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Final Degree Project

Impact of BRCA1/2 somatic mutations in patients with pancreatic cancer in Girona, a population-based study

Research Project

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Acknowledgement

"Vita brevis, Ars longa, Occasio praeceps, experimentum periculosum, ludicium difficile" Hippocrates

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INDEX

1. ABSTRACT

Background and Aims — Pancreatic cancer remains one of the most lethal of malignancies and entails major health burden. The highly heterozygous nature of pancreatic cancer is partially responsible for its late diagnosis, therapeutic ineffectiveness and resistance. BRCA-1 and BRCA-2 gene mutations (which prevent DNA repair by homologous recombination) have been widely studied in patients with hereditary syndromes as predisposing factors for developing Pancreatic Cancer, as well as possible predictive factors for response to platinum-based chemotherapy. Even so, few information concerning BRCA-1 and BRCA-2 somatic mutations in Pancreatic Cancer is available. Knowledge about BRCA1/2 somatic mutations in Pancreatic Cancer may allow us to identify subgroups of patients with distinct biologic and therapeutic outcome. The aim of our study is to analyse the prevalence of Pancreatic Adenocarcinoma with BRCA somatic mutation in Girona's province; to explore if there are significant differences in survival at 5 years between pancreatic cancer patients with or without BRCA1/2 somatic mutations; and, finally, to evaluate if patients treated with platinum-based chemotherapy compared with patients that did not receive platinum agents related with the presence or absence of BRCA1/2 somatic mutations , had an improvement of probability of overall survival at 36 months.

Methods — We present a retrospective cohort trial that will enrol patients diagnosed of pancreatic cancer registered on the Girona's Cancer Registry; 1407 patients will be enrolled from 1994 to 2012 using a non-probabilistic sampling. The mutation analyses will be done through PCR techniques and Next Generation Sequencing in all the available tumour samples. We will estimate the prevalence of BRCA1/2 somatic mutations in Girona's province using a descriptive-univariate analysis. The 5-year survival rate will be studied on patients with pancreatic cancer harbouring BRCA1/2 mutations (n=191) and patients that do not present the tumour mutation (n=191). Besides, we will analyse the probability of survival at 36 months in Pancreatic Cancer patients harbouring BRCA1/2 somatic mutations and patients that do not present the mutation, that had been treated with platinum-based chemotherapy (n=190) or with non-platinum (n=190). The primary outcome measure will be the overall survival which will be analysed through bivariate and multivariate analyses.

Keywords: Pancreatic cancer, BRCA1/2 somatic mutations, prevalence, platinum-based chemotherapy, 5-year survival rate, overall survival rate at 36 months.

Resumen y objetivos — El cáncer de páncreas sigue siendo actualmente uno de los cánceres más letales del mundo. Su mal pronóstico se debe en parte a su naturaleza heterogénea, su diagnóstico tardío debido a la ausencia de métodos de screening eficaces, a la pobre eficacia de los tratamientos disponibles y a la existencia de resistencias a dichos tratamientos. Las mutaciones en los genes BRCA-1 y BRCA-2 (que impiden la adecuada reparación del ADN a través de la recombinación homóloga) han sido ampliamente estudiadas en pacientes con síndromes hereditarios, como factores predisponentes al desarrollo de cáncer de páncreas, además de posibles predictivos a la respuesta a la quimioterapia basada en platinos. Aun así, se dispone de poca información sobre las mutaciones somáticas de BRCA-1 y BRCA-2. En nuestro estudio planteamos los siguientes objetivos para mejorar el conocimiento disponible del cáncer de páncreas: el estudio de la prevalencia de las mutaciones somáticas BRCA1/2 en la provincia de Girona; el análisis de la supervivencia en pacientes con cáncer de páncreas que presentan las mutaciones BRCA1/2 comparado con los que no presentan las mutaciones; y finalmente el análisis de la supervivencia en pacientes tratados o no con quimioterapia basada en platinos según la presencia o la ausencia de las mutaciones somáticas BRCA1/2.

Métodos — Presentamos un estudio de cohortes retrospectivo que incluirá pacientes diagnosticados con cáncer de páncreas y que estén registrados en el Registro de Cáncer de Girona; 1407 pacientes diagnosticados entre 1994 y 2012 serán seleccionados usando un muestreo no probabilístico. El análisis de las mutaciones se realizará en toda muestra disponible a través de técnicas de PCR y de Secuenciación de Nueva Generación. Estimaremos la prevalencia de las mutaciones somáticas BRCA1/2 en la provincia de Girona usando un análisis descriptivo. La tasa de supervivencia a los 5 años se estudiará en los pacientes con cáncer de páncreas que presentan las mutaciones BRCA1/2 (n= 191) y los que no presentan las mutaciones (n= 191). Además, se examinará la probabilidad de supervivencia a los 36 meses de los pacientes que presenten o no la mutación BRCA1/2 pero que hayan sido tratados con quimioterapia basada en platino (n= 190) o no basada en platino (n= 190).

Palabras clave: Cáncer de Páncreas, mutaciones somáticas BRCA1/2, prevalencia, quimioterapia basada en platinos, tasa de supervivencia a los 5 años, tasa de supervivencia a los 36 meses

Resum i objectius ─ El càncer de pàncrees segueix essent actualment un dels càncers més letals del mon, que suposa una important càrrega per a la salut. El seu mal pronòstic es deu en major part a la seva naturalesa heterogènia, el diagnòstic tardiu, al seu tractament ineficaç i a la presència de resistències a dit tractament. Les mutacions dels gens BRCA-1 i BRCA-2 (que impedeixen la reparació del ADN que es fa normalment a través de la recombinació homòloga) han sigut àmpliament estudiades en pacients amb síndromes hereditaris, fent d'ells un factor predisposant al desenvolupament de càncer de pàncrees, a més a més de ser un possible factor predictiu a la resposta als platins. Tot i així, es disposa de poca informació sobre les mutacions BRCA-1 i BRCA-2. Degut a la escassa informació sobre el càncer de pàncrees i les mutacions somàtiques BRCA1/2, es planteja aquest estudi amb els següents objectius: calcular la prevalença de les mutacions somàtiques BRCA1/2 en pacients amb càncer de pàncrees; avaluar la supervivència en pacients que presenten les mutacions comparada amb pacients que no les presenten; i finalment, avaluar la supervivència dels pacients amb càncer de pàncrees tractats amb quimioteràpia basada en platins o no, segons la presència o la absència de les mutacions.

Mètodes ─ Presentem un estudi de cohorts retrospectiu que involucrarà a pacients diagnosticats amb càncer de pàncrees i que estiguin registrats al Registre de Càncer de Girona; 1407 pacients seran seleccionats utilitzant un mostreig no probabilístic durant el període de temps de 1994 a 2012. L'anàlisi de les mutacions es farà en tota mostra disponible a través de tècniques de PCR i de Seqüenciació de pròxima Generació. Estimarem la prevalença de les mutacions somàtiques BRCA1/2 en la província de Girona utilitzant un anàlisi descriptiu. La taxa de supervivència als 5 anys s'estudiarà en pacients amb càncer de pàncrees que presentin les mutacions BRCA1/2 (n= 191) i pacients que no tinguin les mutacions (n= 191). A més a més, s'examinarà la probabilitat de supervivència als 36 mesos en pacients que presentin o no les mutacions BRCA1/2 però que hagin sigut tractats amb platins (n= 190) o que hagin rebut un tractament sense platins (n= 190).

Paraules clau: Càncer de pàncrees, mutacions somàtiques BRCA1/2, prevalença, quimioteràpia basada en platins, taxa de supervivència als 5 anys, taxa de supervivència als 36 mesos.
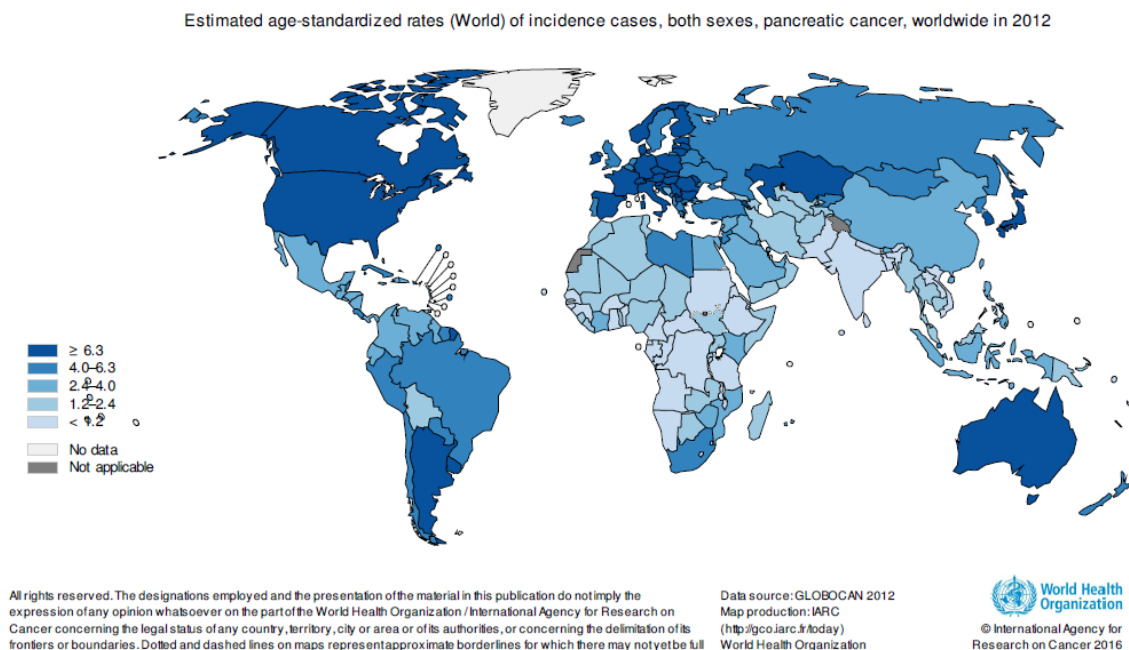
2. INTRODUCTION

2.1 Epidemiology

Pancreatic cancer (PC) is one of the most lethal gastrointestinal disease as it represents the 4th cause of death by cancer and causes 227 000 deaths per year worldwide. Current studies estimate that it will be the second cause of death by cancer in 2020 (1).

