. Zhen DB, Rabe KG, Gallinger S, *et al*. Brca1, BRCA2, PALB2, and CDKN2A mutations in familial pancreatic cancer: a PACGENE study. *Genet Med* 2015;17:569–77.
20. Teo MY, O'Reilly EM. Is it time to split strategies to treat homologous recombinant deficiency in pancreas cancer? *J Gastrointest Oncol* 2016;7:738–49.
21. Kamel D, Gray C, Walia JS, *et al*. Parp inhibitor drugs in the treatment of breast, ovarian, prostate and pancreatic cancers: an update of clinical trials. *Curr Drug Targets* 2018;19:21–37.
22. Cremin C, Howard S, Le L, *et al*. Cdkn2A founder mutation in pancreatic ductal adenocarcinoma patients without cutaneous features of familial atypical multiple mole melanoma (FAMMM) syndrome. *Hered Cancer Clin Pract* 2018;16:7.
23. Witkiewicz AK, McMillan EA, Balaji U, *et al*. Whole-Exome sequencing of pancreatic cancer defines genetic diversity and therapeutic targets. *Nat Commun* 2015;6:6744.
24. Grassi E, Durante S, Astolfi A, *et al*. Mutational burden of resectable pancreatic cancer, as determined by whole transcriptome and whole exome sequencing, predicts a poor prognosis. *Int J Oncol* 2018;52:1972–80.
25. Goodman AM, Kato S, Bazhenova L, *et al*. Tumor mutational burden as an independent predictor of response to immunotherapy in diverse cancers. *Mol Cancer Ther* 2017;16:2598–608.
26. Hellmann MD, Ciuleanu T-E, Pluzanski A, *et al*. Nivolumab plus ipilimumab in lung cancer with a high tumor mutational burden. *N Engl J Med* 2018;378:2093–104.
27. Guo S, Contratto M, Miller G, *et al*. Immunotherapy in pancreatic cancer: Unleash its potential through novel combinations. *World J Clin Oncol* 2017;8:230–40.
28. Blackford A, Serrano OK, Wolfgang CL, *et al*. Smad4 gene mutations are associated with poor prognosis in pancreatic cancer. *Clin Cancer Res* 2009;15:4674–9.
29. Goldstein D, Von Hoff DD, Chiorean EG, *et al*. Nomogram for predicting overall survival (OS) in patients (pts) treated with *nab* -paclitaxel (*nab* -P) plus gemcitabine (Gem) or Gem alone for metastatic pancreatic cancer (MPC). *JCO* 2017;35(15_suppl):4109.
30. Awasthi N, Monahan S, Stefaniak A, *et al*. Inhibition of the MEK/ERK pathway augments nab-paclitaxel-based chemotherapy effects in preclinical models of pancreatic cancer. *Oncotarget* 2018;9:5274–86.