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Research Article

Implementation of a Pancreatic Cancer Treatment Selection Program Based on a Real-time Biomarker Analysis in Available Biopsies

Rodriguez-Pascual J[1,2,*], Plaza JC[3], Alvarez-Gallego R[1,2], Morelli MP[1,4], Angulo B[3], Ugidos L[1,2], de Vicente E[2,5], Lopez de la Guardia U[2,6], Cubillo A[1,2], Hidalgo M[4,7]

[1]Centro Integral Oncológico Clara Campal (HM-CIOCC), Grupo HM Hospitales, Madrid, Spain
[2]CEU San Pablo University, Madrid, Spain
[3]Laboratorio de Dianas Terapéuticas (LDT), Grupo HM Hospitales, Madrid, Spain
[4]Centro Nacional de Investigaciones Oncológicas (CNIO), Madrid, Spain
[5]Division of Surgery, Hospital HM Sanchinarro, Grupo HM Hospitales, Madrid. Spain
[6]Division of Radiology, Hospital HM Sanchinarro, Grupo HM Hospitales, Madrid, Spain
[7]Division of Hematology and Oncology, Beth Israel Deaconess Medical Center, Boston, MA

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ABSTRACT

Background: Systemic chemotherapy is the mainstay for metastatic Pancreatic Ductal Adenocarcinoma (PDCA). There is a paucity of effective predictive markers of drug sensitivity or resistance in this setting, due in large part to marked difficulties in prospectively obtaining baseline tumor. In this study we describe all the clinical and histological limitations to implementing a real-time biomarker panel program for cancer in advanced PDCA patients.

Methods: A retrospective, non-interventional study was conducted using data from the medical records of eligible patients participating in an advanced PDCA treatment selection program based on a real-time biomarker analysis in available tumor tissue. Biomarker Panel (BP) Program was implemented in a single center from 2007 to 2016. To be eligible, patients were required to meet the following inclusion criteria: (i) have histologically-confirmed advanced PDCA, (ii) have started systemic therapy (5´fluouracil-based or gemcitabine-based chemotherapy at single agent or in combination) and (iii) aged ≥18 years at the time of diagnosis of advanced PDCA. A BP consists in a predefined set of 7 molecular targets, including: *KRAS* mutations, *EGFR* amplification (FISH), and Thymidylate Synthase (TS), Thymidine Phosphorylase (TP), Excision Repair Cross-Complementing 1 (ERCC.1), Topoisomerase I (Topo I), and SPARC expression by IHC. Patients treated as part of an ulterior phase II clinical trial were included.

Results: Between January 1st 2007 and January 1st 2017, 111 metastatic PDCA patients were identified as candidates. In 65 patients (58.6%) it was possible to implement a BP. A re-biopsy was performed in only 3 cases (2.7%) to obtain sufficient tumor for molecular analysis. In 31 (47.7%) patients it was feasible to study almost 5 of the 7 planned targets. In registered patients, poor performance status (13.5%) was the most frequent limitation to performing the BP. Other limitations were anatomical limitations to the biopsies (4.5%), incomplete biomarker data (4.5%) or clinical deterioration during procedures (2.7%).

Conclusion: PDCA treatment based on real-time biomarker analysis in available biopsies presents significant limitations due to patient deterioration and sample processing. New approaches are necessary to optimize results in real-time targeted therapy studies.

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Corresponding Author: *Jesus Rodriguez-Pascual, Centro Integral Oncológico Clara Campal (HM-CIOCC)Oña 10, 28050, Madrid, Spain*

INTRODUCTION:

Pancreatic ductal adenocarcinoma (PDCA) is one of the most lethal cancers in humans. A recent publication estimates an incidence of about 9000 new cases in the US[1-2]. Approximately 80% of patients present with locally advanced or metastatic disease, and because pancreatic cancer remains extremely resistant to standard cytotoxic chemotherapies, median survival after diagnosis is only approximately 6 to 8 months[3].