Even though it involves only 3% of all new diagnosed cancers, it entails a huge loss of life expectancy. Survival of patients with metastatic PC goes down to 4.6 months, with modest benefits of non-surgical treatments, compared to 15.1 years for the general population which means a 98% loss of healthy life due to PC (2). Overall its 5-year disease-free survival rate is between 1% and 2%, which is the lowest of the 20 most common adult cancer types (3,4). So, PC is an aggressive cancer that is diagnosed in 85% of patients as disseminated disease.

Its incidence and mortality rates have been increasing year by year throughout the world, which demonstrates that it is becoming an important health problem (5). Data from GLOBOCAN 2012 shown on Figure 1 represent its worldwide incidence estimated by age.

Estimated age-standardized rates (World) of incidence cases, both sexes, pancreatic cancer, worldwide in 2012

≥ 6.3
4.0–6.3
2.4–4.0
1.2–2.4
< 1.2
No data
Not applicable

Data source: GLOBOCAN 2012
Map production: IARC
(http://gco.iarc.fr/today)
World Health Organization

World Health Organization
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<u>Figure 1</u>: Estimated age-standardized rates (World) of incidence cases, both sexes, pancreatic cancer, worldwide in 2012.

Incidence ranges from 1 to 10 cases per 100 000 inhabitants (6). In 2012, incidence of PC was 8-10 cases per 100 000 inhabitants per year and in Spain there were 6367 new cases diagnosed which represents 3% of cancers diagnosed that year (7,8). This incidence increases with age, starting from 45 years old with a peak incidence between 60 and 80 years old. It is more

frequent in men than in women (ratio 1,3:1) (9). This has been reflected on a worldwide basis, with approximately 120 000 yearly male deaths compared to 107 000 female deaths (10). Figure 2 offers a view of a dual bar chart comparing incidence and mortality by the estimated age-standardized rates in both sexes in 2012 and in Spain.
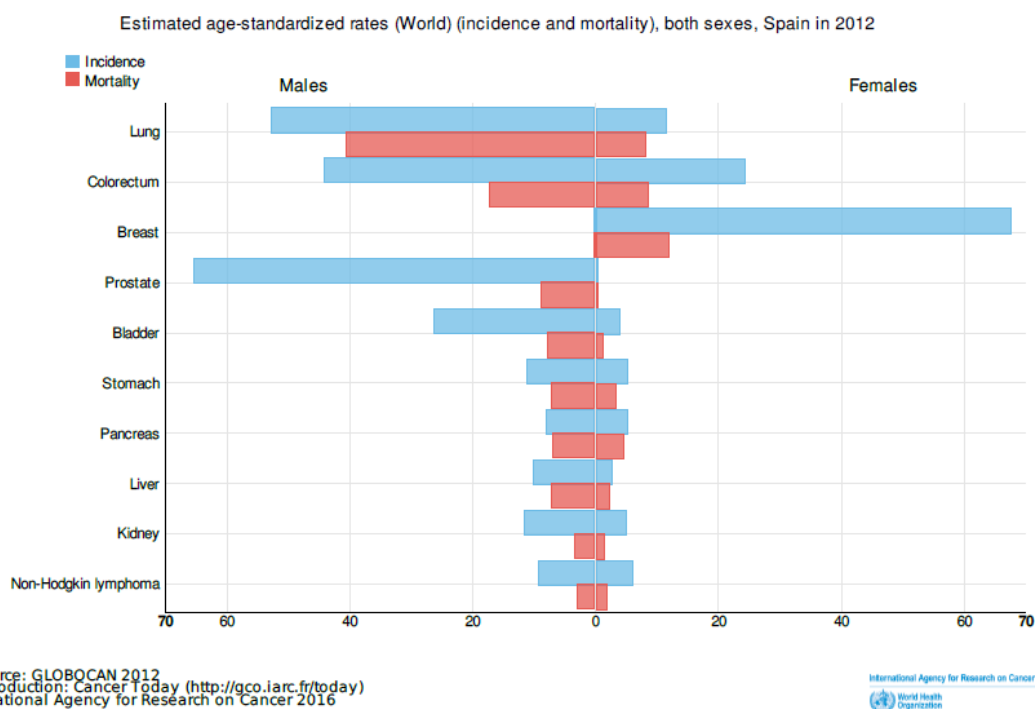
Figure 2: Estimated age-standardized rate (World) incidence and mortality, both sexes, Spain in 2012.

Focusing on data from the Cancer Registry of Girona, PC incidence between 2010 and 2012 was about 2.5% in men and 2.7% in women which represents the 7th cancer in men (4.47% of all cancers) and the 4th in women (6.48%). Incidence in women may grow in forthcoming years because of the increasing tobacco use in this population.

Mortality rate approaches 100%. It is surprising how patients with other malignant gastrointestinal tumours have had an increasing on their survival rate in the past thirty years, whereas this cannot be extrapolated to pancreatic cancer as this rate remains the same despite the last century's therapeutic innovations (1). Only 15-20% of PC cases can be treated by its unique curative treatment: surgery, achieving with it a 5-year survival rate of 20% (9,11). Data extracted from GLOBOCAN 2012 underline that mortality rates are also high in developed countries and do not differ from incidence rates, which is alarming. In Spain, in 2012, its mortality rate was of 5.5 whereas incidence rate was of 6.3. Figure 3 shows the mortality rate worldwide in both sexes.

Estimated age-standardized rates (World) of deaths, both sexes, pancreatic cancer, worldwide in 2012

≥ 6.2
3.9–6.2
2.4–3.9
1.2–2.4
< 1.2
No data
Not applicable

Data source: GLOBOCAN 2012
Map production: IARC
(http://gco.iarc.fr/today)
World Health Organization

World Health Organization
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Figure 3: Estimated age-standardized rates (World) of deaths, both sexes, pancreatic cancer, worldwide in 2012.

The 5-year relative survival rate between 2000 and 2007 in Girona was of 5.4% in men and 1.5% in women, and the global survival rate in this same period was 4.6% in men and 1.4% in women.

2.2 Risk factors

The origin of all cancers is genetic. A normal cell, after one or several linked mutations that confer aggressive proliferative proprieties, is transformed into a tumour stem cell and lately into a malignant tumour. This implies that cancer stem cell could be developed from germinal mutations as well as from somatic mutations. Over 80% of PCs are caused by sporadic mutations with only 10% of them being hereditary cancers (12).

Until now, there is no useful screening method for PC detection in the general population, as it develops silently and does not show any specific clinical syndrome. It is a priority to find the risk factors producing PC in order to define population subgroups that could benefit from prevention strategies and/or a more intensive follow-up. PC risk factors vary according to the country and involve demographic, genetic, medical conditions, environmental and lifestyle factors (13). Table 1, adapted from a review of meta-analytical studies (14) summarizes the most important risk and protective factors that are linked with PC.

Table 1: Summary of the associations between risk factors and pancreatic cancer reported in published 86 meta- and 34 pooled-analyses (14).

Risk Factors (RF)	Degree of association	Strength of association	Number of published meta/ pooled-analyses
History of chronic pancreatitis	High risk	++	1/1
History of idiopathic thrombosis		0	1/0
Tobacco smoking	Moderate risk	++	3/5
Diabetes mellitus		++	7/7
Genetic factors (family history) *		++	1/1
Metabolic syndrome		0	2/0
Obesity	Low risk	++	5/5
Height		++	1/3
Non-O blood group		++	2/1
Heavy alcohol intake		++	1/3
Elevated sugar intake		0	1/0
Processed meat		0	1/0
Helicobacter Pylori infection		+	4/0
Hepatitis B virus infection		++	5/0
Hepatitis C virus infection		0	2/0
Coffee consumption	No association	++	2/1
Tea consumption		++	1/1
Aspirin / NSAIDS use		++	4/1
Statin use		++	2/0
Environmental tobacco exposure		0	1/0

Allergy	Low to moderate protection	++	1/1
Metformine use for diabetics		+	4/0
High adiponectin level		+	0/1
Intense physical activity		+	2/0
High fruit consumption		+	21
High dietary folate intake		+	3/1
High vegetables consumption		0	1/1

(++) strong evidence; (+) moderate evidence; (0) poor evidence (14)

Regarding environmental and lifestyle factors, tobacco is the strongest risk factor related with pancreatic cancer as it increases in 75% its risk and even with the cessation of smoking, PC risk could last for a minimum of 10 years (15).

Research in germline mutation has also been made in the last decade and some mutations have been associated to PC, often as a part of a familial cancer syndrome. Table 2 exposes some of the most important hereditary diseases related to PC with their respective mutations (7,11,16,17). Germline mutation BRCA-1 and BRCA-2 were carried by 10-15% of families with a hereditary disease and are the most common mutations in theses hereditary syndromes. They increase the risk of PC 3.5 to 10 times compared with the general population. Even so, germline mutations entail a small amount of PC. These mutated genes cause dysregulation on the cycle cell which could lead to invasion and metastasis.

Table 2: Inherited disorders with increased risk of pancreatic cancer.

Hereditary Disease	Genes	Chromosome	Risk Ratio
Familial Atypical Mole-Multiple Melanoma	CDKN2A	9	3,5-10
Hereditary Pancreatitis	PRSS1, SPINK1, PRSS2, CTRC	7;5;7;1	50-80
Peutz-Jeghers Syndrome	STK11 (LKB1)	19	132
Familial pancreatic Cancer	PALLD	4	5

Cystic Fibrosis	CFTR	7	5.3
Fanconi anemia and Familial breast or ovarian cancer	BRCA-1/ BRCA-2, PALB2	13;16	3-6
Familial adenomatous polyposis	APC	5	4,46
Li-Fraumeni Syndrome	TP53	17	1.3%
Lynch Syndrome	MSH2 / MSH6, MLH1	2; 3	9

2.3 Pathogenesis

Pathogenesis is based on hereditary or acquired inactivation of tumour suppressor genes, activation of oncogenes and deregulation of the cell cycle; because of that, the normal pancreatic epithelium is deranged with a successive accumulation of genetic mutations.

The grand majority of pancreatic tumours are originated from the exocrine cells of the pancreas. That includes pancreatic ductal adenocarcinoma (PDAC), acinar cell carcinoma, cystadenocarcinoma, adenosquamous carcinoma, signet ring cell carcinoma, hepatoid carcinoma, colloid carcinoma, undifferentiated carcinoma, pancreatoblastoma (more frequent in children, with a poor prognosis in adults) and pancreatic mucinous cystic neoplasm. Neuroendocrine pancreatic tumours represent only 1% of all pancreatic tumours. You can find in ANNEX 1 the WHO histological classification of pancreatic exocrine tumours.
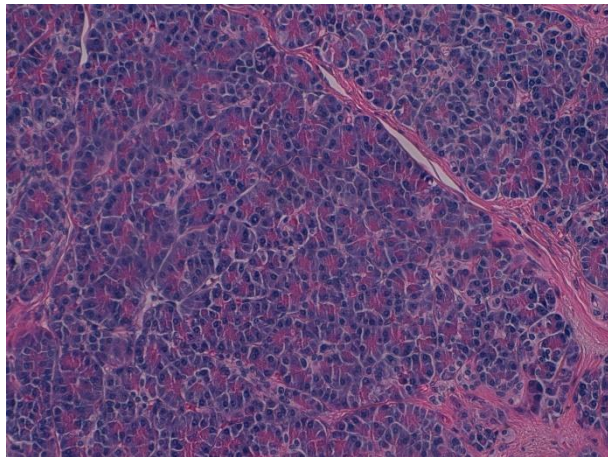
Figure 4: Normal pancreatic parenchyma. Magnification x20. With permission of Dr. Melendez
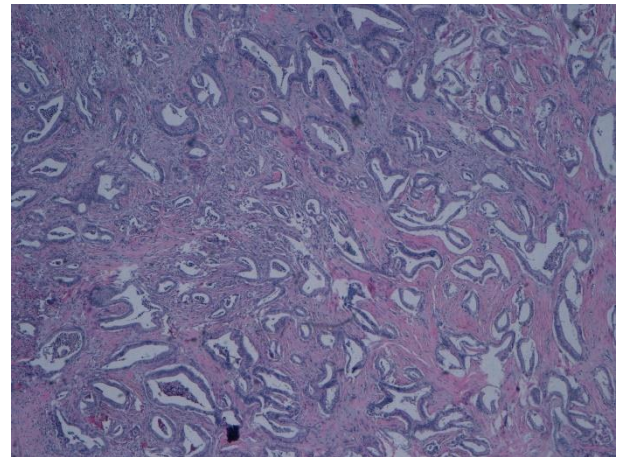
Figure 5: Pancreatic ductal Adenocarcinoma Magnification x4. With permission of Dr. Melendez

Among all types of pancreatic cancer, PDAC entails more than 85% of all them. It is characterized by moderate to poor differentiate mucin-producing glandular cells. The higher the grade, the more genetic alterations present. Figure 4 and 5 shows the pathological differences

that could be found between the normal pancreatic parenchyma and PDAC. Three morphologic preforms of PC are observed (5,9,18):

Intraductal papillary mucinous neoplasm (IPMN), integrated by mucin-producing cyst cells. Its first site is the main pancreatic duct or one of its major branches. It is diagnosed more often in men than in women, and usually on the pancreas head. SMAD4 inactivation has not been described.

Mucinous cystic neoplasm, frequently sited on pancreas' body or tail, is characterized by its mucinous cystic cells and can be divided into three other categories: malignant, borderline and benign.

Pancreatic intraepithelial neoplasia (PanIN) is considered the precursor lesion of PDAC because it is found on the boundaries of PDAC and its shares genetic and epigenetic mutations with the malignant lesion. According to molecular changes and cell aggressiveness, it has been subdivided into three grades:

- Low grade (stage PanIN- 1A and -1B): presents telomere shortening with KRAS mutation in more than 90% of the cells. Figure 6 shows the transition between the normal epithelium to the PanIN-1A.
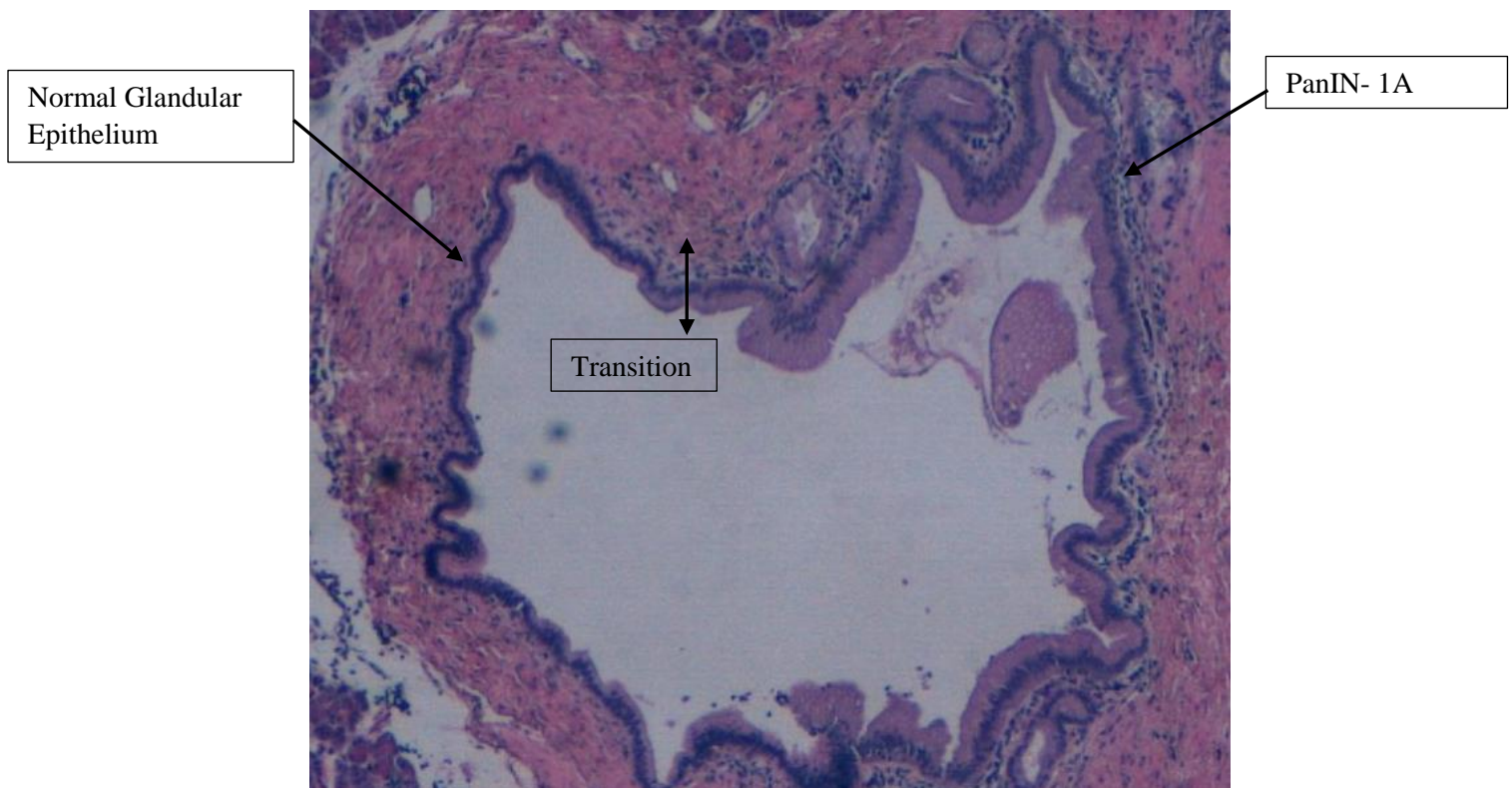
Figure 6: Transition between a normal glandular epithelium to PanIN-1A. Magnification x4. With permission of Dr. Melendez

- Moderate grade (PanIN-2): where an inactivation of p16, a tumour suppressor gene, is found in 95% of cases. Figure 7 shows the moderate grade lesion.
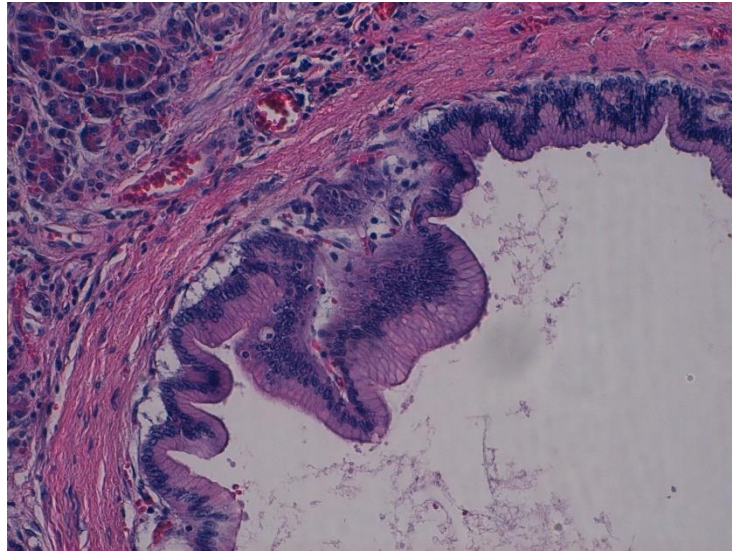
Figure 7: PanIN-2. Magnification x20.With permission of Dr. Melendez

- High grade (PanIN-3): the last grade before PDAC. It starts with an inactivation of CDKN2A (95%), p53 (50-75% cases), SMAD4 (55%) and BRCA-2 all of them tumour suppressor genes. BRCA-2 mutation seems to be one of the last mutations to appear in the neoplasm (5).

Figure 8 resumes the different stages of PanIN related with their specific mutations on each stage.
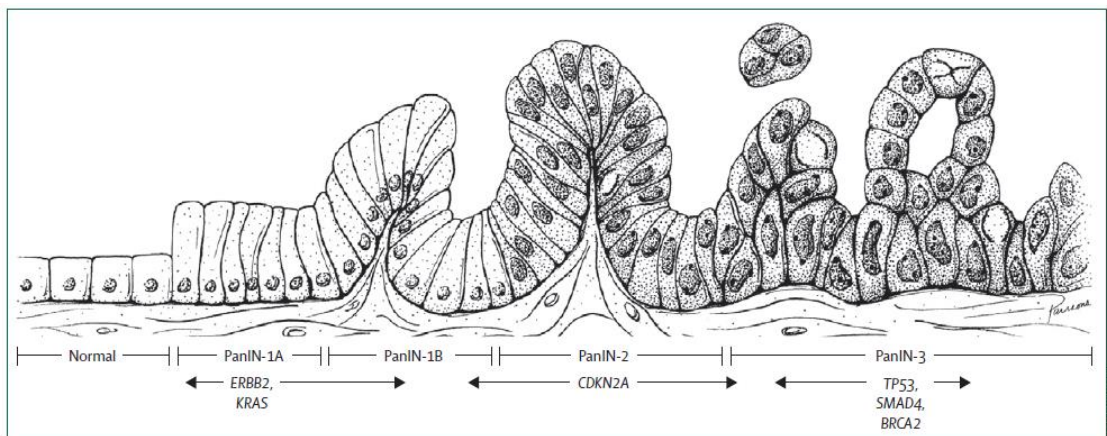
Figure 8: Pancreatic intraepithelial neoplasia (PanIN) plus genetic alterations (1).