Systemic chemotherapy is the mainstay for PDCA. In recent years, a few novel agents have demonstrated promising clinical activity and overall survival advantage in this setting, both Gemcitabine-based chemotherapy with Erlotibin[4], Capecitabine[5] and Nab-Paclitaxel[6] or 5´Fluouracil (5´FU) based chemotherapy with Leucovorin, Oxaliplatin and Irinotecan (FOLFIRINOX)[7]. However, none of these chemotherapy combinations have been compared with each other. On the other hand, Nanoliposomal Irinotecan (Nal-Iri) plus 5´FU has been incorporated as a new treatment option in second line chemotherapy[8].

There is a paucity of effective predictive markers of drug sensitivity or resistance due, in large part, to difficulties in prospectively obtaining baseline tumor tissue in patients with PDCA or attributable to inconsistent clinical results[9-14]. In the Centro Integral Oncologico Clara Campal (CIOCC) Comprehensive Cancer Center program of personalized treatment for PDCA reported in this article, patients were invited to participate in a novel program to evaluate a predefined panel of 7 biomarkers [see Table 1] in available tumor tissue to elucidate the most appropriate chemotherapy combo. Results were evaluated by medical oncologists to decide the most convenient therapeutic approach among all the standard chemotherapy options.

In this study, we further evaluate the feasibility of real-time biomarker-tailored treatment implementation describing all the clinical and histological limitations recorded in this clinical program. For this purpose, we recorded the impediments to participation in the entire cohort of potential candidates and, in enrolled patients, clinical and histological limitations to guiding treatment using our Biomarker Panel (BP) [see Figure 1].

MATERIALS AND METHODS

Study Design

This was a retrospective non-interventional study to test the feasibility of implementing a real-time biomarker panel (BP) program in treatment selection of standard chemotherapy for advanced PDCA patients. The specific study objectives were 1) to determine the proportion of patients in which the panel is performed, 2) to record the impediments to the BP implementation. To be eligible in the program, patients were required to meet the following inclusion criteria: (i) have histologically-confirmed locally advanced or unresectable adenocarcinoma of the pancreas, (ii) to have started systemic therapy (5´fluouracil-based or gemcitabine-based chemotherapy at single agent or in combination) and (iii) aged ≥18 years at the time of diagnosis of adenocarcinoma of the pancreas. All the potential candidates were invited to participate in the program, considering the available chemotherapy options at the beginning of the study program and the most attractive potential biomarkers at this time: Oxaliplatin, Irinotecan, 5´Fluouracil, Gemcitabine, Nab-Paclitaxel and Erlotinib. For this purpose, a Biomarker Panel (BP) was composed of Kirsten Rat Sarcoma Viral Oncogene (*KRAS)* mutation and Epidermal Growth Factor Receptor (*EGFR*) amplification[13] for Erlotinib utilization and *Thymidylate Synthase* (TS)[9], *Thymidine Phosphorylase* (TP)[10], *Excision Repair Cross-Complementing 1* (ERCC-1)[11], *Topoisomerase I* (Topo I)[12], and *Secreted Protein Acidic and Rich in Cysteine* (SPARC) expression[14] for 5´Fluouracil, Capecitabine, Oxaliplatin, Irininotecan and Nab-Paclitaxel use respectively [see Figure 1]. PDCA participating patients between January 2007 and January 2016 at CIOCC were registered for the study. Patients recruited as part of a phase II trial developed to test the clinical effects of using this PB in treatment selection of standard chemotherapy for advanced PDCA (clinicaltrials.gov Identifier: NCT01394120) were included.