As stated before, more than 90% of PC presents a KRAS mutation in the lower grade stage which possibly means that it might be the first step for the tumorigenic growth. KRAS pathway regulates division, differentiation and apoptosis of pancreatic cells, so when mutated, the cell to becomes anarchic and proliferate without any barriers that could stop it, which means that

KRAS is essential for the maintenance of PC and acts as a prognosis factor as it has been shown in several studies (19). Loss of KRAS expression results in massive cell death and arrested proliferation, leading to rapid tumour regression. Despite the importance of KRAS in PC, targeting it in order to treat PC in an earlier stage has shown non-significant clinical benefit (20).

BRCA-1 and BRCA-2 are tumour gene suppressors, their correspondent proteins regulate the cycle checkpoints when DNA is damaged and even repair it when there are double-strand breaks via homologous recombination (HR). When mutated, the subsequent lack of DNA-damaged repair provokes genome instability which is the chief point to a malignant transformation. This makes of BRCA-1 and BRCA-2 critical points in the maintenance of genomic integrity (5,21).

A special feature found in PDAC is that malignant cells are often surrounded by a hypovascular desmoplastic reaction which can disturb the cytopathological diagnosis by fine-needle aspiration and makes difficult the arriving of chemotherapy treatment. PC rapidly metastasizes to regional lymph nodes, then to the liver and the peritoneal cavity. Neural invasion is prevalent and may be associated with abdominal pain. Less frequently PC metastasizes to the lungs, bones, brain or skin. Figure 9 demonstrate the presence of desmoplastic reaction and the importance of peri- and intraneural invasion.
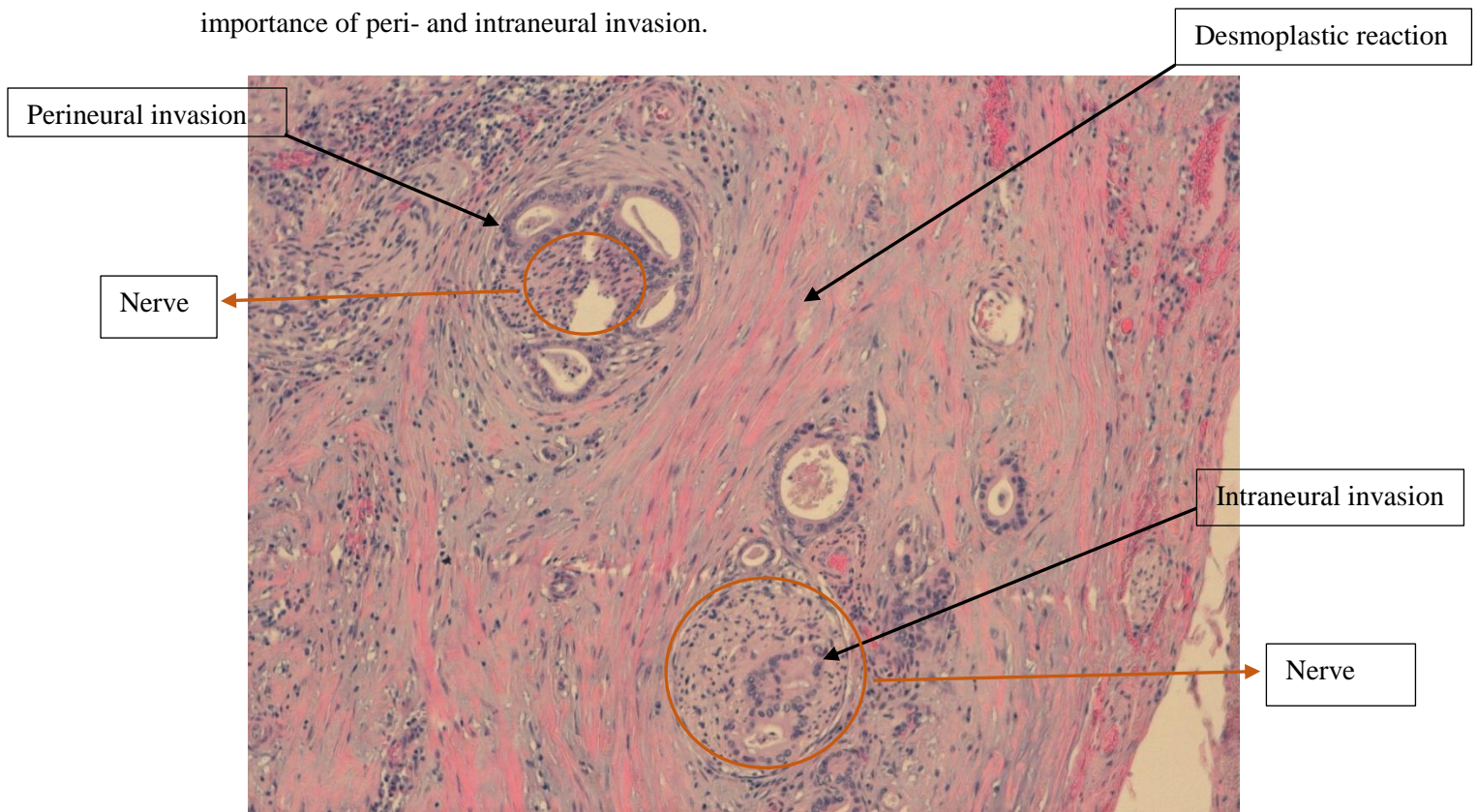
Figure 9: Neural invasion and Desmoplastic reaction. Magnification x10. With permission of Dr. Melendez

2.4 Clinical course

PC is usually a silent disease in earlier stages, and produces no symptoms until the disease has spread to surrounding tissues or metastasises to other organs. That is why most patients are diagnosed in later stages.

The classical symptom of PC is abdominal pain, usually focused in the superior abdomen and often irradiated to the back, causing backache. Obstructive jaundice can be present if the tumour is sited on the head of the pancreas, and it can quickly progress causing choluria, acholia and itching. It could be painless at the beginning, but painful in a later stage. Loss of weight is often present driven either by anorexia or by maldigestion because of pancreatic insufficiency due to pancreatic ductal obstruction. In that case, steatorrhea or diarrhoea can appear and lead to cachexia because of fat malabsortion. Furthermore, when the tumour invades the duodenum, it produces nausea and vomiting as symptoms of intestinal obstruction and even high digestive haemorrhage (22).

Some studies reveal the relation between early diabetes mellitus (DM) and PC; Pannala et al. reports that DM of new-onset is present in nearly half the patients with PC in his study, which could be a consequence of the infiltration of the pancreatic parenchyma by the malignant cells. After surgery, when resection is possible, DM disappears (23). In spite of these findings, new-onset DM is not to be always found in PC patients even if Chari et al. study suggest that new-onset DM would benefit from a PC screening (24).

The symptoms presented above are directly related with the tumour site within the pancreas'. Indeed, if tumour invades the pancreas' head, which represents 60 to 70% of PC, it would produce more weight loss and jaundice whereas if it is located in pancreas' tail or body, which embodies a 20 to 25% of cases, weight loss and pain would be more frequently seen.

2.5 Diagnosis

Diagnosing an earlier stage of PC can be challenging but, in a metastatic stage, it remains important to put all our diagnostic methods in order to identify PC. (7,25,26)

2.5.1 Haematological, biochemical, liver function and coagulation tests:

Blood and biochemical tests may reveal non-specific PC results as: anaemia, hypoalbuminaemia and prolonged prothrombin time. Liver function may be affected with the presence of hyperbilirrubinaemia, increased transaminases concentration in serum, and increased gamma glutamyl transferase (GGT), which can be related to extensive liver metastasis if it rises exponentially. Besides, glycaemia must be controlled as patients with PC could present DM.

2.5.2 Tumour markers

Tumour markers are very sensitive (70 to 92%) to the progression of PC but none specific (68-92%). Par excellence CA19.9 is the most extensively tumour marker used in PC. Its increase level is related to the PC volume which results useful for the patient's follow-up. When levels are increased more than 100-200 U/mL, it means that it is an unresectable PC. When levels are reduced with chemotherapy, it is considered to be a predictive factor.

2.5.3 Imaging techniques

2.5.3.1 Abdominal ultrasound (AUS)

The first approach in a patient with jaundice should include an AUS. It is a non-invasive and non-expensive technique that delivers information about the pancreas' and duct morphologies, tumour's location and pancreas surrounding structures as lymph nodes, liver and vessels. Even so, it is an operator-dependent procedure and is affected by obesity, ascites or bowel gas which may cause erroneous imaging. In AUS PC is seen as a hypoechoic, hypovascular solid mass with irregular margins. Lesions bigger than 3 cm are well-detected with AUS as well as ducts dilations. AUS could also be used with Doppler to define vascular invasion.

2.5.3.2 Contrast-Enhanced multidetector CT (MDCT)

MDCT gives thin slice cuts with high image resolution and fast image acquisition which is usually made to diagnose suspicious pancreatic lesions and assess the accurate staging of PC.

Malignant tumours are generally hypovascular in comparison to surrounding pancreatic parenchyma. They are better visualised with contrast imaging than without contrast as it increases sensitivity and specificity.

MDCT has three main phases: non-contrast, pancreatic parenchyma or arterial phase and portal vein phases, and it is recommended to use a minimum of two phases for an adequate assessment of the neoplasm (22,26).