Tissue handling and laboratory studies

Pancreatic tissue was obtained from resection specimens and inspected macroscopically and by means of frozen sections to select cancer-containing tissue. Sections from paraffin-embedded tissues were cut into silane-treated slides and left to dry at 65ºC for 2 hours, an EDTA buffer CC1 for 36 minutes for ERCC1 and TS, 44 minutes for SPARC and 20 minutes for TP in the Benchmark ULTRA Antigen retrieval for Topo I was done using citrate buffer CC2 for 8 minutes in the Benchmark ULTRA. All tissues were immunostained using ERCC1 monoclonal D-10 antibody clone, monoclonal anti-Thymidylate Synthase antibody cocktail 106/4H4B1 clones, monoclonal Anti-Topoisomerase I, monoclonal anti-Osteonectin/SPARC clone ON 1-1, monoclonal antibody TP clone PD-ECGF ab-1. The antibody incubation was carried out for 60 minutes for ERCC1 and TS, 32 minutes for SPARC and TP, and 16 minutes for Topo I, all at 37ºC temperature. Sections were counterstained with hematoxylin for 8 minutes. EGFR FISH analysis was performed using the standard method with the dual-color EGFR SpectrumOrange/CEP7 SpectrumGreen probe (Vysis, Downers Grove, IL) and paraffin pretreatment reagent kit (Vysis, Inc, Downers Grove, IL). In brief, paraffin sections were incubated overnight at 56ºC and deparaffinized in xylol and rehydrated in ethanol. Sections were digested with protease K (0.5 mg/mL) at 37°C for 28 minutes. The slides and the probes were denatured at 80°C for 15 minutes before hybridization. Slides were hybridized overnight at 37°C and washed in "stringent wash buffer" at 65°C for 10 minutes. Nuclei were counterstained with haematoxylin II. Exon 2 codon 12 and 13 mutations and exon 3 codon 61 of the KRAS gene were assessed using the Real-time PCR-based as previously described[15].

RESULTS

Patients' characteristics.

Between January 2007 and January 2017, one hundred and eleven (111) cases were identified. Table 2 showed the principal patients´ characteristics. The age of patients screened ranged from 39 to 81 years old (median 65) with an equivalent male/female ratio. Performance status was ECOG ≤1 in more than 95% of cases. 90 patients (81.1%) had undergone a previous pancreatectomy. The majority of cases presented one or two different organs affected.

Biomarker Panel Feasibility.

Table 3 showed our feasibility study results. Of the 41 patients who did not enroll in the Biomarker Program, the majority were due to poor performance status (13.5%). In good-performance status patients, the main limitation to participating in the program was an anatomical impediment to performing a good quality tumor tissue (4.5%). Only in three cases (2.7%), the biopsy was repeated to obtain sufficient tumor tissue for molecular analysis and in all of these the new biopsy obtained good quality tissue. A BP was applied in 65 cases (58.6%). BP information was irrelevant or not useful (mainly due to the absence of a theoretical responsive drug or an incomplete biomarker panel) in 15 cases (13.5%).

Table 4 shows the BP results. The main histological source to perform BP was a pancreatectomy (67.7%). In 12 of the 44 pancreatectomy specimens (27.3%), patients were treated with neoadjuvant radio and/or chemotherapy before surgical primary resection. ERCC-1 and TS were the most frequently determined biomarkers, while Topo I was the least frequently performed biomarker. In 31 of the 66 cases (47%) it was possible to make at least 5 of the 7 planned target determinations per panel.

Inclusion of the prospective phase II trial was stopped due to poor recruitment. Between September 2011 and February 2014, 33 patients were recruited from 4 hospitals in Spain. Ten (32%) patients were ineligible because of failure to meet selection criteria.

Figure 1.- Consort Diagram. [PDCA - Pancreatic Ductal Adenocarcinoma, BP – Biomarker Panel]