In the arterial phase (the first one), PC appears poorly highlighted compared to the normal pancreatic parenchyma because of the desmoplastic reaction. This phase gives a pancreatic and liver vascularisation's map. Portal venous phase evaluates the veins of this particular location which are portal, splenic and superior mesenteric veins as much as the abdomen's extension study which will highlight adenopathies or tumour implants. To detect liver metastatic lesions, the three phases are required. PC's features that could be seen in MDCT are (16,25):

- Hypoattenuation (sensitivity 75% and specificity 84%)
- Ductal pancreatic or bile dilation (sensitivity 50% and specificity 78%) with possible double duct sign.
- Ductal interruption (sensitivity 45% and specificity 82%)
- Distal pancreatic atrophy (sensitivity 45% and specificity 96%)
- Pancreatic contour anomalies (sensitivity 15% and specificity 92%)
- Common bile duct dilation (sensitivity 5% and specificity 92%)

Even so, at least 10% of PC do not present hypoattenuation but are isodense with the normal pancreatic parenchyma, which result in an added difficulty to its detection(22).

MDCT's sensitivity goes from 65% for tumour lesions smaller than 1.5cm, to 100% when the lesions are bigger. But MDCT has limitations. Both focal and autoimmune pancreatitis may mimic PC and also present some PC's features, making them indistinguishable without biopsy.

2.5.3.3 Magnetic Resonance Imaging (MRI) and magnetic resonance cholangiopancreatography (MRCP)

MRI is the most useful technique for imaging pancreas lesions for defining vascular invasion of PC, MDCT is more accurate. MRCP allows the study of the pancreaticobiliary tree, liver parenchyma and vascular structures, and gives a three-dimensional image which is an interesting and harmless alternative to endoscopic retrograde cholangiopancreatography(25).

2.5.3.4   Endoscopic retrograde cholangiopancreatography (ERCP) and percutaneous transhepatic cholangriography (PTC)

These two techniques enable to take directly cytopathological samples and to treat patients with obstructive jaundice due to tumour obstruction of the pancreaticobiliary tree with a biliary stenting. Besides for this, endoscopic ultrasound remains more reliable (5).

2.5.3.5   Endoscopic ultrasound (EUS)

Some studies reviewed the superiority of EUS to MDCT, and conclude that EUS is more accurate for detecting and staging PC (27,28). Nowadays, this radiology technique is used to complete CT and MRI studies in order to define lymph nodes and vascular invasion of PC. It is the main technique used in order to obtain cytological samples and biopsies, using an endoscope attached with an ultrasound transducer that has a small-diameter aspiration needle which can be placed where the tumour is located (25,29). This is called EUS-guided fine-needle aspiration (EUS-FNA). A cytopathological diagnose is required in unresectable tumours before neoadjuvant chemo- and/or radiotherapy treatment. However, results could be disturbed by the presence of chronic pancreatitis, diffuse infiltration of PC, recent acute pancreatitis (less than 4 weeks), etc. Its complication rates are lower than ERCP.

2.5.3.6   Positron emission tomography (PET)

The use of PET is limited to situations when no conventional imaging technique could solve PC's suspicion or when biopsy has not been diagnostic.

2.5.3.7   Laparoscopy

This surgery is not generally done for achieving a diagnose facing a PC suspicion. Its use as staging tool before neoadjuvant treatment in borderline resectable or unresectable tumours, in order to detect peritoneal carcinomatosis, is controversial.

2.6 Staging

Staging is crucial for the treatment and prognosis of PC. AJCC Cancer Staging system is used to divide pancreatic cancer into different prognostic stages that are shown in Table 3 and 4.

Table 3: Tumour / node / metastasis staging of pancreatic cancer.

Primary Tumour (T)	Regional Lymph Nodes (N)	Distant Metastasis (M)
TX: Primary tumour cannot be assessed	NX: Regional lymph nodes cannot be assessed	M0: No distant metastasis
T0: No evidence of primary tumour	N0: No regional lymph node metastasis	M1: Distant metastasis
Tis: Carcinoma in situ	N1: Regional lymph node metastasis	
T1: Tumour limited to the pancreas, 2cm or less in greatest dimension		
T2: Tumour limited to the pancreas, more than 2cm in greatest dimension		
T3: Tumour extends beyond the pancreas but without involvement of the celiac axis or the superior mesenteric artery		
T4: Tumour involves the celiac axis or the superior mesenteric artery (unresectable primary tumour)		

Table 4: Staging of pancreatic cancer by AJCC.

Stage	T	N	M
Stage 0	Tis	N0	M0
Stage IA	T1	N0	M0
Stage IB	T2	N0	M0
Stage IIA	T3	N0	M0
Stage IIB	T1	N0	M0
	T2	N1	
	T3		
Stage III	T4	Any N	M0
Stage IV	Any T	Any N	M1

For locally advanced tumours a specific pro-therapeutic approach categorizes PC into resectable, borderline resectable and unresectable tumours. Resectability criteria are

summarized in ANNEX 2, took from the National Comprehensive Cancer Network (NCCN) Guidelines version 2. 2015.

2.7 Management and treatment

Treatment decision would be taken according to staging. Surgery is the only curative treatment for PC but it can only be used for resectable disease which corresponds to 10-20% of patients (25). When PC affects the pancreas' head or has a periampullar location, cephalic pancreatoduodenectomy, known as Whipple procedure, is done. Even so, the 5-year survival rate after this treatment is only 10 to 25% with a median survival of 15-19 months (5,25). If PC affects pancreatic body or tail, distal or subtotal pancreatectomy is recommended. In these cases, adjuvant therapy has to be administered with gemcitabine $1000mg/m^2$ in monotherapy when chemotherapy can be tolerated. As shown on the ESPAC-03 trial, this treatment increases the median survival rate to 22.8 months and the 5-year overall survival rate to 21%, vs 9% without chemotherapy. According to those results, adjuvant chemotherapy with gemcitabine is the standard treatment for resected pancreatic cancer in our centre.

Locally advanced disease is present in up to 40% of patients at the time of diagnosis. These patients have to be evaluated in interdisciplinary tumour board in order decide the best therapeutical option in each case.

Concerning borderline resectable tumours, neoadjuvant therapy with induction chemotherapy, preoperative chemoradiation or a combination of both is the gold standard treatment, in order to have better systemic control and to increase the probability of a posteriori resection. International guidelines recommend at least three months of chemotherapy (platin- or gemcitabine-based) followed by a full-dose radiotherapy treatment course in combination with capecitabine, 5-fluoruracil or weekly gemcitabine. After this, and before taking the next step, surgery, new imaging techniques should be performed and the final resolution should be taken on tumour board. After surgery, adjuvant chemotherapy could be offered. If the tumour is considered definitely unresectable, treatment should be stopped after radiotherapy.

Even with this case-directed approach, the average overall survival rate remains low. There is not an established standard chemotherapy regimen in locally advanced pancreatic cancer (12). Possible therapies recommended by international guidelines are monotherapy with gemcitabine, the combination of gemcitabine plus nab-paclitaxel, 5-FU with oxaliplatin and irinotecan (FOLFIRINOX) and even, gemcitabine plus erlotinib. In our institution, three months chemotherapy treatment with gemcitabine and oxaliplatin or FOLFIRINOX, followed by radiotherapy is the standard.

Chemotherapy is the treatment of choice for metastatic or recurrent disease. It should only be offered to the patient if a good performance status (PS) is maintained. PS is summarized in Table 6 which represents the Eastern Cooperative Oncology Group or ECOG's scale and it includes the related treatment that is recommended for each PS grade (30).

Table 6: ECOG Performance Status and treatment recommendations according to PS.

Grade	ECOG Performance Status	Treatment
0	Fully active, able to carry on al pre-disease performance without restriction	FOLFIRINOX [Folinic acid (leucovorin)/5-FU/irinotecan/ oxaliplatin]
1	Restricted physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature	
2	Ambulatory and capable of self-care but unable to carry out any work activities. Up and about more than 50% of waking hours	─Gemcitabine in monotherapy if level of bilirubin is higher than 1.5 x Upper Limit Normal ─Gemcitabine + Nab-paclitaxel could be considered in this patients with large tumour ─ Gemcitabine + erlotinib ─Gemcitabine + capecitabine ─GTX(gemcitabine, docetaxel and capecitabine)
3	Capable of only limited selfcare; confined to bed or chair more than 50% of waking hours	Patients with significant morbidities and short life expectancy: Palliative care
4	Completely disabled; cannot carry on any selfcare; totally confined to bed or chair	
5	Dead	

Treatment with FOLFIRINOX [Folinic acid (leucovorin) / 5-FU / irinotecan / oxaliplatin] or gemcitabine plus nab-paclitaxel in first line of metastatic PC have shown significant benefit in terms of survival. According to the results of the PRODIGE and MPACT trials(31,32), treatment with either of these chemotherapy regimens increase the overall survival rates, median survival time and disease-free survival time. It is an attractive choice for those patients

with good PS (ECOG 0-1). For ECOG 2 patients, gemcitabine is the preferred option because its low toxicity profile.

The aim of palliative and supportive care is to increase the quality-adjusted life year (QALY) of patients with metastatic or recidivant disease with ECOG 3 or 4. It includes placement of an expandable stent if presence of a duodenal obstruction or an anaesthetic blockade of the celiac plexus for treating the abdominal pain.

Summarized evidence concerning nowadays therapy of PC related to the criteria of resectability, extracted from the NCCN Guidelines of PC version 2.2015, will be found in ANNEX 3.

2.8 BRCA-1 and BRCA-2 mutations. Why are they so important?

─ Mutations in genes involved in DNA-repairing and its synergy with chemotherapy

Besides hereditary syndromes, investigation on tumour somatic mutations in DNA-repairing genes could lead to new ways to treat PC. Knowing the mutational signatures of a specific cancer is a way to personalize treatment, in example through synthetic-lethality approaches preventing normal cells from drug toxicity.

As stated before, BRCA-1 and BRCA-2 are implied in the cell cycle maintenance. When the biallelic and somatic BRCA-1 and BRCA-2 genes are inactivated, there is a lack of DNA-damaged repair via HR, which causes genomic instability. It is at that level where the platinum agents act, creating covalent links. The platination of the cell leads to apoptosis. Even so, the cell may use other ways to avoid apoptosis; Poly (ADP-Ribose) Polymerase is one of those cells mechanisms. It is known that BRCA-defective cells are more sensitive to Poly (ADP-Ribose) Polymerase inhibitors. Therefore, using Poly (ADP-Ribose) Polymerase inhibitors with the platinum agents, which allow the loss of both HR and PARP1 pathways inhibition, will selectively cause apoptosis with non-repair possibility. This is known as synthetic lethality that is produced during DNA replication of mutated cells (21).

As we have seen, gemcitabine is the milestone in PC treatment, and it can be combined with other therapies in order to improve tumour response. One of these combination regimes, gemcitabine plus cisplatin, has demonstrated a striking benefit in patients with breast and ovarian cancers who carry BRCA1/2 mutations. Patients with PC harbouring BRCA germline or somatic mutations may also present some benefits with this therapy (21,33,34). Indeed, Oliver et al conducted a clinical trial in patients with a family history of PC alone and demonstrated a large survival advantage when treated with platinum-based chemotherapy (33).

Currently this therapy is being studied in association of Poly (ADP-Ribose) Polymerase 1 inhibitors in order to increase, on a synergistic basis, tumour response to platinum-based chemotherapy.

─ Poly (ADP-Ribose) Polymerase 1 inhibitors (PARPi): demonstrated efficacy in platinum-sensitive tumours

PARP are a large family of multifunctional enzymes located in chromosome 1 that can repair DNA single-strand breaks. The most common enzyme is PARP1. Its inhibition prevents malignant cells from repairing DNA damage; they accumulate single-strand breaks and lately double-strand breaks, leading the cell to apoptosis. PARPi were developed in order to specifically act in PARP-repair DNA pathways in carriers of BRCA1/2 mutations, which already have a defect on the DNA mismatch repair system. It represents a potential synthetic lethal therapeutic strategy. McLornan et al. reviewed several studies that have been testing PARPi in BRCA-mutated tumours revealing an increase in overall survival rate in those cohorts of patients treated with PARPi. Even so, malignant cells can develop resistantce to PARPi, restoring their cell malignant function(35).

PARPi have also been studied in other epithelial cancers. The first study to be published was conducted in ovarian cancer after platinum-based chemotherapy. Ovarian cancer is characterized by frequent relapses and short life expectancy even with response to platinum therapy was up to 80%. Maintenance treatment with conventional chemotherapy has been studied, but it ends up with relapses too. That is why research on new treatments has been conducted. Ledermann et al has evaluated the efficacy of the PARPi olaparib as maintenance treatment in ovarian cancer and found a significant improvement in progression-free survival among them without a clear benefit in overall survival. Even so, when BRCA1/2 mutations were present, the benefit seemed to be significant (36). O'Shaughnessy et al conducted as well a phase 2 clinical trial comparing the efficacy and safety of the combination of gemcitabine and carboplatin with the PARPi iniparib in metastatic triple-negative breast cancer. Results were positive with a significant clinical and survival benefit for these patients (37). Recent studies in prostatic cancer have also evidenced this beneficial trend, with good treatment response with olaparib (38). Fong et al. studied in the clinical setting, the differential response to PARPi in patients who carried BRCA1/2 mutations and found in early-stage studies that individuals treated with PARP1 inhibitors present a real improvement in their cancer treatment (39). So, all these studies show the increasing importance of synthetic lethal therapeutic strategy that could be employed in PC's with BRCAs/2somatic mutations.

─ BRCA1/2 mutations and PARPi therapy in PC

BRCA1/2 mutations are present in 9.9% tumour samples of PC (40) and confer a high risk for developing PC. According to Waddel et al. study, BRCA-1 mutation was present in 3% of tested tumours whereas BRCA-2 was in 2% (41), representing an small proportion of cases. Moreover, in the same study, they evidenced an 80% response-rate (4/5) when a patient with this defective DNA repairing features where treated with platinum-based treatment. Some studies are investigating the use of PARPi in PC harbouring BRCA1/2 mutation as monotherapy or in combination with platinum compounds. These phase II trials are showing encouraging results, achieving meaningful partial responses for 1-2 years(34,42,43). Indeed, Lowery et al. conducted a phase II trial with PARPi olaparib in monotherapy for BRCA mutation associated to solid tumours. Three patients received the PARPi in combination with chemotherapy resulting in two patients with an initial radiographic partial response by the Response Evaluation Criteria in solid Tumours (RECIST) but progressed at 5 and 6 months whereas the third one shown stable disease as a best response. Pishvaian et al. in their phase I/II trial revealed that the combination of PARPi (ABT-888), 5-FU and oxaliplatin had a promising efficacy in metastatic PC and particularly BRCA2 gene mutation. PARPi may improve survival on these patients with PC harbouring BRCA1/2 mutations.

3. JUSTIFICATION

Throughout all our introduction we have shown that PC remains an important health problem that is far from being solved. We do not have enough information to fight against PC, to detect it in early stages or to treat PC patients with effective therapies in terms of survival. It is one of the most lethal cancers where the only way to cure it, is surgery, and this can be done in only a 20% of cases. Research in somatic mutations will find targets to develop or to create a new schema of treatment that will permit to those patients to have a larger survival. We focus in BRCA1/2 somatic mutations even if they represent a small proportion of cases because these groups of patients could benefit from a personalised and optimized treatment. We will investigate the prevalence, prognostic value and also if patients with mutated-PC treated with platinum-based chemotherapy have a better overall survival rate than patients with non-mutated PC (predictive value). With the results, we hope we will be able to define subgroups of PC patients that could benefit from targeted therapies in prospective clinical trials (for example with PARPi in monotherapy or in combination with platinum compounds) in order to develop personalized treatment strategies (34).

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5. HYPOTHESES

H1: Prevalence of Pancreatic Cancer with BRCA1/2 somatic mutations in patients coming from Girona's province is like previously reported prevalence in other Caucasian population studies, between 2-3% of cases.

H2: The 5-year survival rate of patients with Pancreatic Ductal Adenocarcinoma harbouring BRCA1/2 somatic mutations are not different from patients with non-mutated PDAC.

H3: The overall survival rate at 36 months of patients with Pancreatic Ductal Adenocarcinoma harbouring BRCA1/2 somatic mutations treated with platinum-based chemotherapy differs from the overall survival rate of patients with mutated pancreatic cancer treated without platinum-based chemotherapy.

6. OBJECTIVES

1) To analyse the prevalence of Pancreatic Adenocarcinoma with BRCA somatic mutation in Girona's province.

2) To explore if there are significant differences in 5-year survival rates of patients with Pancreatic Adenocarcinoma harbouring somatic BRCA1/2 mutations and patients with non-mutated Pancreatic Adenocarcinoma.

3) To evaluate if treatment with platinum-based chemotherapy is associated with an improvement of probability of overall survival rate at 36 months in Pancreatic Adenocarcinoma's patients with BRCA1/2 somatic mutations.

7. SUBJECTS AND METHODS

7.1 Study design

We will conduct a retrospective cohort study with patients diagnosed of pancreatic cancer in Girona's province registered on the Cancer Registry of Girona between 1994 and 2012.

7.2 Population in study

Our study will include all patients with diagnoses of Pancreatic Adenocarcinoma between 1994 and 2012 that will be identified by the Cancer Registry of Girona with the ICD code 25.0 to 25.9.

7.3 Inclusion Criteria

- Patients with PDAC registered with ICD codes 25.0 to 25.9 between 1994 and 2012
- Inhabitant of Girona's province
- Available histological or cytological sample for genetic analyses
- Signed informed consent for tissue analyses (endorsed by the patient, family or ethical committee of the institution in case of exitus)

7.4 Exclusion Criteria

- Patients with radiological diagnose of Pancreatic Adenocarcinoma without available tumour sample.
- Non-carcinoma or mixed histology pancreatic neoplasms.
- Concurrent malignancies or diagnosed in the previous 5 years that require simultaneous treatment with any other anti-cancer therapy.

7.5 Sample selection

Non-probabilistic sampling will be done for selection. The sample recruitment will include patients with Pancreatic Ductal Carcinoma registered in the Cancer Registry of Girona's province with the ICD code 25.0 to 25.9 between 1994 and 2012. Within them, patients with available histological or cytological tumour sample will be selected.

In order to detect BRCA1/2 somatic mutations. With these data, we will find out the prevalence of BRCA1/2 somatic mutation in Girona's province.

For the second objective, after determining BRCA1/2 somatic mutations, we will analyse retrospectively the 5-year survival rates. First for the whole sample, and then for the two studied cohorts: patients with BRCA1/2 mutated and non-mutated PC, in order to determine the prognostic value of the mutations.

Finally, for the third objective (predictive value), we will select mutated patients and compare the overall survival rate at 36 months of those treated with platinum-based chemotherapy with those patients treated with non-platinum regimens.

7.6 Sample size

In order to evaluate prevalence of BRCA1/2 somatic mutations in Girona's province, it will be necessary at least 125 subjects to estimate with a 95% confidence and a precision of 3 percent units.

Concerning our second objective, GRANMO was used in order to calculate the sample size. Accepting an alpha risk of 0.05 and a beta risk less than 0.2 in a bilateral contrast, we will need 191 patients with mutated-PC in one group and 191 patients with non-mutated PC in the other group, in order to detect significant statistically difference concerning the 5-year survival rate between the two groups, which is expected to be in the first one of 0.05 and in the second one of 0.13 (44). It has been estimated a follow-up loss rate of 0%. So, 382 are required in order to come across the second part of the study.

The sample size of the third objective has been calculated as well using GRANMO, based on the scarce bibliography available (21). Accepting an alpha risk of 0.05 and a beta risk less than 0.2 in a bilateral contrast, we will need 190 patients harbouring BRCA1/2 mutation treated with platinum-based chemotherapy in one group and 190 patients harbouring BRCA1/2 mutation with non-platinum chemotherapy in the other group, in order to detect significant statistically difference concerning the overall survival rate at 36 months between those two groups, which is expected to be in the first one of 0.16 and in the second one of 0.07. It has been estimated a follow-up loss rate of 0%. We will require 380 in order to come across the third part of the study.

Indeed, for these objectives, our sample is made up of 1407 individuals with PC registered in Cancer Registry of Girona's province between 1994 and 2012. We expect that in this 1407

individuals, a 30% will present histological sample which means that we may have 422 cases with histological sample that will be used to evaluate the presence of BRCA1/2 mutations.

7.7 Variables analyses

─ First Objective

Prevalence of BRCA1/2 somatic mutation represents a quantitative variable, and will be expressed as percentage.

─ Second Objective

BRCA1/2 somatic mutation represents the independent nominal qualitative variable, and will be transformed in a dichotomous variable (presence or not presence of BRCA1/2 somatic mutation) in order to facilitate the statistical analyses.