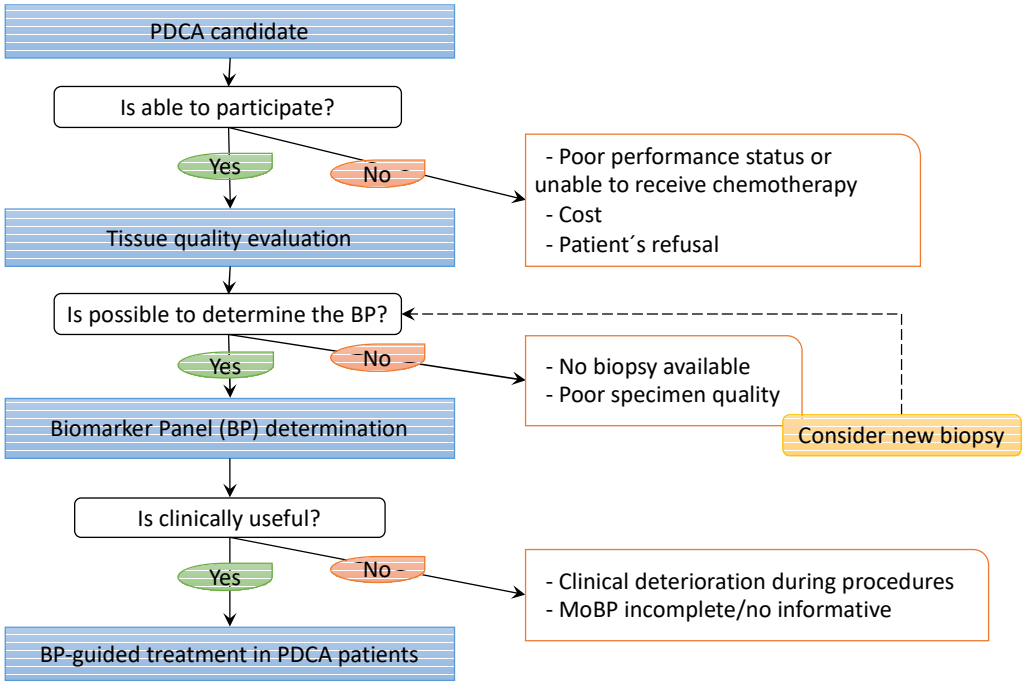

Table 1.- Predictive biomarker panel in pancreatic cancer patients and chemotherapy options in the study program. [IHQ – immunohistochemistry, PCR - Protein Chain Reaction, FISH – fluorescence in situ hybridization]

Biomarker	Drug	Technique	Potential responder	Potential nonresponder
Excission Repair Cross-Complementing 1 (ERCC-1)	Oxaliplatin	Tumor staining IHQ	Semiquantitative score	Semiquantitative score
Topoisomerase I (Topo I)	Irinotecan	Tumor staining IHQ	Semiquantitative score	Semiquantitative score
Thymidylate Synthase (TS)	5´Fluouracil	Tumor staining IHQ	Semiquantitative score	Semiquantitative score
Thymidine Phosphorylase (TP)	Capecitabine	Tumor staining IHQ	Semiquantitative score	Semiquantitative score
Secreted Protein Acidic and Rich in Cysteine (SPARC)	Nab-paclitaxel	Tumor and stromal staining IHQ	Semiquantitative score	Semiquantitative score
Kirsten Rat Sarcoma Viral Oncogene Homolog (KRAS)	Erlotinib	PCR	No mutation	Mutation
Epidermal Growth Factor Receptor (EGFR)	Erlotinib	FISH	Amplification	No amplification

Table 2.- Patient´s characteristics. [ECOG – Eastern Cooperative Oncology Group, BP – Biomarker Panel]

		Patients with BP (n=65)	Patients without BP (n=46)	All patients (n=111)
Age				
	≤ 50	6 (9,2%)	2 (4,4%)	7 (6,3%)
	51-60	10 (15,4%)	5 (10,9%)	15 (13,5%)
	61-70	31 (47,7%)	22 (47,8%)	53 (47,8%)
	>70	18 (27,7%)	17 (36,9%)	36 (32,4%)
Gender				
	Male	37 (56,9%)	23 (50,0%)	59 (53,2%)
	Female	29 (43,1%)	23 (50,0%)	52 (46,8%)
ECOG				
	0	15 (23,1%)	7 (15,2%)	22 (19,8%)
	1	49 (75,4%)	37 (80,4%)	86 (77,5%)
	2	1 (1,5%)	2 (4,4%)	3 (2,7%)
Previous pancreatectomy				
	Yes	54 (83,1%)	36 (78,3%)	90 (81,1%)
	No	11 (16,9%)	10 (21,7%)	21 (18,9%)
Metastasis: no. organs affected				
	1	37 (56,9%)	23 (50,0%)	61 (55,0%)
	2	23 (35,4%)	22 (47,9%)	46 (41,4%)
	≥3	5 (7,7%)	1 (2,1%)	4 (3,6%)