The **5-year survival rate** will be the dependent nominal qualitative variable that will be also transformed as a binomial variable (5-year survival rate is different or is not different). This rate defines the percentage of people diagnosed with cancer or treated for their cancer that will be alive 5 years after the diagnosis/ beginning of the treatment. We will use it to see if BRCA1/2 somatic mutation in pancreatic cancer is a prognostic factor.

─ Third objective

For this objective, we will study the overall survival rate at 36 months in individuals with BRCA1/2 mutation and in non-mutated individuals. And then we will compare the survival of patients treated with platinum-based chemotherapy and non-platinum based chemotherapy in each group.

The **overall survival rate at 36 months** will be the dependent nominal qualitative variable, that will be transformed to a dichotomic variable:

- The overall survival rate at 36 months in patients with BRCA1/2 mutation will differ (which means that it will increase or decrease)
- The overall survival rate at 36 months in patients with BRCA1/2 mutation won't differ

We will see through it if patients with pancreatic cancer harbouring BRCA1/2 mutation were responding to platinum-based chemotherapy and if it was a successful therapy to increase **the overall survival rate at 36 months** of the person with pancreatic cancer. It will allow us to see if BRCA1/2 somatic mutation is a predictive factor.

Thus, **Platinum-based chemotherapy** will be our independent qualitative nominal variable that will be also exposed as a binomial variable (platinum-based chemotherapy yes/no).

─ Covariable that we will analyse:

- Age (years)
- Gender (male/female)
- Family history of cancer (yes/no)
- Tumour specific variables: histological grade (reflected in the pathology report: well-differentiated, moderately differentiated or poorly differentiated/undifferentiated), location (pancreas' head, body or tail).
- Staging
- ECOG performance status

7.8 Instrumentation

─ Cancer Registry of Girona's province

The Cancer Registry will allow us to identify patients with PC diagnosed between 1994 and 2012. Registry will provide:

- Demographic data: age, residency
- Tumour data: date of diagnose, histological type, anatomic sublocation, morphology and diagnosis methodology (biopsy, cytological sample or clinical diagnosis)
- Information about where the patients were identified
- Last contact with the patient
- Vital status (alive/dead)

─ Clinical Registry

Taking this basic information, complementary data will be registered through a detailed revision of clinical documentation of all the different hospitals of Girona's province. We will extract data from those patients with histological (cytological o biopsy) samples from each Hospital of Girona's province. This complementary data will include:

- First symptomatology presented by the patient
- Tumour markers CA19.9
- ECOG or Karnofsy index at diagnosis
- Diagnosis technique and year of diagnosis

- Imaging techniques that were realised when the diagnosis was made, plus extension study: abdominal CT, MDCT, thoracic CT, IRM, AUS
- Patient presented to the multidisciplinary committee
- Staging when diagnose (cTNM)
- Pathological staging (pTNM) that will include regional and positive lymph node that will be examined
- Tumour size
- First treatment received:
    o Biliary drainage (yes/no) previous at the surgery/palliative care, plastic/metallic prosthesis
    o Surgery data: surgery (yes/no), resectability, surgery type, surgery date
    o Chemotherapy: neoadjuvant, adjuvant or first line of metastatic disease. Beginning of chemotherapy. Type of chemotherapy drugs. Discontinuation or not of the treatment due to toxicity or tumour progression.
    o Radiotherapy: neoadjuvant, adjuvant or palliative. Date of first radiation, dose of radiation, date of final radiation, PTV. Discontinuation or not of the treatment due to toxicity or tumour progression.
    o Recurrent disease or tumour progression: Progression's date. Type of progression or recurrent (local, locorregional or distant recurrent or progression). Metastasis location if disseminated disease (lymph node and/or liver and/or lung metastasis)
    o No treatment realised: specify why the patient has not received treatment
- Second neoplasm during the treatment or follow-up of PC and date of the diagnosis of this new neoplasm.
- Molecular and genetic markers determination: presence or absence and BRCA1/2 somatic mutation
- Vital status and last-contact date

─ Measures:

1. The 5-year survival rate and the overall survival rate at 36 months
2. Vital status will be obtained through:
    a. Basic information of the Cancer Registry of Girona's province
    b. Mortality Registry of Catalonia
    c. Deceased National Index will provide information about patient that had moved on outside Catalonia
    d. Active follow-up in health centres will be automatic through discharges and consulting the patient clinical history.

─ BRCA1/2 somatic mutation analyses:

All the available samples will be examined by a central pathologist in Josep Trueta's Hospital that will prepare them for the genetic test. Those samples will be sent to the Laboratory and Pathology Department of Bellvitge University Hospital where they will determine BRCA1/2 somatic mutation. An extended bibliographic review will be done in order to identify these mutations that could represent a prognostic and predictive factor.

Formalin-fixed, paraffin-embedded (FFPE) tumour samples will be analysed in order to find out BRCA1/2 somatic mutation through the Next Generation Sequencing (NGS). In order to do so, BRCA tumour MASTR (Multiplex Amplification of Specific Targets for Resequencing) Plus commercial assay (Multiplicom) will be used. This molecular technique allows us to identify somatic mutations on BRCA1/2 codifying regions through the creation of genomic library based on amplicons that will permit the targeted resequencing. To prepare the genomic library, we will do four specific multiplex Polymerase Chain Reaction (PCR) amplifications on the regions of interest that are the BRCA1/2 gene. This will allow us to generate a total of 181 short amplicons (123-130 bp) that overlap each other. Sequencing of the genomic library will be done using MiSeq that integrates cluster generation, amplification, sequencing and data analysis into a single instrument. MiSeq uses the Sequencing by Synthesis (SBS) technique. To analyse the variants of BRCA1/2 mutations we will use the SeqNext software (JSI medical systems) which permit us to do a simple mapping and observation of the variants. ANNEX 4 contains specific information about the MiSeq system and SBS.

7.9 Methods of data collection

The data will be collected from the clinical medical records of each participating patient and will then be reflected on the study database.

8. STATISTICAL ANALYSIS

8.1 Descriptive analysis

First of all, we will do a descriptive analysis. The prevalence of BRCA1/2 somatic mutations in pancreatic cancer will be measured like a proportion and the results will be presented in percentage.

8.2 Survival analysis

For the second and third objectives, results will be expressed as percentages for all categorical variables. Proportions will be compared with the χ^2 test. We will calculate the relative risks for each of the objectives:

- Objective 2: Relative risk of patients with Pancreatic Cancer harbouring BRCA1/2 mutation compared to patients that do not have the mutation related to the 5-year survival rate.
- Objective 3: Relative risk of patients with Pancreatic Cancer harbouring BRCA1/2 mutations and treated with platinum-based chemotherapy compared to those that do not received platinum related to the overall survival rate at 36 months.

For the survival analysis, a Kaplan Meyer test will be done. The differences between the Kaplan-Meier curves will be tested for significance with a log-rank test.

8.3 Multivariate analysis

For these analyses, the 5-year relative survival rate and the overall survival rate at 36 months will be stratified according to staging. A multivariate analysis will be done using a Cox proportional hazard regression model.

Significance for these analyses will be set at P values ≤ 0.05. The Social Sciences (SPSS) version 22 for Windows® will be used for all the statistical analyses.

9. ETHICAL CONSIDERATIONS

This research will be carried out, reviewed and undertaken to guarantee integrity, quality and transparency, according to the medical ethics requirements defined in the World Medical Association Declaration of Helsinki of Ethical Principles for Medical Research Involving Humans Subjects created in 1964 and reviewed for the last time in 2013.

This protocol will be presented to and considered by the Ethics Review of the Clinical Research Ethics Committee (CEIC) of the Dr. Josep Trueta University Hospital in Girona.

All individuals, patients or family, involved in this study will be informed entirely about the purpose, methods and intended use of the research, about what their participation entails. Participants will take part voluntarily, free from any explicit or implicit coercion. The confidentiality of information supplied by participants will be respected as well as anonymity along all the study and then after. Before including individuals on the project and at the time the study is carried out, participants that are not registered in biobank research will receive a written and spoken valid informed consent concerning the research, which will be in a comprehensible and accessible language, avoiding all kind of deception. Participants will have time to consider the entrance to the study and can deny their consent at any moment without any consequence on posterior follow-up. The informed consent, that is included in ANNEX 5, will be compliant with the International Conference on Harmonisation (ICH) guideline on Good Clinical Practice. Compiled data will be used by the investigational team and stored in the data-managing department of the Institut Català d'Oncologia (ICO) in Girona for fifteen years. Outcomes will be informed to all participants in this study. All this part of the study will be completed according to the "Ley 41/2002, de 14 de noviembre" that specifically concerns autonomy and rights of patients concerning information and clinical documentation.

Confidentiality will be respected according to the "Ley Orgánica 15/1999, de 13 de diciembre, de Protección de Datos de Carácter Personal" of Spain and approved by the "Real Decreto 1720/2007, de 21 de diciembre", providing the anonymity of the involved patients and allowing the access, modification or destruction of the collected data.

We will act in conformity with the "Real Decreto 411/1996, de 1 de marzo" that regulates all activity related to human tissues, and also with the "Real Decreto 2070/1999, de 30 de diciembre" that controls the extraction and use of human tissues besides organ transplantation.

10. LIMITATIONS

■ Data access and location of clinical histories in Documentation Service of each hospital. Clinical histories of deceased patients will be the major difficulty

■ Obtaining tumour tissue sample will be difficult. We expect that 30% of all patients that will be included in our project will have tumour tissue sample because of the diagnosis is currently done in a late stage that could not be treated with surgery and is treated directly with chemotherapy without knowing the cancer's histology.

■ Samples' viability: bad conservation or bad quality of tissue sample may difficult the process of DNA extraction and therefore somatic mutation's determination through the NGS. To establish the quality of the samples we will realise a quality control previous of the generation of genomic library that will determine if the sample will be able to be analysed with NGS.

■ Different healthcare professionals will be involved in patient care: measurement of RF and outcomes throughout the database would probably be less accurate and consistent than that achieved with a prospective cohort study design.

■ Using records that were not designed for the study may decrease the quality of this study.

■ Not possible to establish a causal effect: no randomisation which can imply the possibility of another variable that can interfere with our hypothetic association (confusion factor).

■ Selection bias: We expect a selection bias secondary to availability of tumour tissue.