Table 3.- Feasibility study results. [PDCA – Pancreatic Ductal Adenocarcinoma]

PDCA candidates					111 (100%)
	Unable to participate				41 (36,9%)
		Poor performance status			15 (13,5%)
		Patient´s refusal			2 (1,8%)
		Not registtered (missing data)			17 (15,3%)
	Able to participate				70 (63,1%)
		Anatomical limitation to biopsies			5 (4,5%)
		Poor quality tissue and re-biopsy			3 (2,7%)
		Good quality tissue			62 (55,6%)
		Molecular Biomarker Panel*			65 (58,6%)
			Clinically not useful		15 (13,5%)
				PS deterioration during procedures	3 (2,7%)
				MoBP incomplete/uninformative	5 (4,5%)
				Not registered (missing data)	7 (6,3%)
		Clinically useful	Clinically useful		50 (45,1%)

DISCUSSION

Standard chemotherapy for the vast majority of advanced-stage solid tumors is ineffective in a very high proportion of cases: this is especially remarkable in advanced pancreatic cancer. The development of tools to distinguish potential responders from non-responders in this setting would improve our results: optimizing use of the same drugs leading to better therapeutic expectations in responders and avoiding unnecessary side effects in non-responders is the cornerstone of precision medicine. However, despite a large number of publications, none of these biomarkers is part of routine clinical practice. The lack of indicators for targeted therapy has multiple explanations: limitations in obtaining optimum tissue samples, multiple biomarker analysis methods, high diversity of potential biomarkers for a single drug, use of chemotherapy combinations that can modify biomarker prediction capacity and the lack of prospective clinical studies that demonstrate feasibility and clinical utility.

Our study reflects the difficulty of performing real-time use of biomarkers based on tumor biopsies in PDCA. The basic principle of our study is the possibility of determining the potential resistance or sensitivity to each drug in each person individually on the basis of information obtained from a panel of biomarkers. Our key goal in this work was to demonstrate that the real-time biomarker panel (BP) warrants the launching of a clinical development program in advanced pancreatic cancer.

An obvious limitation during the program design was caused by the absence of biomarkers of proven clinical use for each available drug. We also did not know if the potential predictive value of a biomarker postulated for a chemotherapeutic agent in a specific tumor could be extrapolated to other tumors: SPARC determination, for example, only presents studies in pancreatic cancer, others like TP or TS determination present extrapolated data of clinical use in colorectal cancer patients. In addition, the scientific validity in choosing biomarkers for each chemotherapy option is highly variable; in fact, during the program implementation, none of these biomarkers have been established in routine clinical practice due to subsequent negative results or a lack of further studies.

The quality of the histological samples has also been of great importance. A significant proportion of surgical pancreatectomy samples presenting abundant histological material have been treated with preoperative chemo and/or radiotherapy. This fact could have notably interfered in the biomarker analysis. On the other hand, the location of non-hepatic patterns of dissemination (retroperitoneal nodes, peritoneal implants…) decrease the probability of obtaining high quality histological samples. In certain cases, some biomarkers (such as Topoisomerase I), were not available during part of the study. For this reason, some patients could not be assigned as potentially sensitive or resistant to Irinotecan

Physical worsening of PDCA patients was the most relevant clinical problem during the implementation of our BP program. In a phase II Biomarker-Integrated Approaches of Targeted Therapy for Lung Cancer Elimination (BATTLE) program of personalized medicine, Non-Small Cell Lung Cancer (NSCLC) patients were prospectively biopsied and, based on tumor markers, adaptive randomization was used [16] to assign patients to the treatment with the greatest potential benefit based on current data [17]. In this trial, Eighty-six patients (25.2%) of the series could not be randomly assigned because of intercurrent illnesses, worsening overall conditions, conditions preventing a biopsy or choosing an alternative treatment. In PDCA patients, similar to those of NSCLC, clinical deterioration has to be taken into account for the design of clinical trials.

Unfortunately, we were unable to demonstrate the utility of the BP in the prospective study due to poor recruitment. This is a common problem in clinical trials for pancreatic cancer; moreover, patient enrollment in our phase II trial presented the added difficulty of having to obtain sufficient tissue samples. After the first 18 patients were enrolled no differences were found in response or survival advantage and the study trial management group decided to halt recruitment.

To our knowledge, ours is the first study to test the feasibility of implementing a real-time predictive biomarker panel in pancreatic cancer patients treated with standard systemic chemotherapy. Further studies

are mandatory to enhance the applicability of this approach and to confirm our results.

BIBLIOGRAPHY

1) Siegel, R. L., Miller, K. D. & Jemal, A. Cancer statistics, 2018. CA. Cancer J. Clin. 68, 7–30 (2018).
2) Bray, F. et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA. Cancer J. Clin. 68, 394–424 (2018).
3) Ilic, M. & Ilic, I. Epidemiology of pancreatic cancer. World J. Gastroenterol. 22, 9694–9705 (2016).
4) Moore, M. 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. Off. J. Am. Soc. Clin. Oncol. 25, 1960–1966 (2007).
5) Cunningham, D. et al. Phase III randomized comparison of gemcitabine versus gemcitabine plus capecitabine in patients with advanced pancreatic cancer. J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol. 27, 5513–5518 (2009).
6) Von Hoff, D. D. et al. Increased survival in pancreatic cancer with nab-paclitaxel plus gemcitabine. N. Engl. J. Med. 369, 1691–1703 (2013).
7) Conroy, T. et al. FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. N. Engl. J. Med. 364, 1817–1825 (2011).
8) Wang-Gillam, A. et al. Nanoliposomal irinotecan with fluorouracil and folinic acid in metastatic pancreatic cancer after previous gemcitabine-based therapy (NAPOLI-1): a global, randomised, open-label, phase 3 trial. Lancet Lond. Engl. 387, 545–557 (2016).
9) Leichman, C. G. et al. Quantitation of intratumoral thymidylate synthase expression predicts for disseminated colorectal cancer response and resistance to protracted-infusion fluorouracil and weekly leucovorin. J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol. 15, 3223–3229 (1997).
10) Meropol, N. J. et al. Thymidine phosphorylase expression is associated with response to capecitabine plus irinotecan in patients with metastatic colorectal cancer. J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol. 24, 4069–4077 (2006).
11) Kim, S.-H. et al. Prognostic value of ERCC1, thymidylate synthase, and glutathione S-transferase pi for 5-FU/oxaliplatin chemotherapy in advanced colorectal cancer. Am. J. Clin. Oncol. 32, 38–43 (2009).
12) Braun, M. S. et al. Predictive biomarkers of chemotherapy efficacy in colorectal cancer: results from the UK MRC FOCUS trial. J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol. 26, 2690–2698 (2008).
13) da Cunha Santos, G. et al. Molecular predictors of outcome in a phase 3 study of gemcitabine and erlotinib therapy in patients with advanced pancreatic cancer: National Cancer Institute of Canada Clinical Trials Group Study PA.3. Cancer 116, 5599–5607 (2010).
14) Infante, J. R. et al. Peritumoral fibroblast SPARC expression and patient outcome with resectable pancreatic adenocarcinoma. J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol. 25, 319–325 (2007).
15) Angulo, B. et al. A commercial real-time PCR kit provides greater sensitivity than direct sequencing to detect KRAS mutations: a morphology-based approach in colorectal carcinoma. J. Mol. Diagn. JMD 12, 292–299 (2010).
16) Zhou, X., Liu, S., Kim, E. S., Herbst, R. S. & Lee, J. J. Bayesian adaptive design for targeted therapy development in lung cancer--a step toward personalized medicine. Clin. Trials Lond. Engl. 5, 181–193 (2008).
17) Kim, E. S. et al. The BATTLE trial: personalizing therapy for lung cancer. Cancer Discov. 1, 44–53 (2011).

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