11. VIABILITY

This research project will be carried out using the Cancer Registry of Girona resources that will provide us all the necessary data for our study. All the professionals that will be involved (physicians, medical collaborators, fellowship researcher, pathologist, geneticist and statistician) are qualified and able to understand the goal of this project. We will have regular meetings with the laboratory staff of the different centres that will be implied in this research study, and with the Laboratory of Bellvitge's Hospital.

12. WORK PLAN

Personnel involved:

- Investigators: Physicians from ICO
- Collaborators: laboratory team, geneticist, pathologist
- Statistical consultant
- Data manager: fellowship researcher

The approximated length of this project will be 10 months. This study has been designed in 4 main stages:

Stage 1: Preparation and coordination phase (2 months)

─ Project research elaboration, bibliographic research and evaluation

─ Coordination of the research team: Meetings with all the study's team members will be realised to clarify and understand all the objectives of this project. We will plan the timeline of the data collection and of all the study. Besides, we will create a specific study database to facilitate the recognition of the participants and of the variables that we will study.

─ Presentation and Approval of the Clinical Research Ethics Committee: To conduct our research project, we will present our study tothe Clinical Research Ethic Committees. When approved, we will begin with the study.

Stage 2: Field work and data collection (5 months)

─ PC's Identification: We will identify all the patients with pancreatic cancer through the Cancer Registry of Girona and proceed to recollect the data in our study database. When

identified, we will talk with the possible participants in this research project and give them the informed consent that they will or won't sign if they are willing to participate.

─ Exhaustive revision of clinical histories of the cases with missing data: This phase will be managed by a fellowship researcher and/or resident that will exhaustively review all the patient that had been diagnosed of pancreatic cancer between 1994 and 2012 in order to complete the database.

─ Database completion and recording: The Data Manager will oversee all the completion of the database.

─ Database preparation for statistical analysis through the creation of new variables in order to realise descriptive and survival analyse: Statistician consultant will review the database before descriptive and survival analyses. IBM Statistical Package for the Social Sciences (SPSS) version 22 for Windows® will be used.

─ Detection and recollection of tumour samples in order to determine the possible presence of BRCA1/2 somatic mutations in those samples: The pathologist will have to identify and recollect all the tumour samples that are available to proceed with our research project.

─ Tumour tissue sample will be sent to Bellvitge Hospital in order to determinate BRCA1/2 somatic mutations: The Translational Investigation Laboratory that is included in the Pathology Service of Bellvitge Hospital will examine the different tissue samples in order to identify all BRCA1/2 somatic mutations. With this different data, we will examine the frequency of BRCA1/2 somatic mutation and we will be able to calculate the 5-year relative survival rate.

Stage 3: Data Analyses and Final evaluation (2 months)

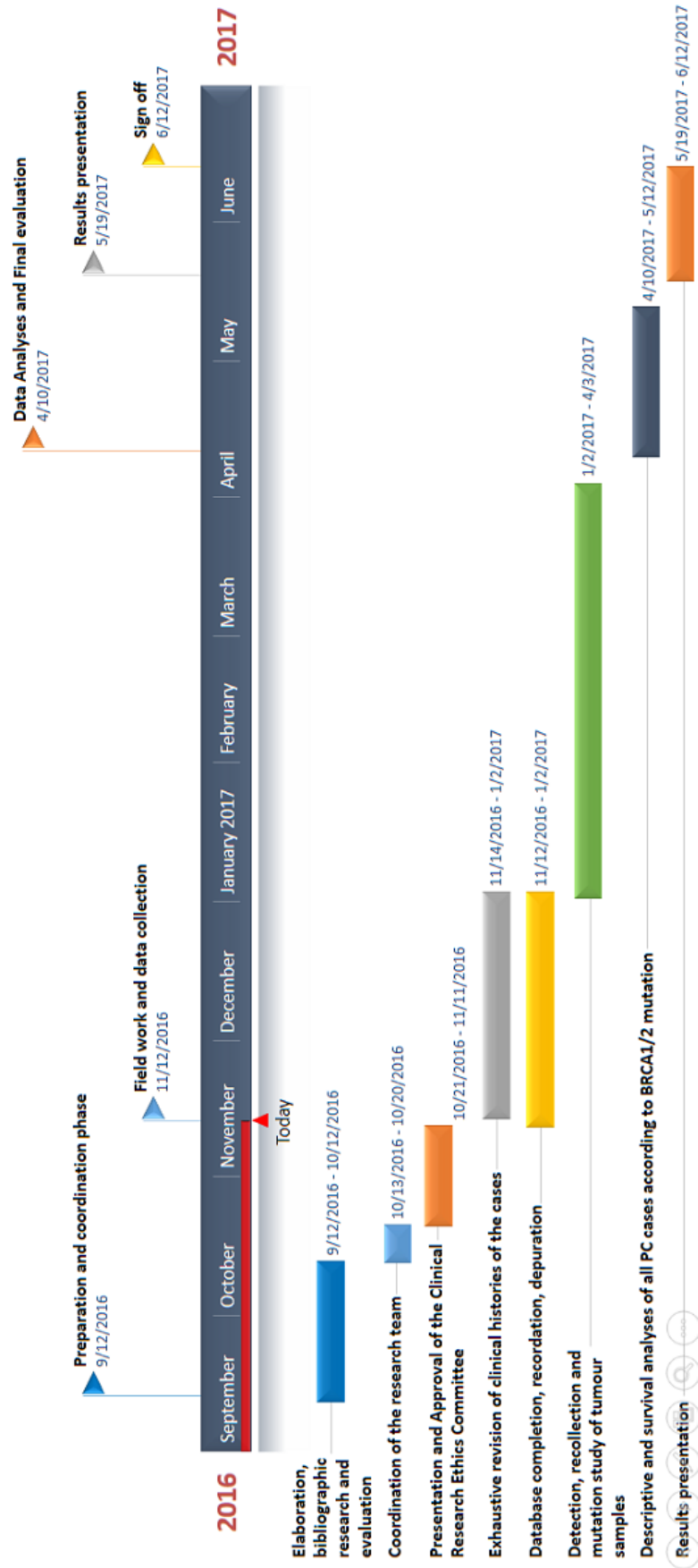─ Descriptive and survival analyses of all PC cases according to BRCA1/2 mutation

Stage 4: Results presentation in international and national clinical meetings and congresses (1 month)

─ Publication

─ National Congresses

─ European Society of Medical Oncology (ESMO)

13.   CHRONOGRAM

14.   AVAILABLE MEANS

	Costs (€)
PERSONAL COSTS	
─ Data manager or fellowship researcher (800x4 months)	3200
SUBTOTAL	**3200**
EXECUTION COSTS	
Goods acquisition and procurement of services:	
─ Samples sending to Bellvitge's Hospital	600
─ Statistical analysis (1100x2 months)	2200
─ Prints, Fax, Photocopies, toners	300
─ BRCA1/2 somatic mutation analysis (500x422)	211000
SUBTOTAL	**214100**
Travel and subsistence costs:	
─ Trip to the others Hospitals in order to review all the clinical histories and Meetings with the investigation staff	150
─ National Congress expenses	800
─ International Congress expenses	1500
SUBTOTAL	**2450**
PUBLICATION	
─ *Paper publication*	500
SUBTOTAL	**500**
TOTAL AMOUNT	**220250**

15.   ANNEXES

ANNEX 1:

WHO histological classification of tumours of the exocrine pancreas

Epithelial tumours

Benign

Serous cystadenoma	8441/0[1]	Serous cystadenocarcinoma	8441/3
Mucinous cystadenoma	8470/0	Mucinous cystadenocarcinoma	8470/3
Intraductal papillary-mucinous adenoma	8453/0	– non-invasive	8470/2
Mature teratoma	9080/0	– invasive	8470/3
		Intraductal papillary-mucinous carcinoma	8453/3
Borderline (uncertain malignant potential)		– non-invasive	8453/2
Mucinous cystic neoplasm with moderate dysplasia	8470/1	– invasive (papillary-mucinous carcinoma)	8453/3
Intraductal papillary-mucinous neoplasm with moderate dysplasia	8453/1	Acinar cell carcinoma	8550/3
Solid-pseudopapillary neoplasm	8452/1	Acinar cell cystadenocarcinoma	8551/3
		Mixed acinar-endocrine carcinoma	8154/3
Malignant		Pancreatoblastoma	8971/3
Ductal adenocarcinoma	8500/3	Solid-pseudopapillary carcinoma	8452/3
Mucinous noncystic carcinoma	8480/3	Others	
Signet ring cell carcinoma	8490/3		
Adenosquamous carcinoma	8560/3	**Non-epithelial tumours**	
Undifferentiated (anaplastic) carcinoma	8020/3		
Undifferentiated carcinoma with osteoclast-like giant cells	8035/3	**Secondary tumours**	
Mixed ductal-endocrine carcinoma	8154/3		

[1] Morphology code of the International Classification of Diseases for Oncology (ICD-O) {542} and the Systematized Nomenclature of Medicine (http://snomed.org). Behaviour is coded /0 for benign tumours, /1 for unspecified, borderline or uncertain behaviour, (/2 for in situ carcinomas) and /3 for malignant tumours.

ANNEX 2: <u>NCCN Guidelines Resectability Criteria</u>

Resectability Status	Arterial	Venous
Resectable	No arterial tumour contact (celiac axis [CA], Superior Mesenteric Artery [SMA], or common hepatic artery [CHA])	No tumour contact with the Superior Mesenteric Vein (SMV) or portal vein (PV) or ≤ 180º contact without vein contour irregularity
Borderline Resectable	<u>Pancreatic head/uncinated process</u>: −Solid tumour contact with CHA without extension to celiac axis or hepatic artery bifurcation allowing for safe and complete resection and reconstruction −Solid tumour contact with the SMA ≤180º −Presence of variant arterial anatomy (ex: accessory right hepatic artery, replaced right hepatic artery, replaced CHA and the origin of replaced or accessory artery), and the presence and degree of tumour contact should be noted if present as it may affect surgical planning. <u>Pancreatic body/tail</u>: −Solid tumour contact with the CA of ≤ 180º −Solid tumour contact with the CA of > 180º without involvement of the aorta and with intact and uninvolved gastroduodenal artery [some members prefer these criteria to be in the unresectable category].	−Solid tumour contact with the SMV or PV of > 180º, contact of ≤ 180º with contour irregularity of the vein or thrombosis of the vein but with suitable vessel proximal and distal to the site of involvement allowing for safe and complete resection and vein reconstruction. Solid tumour contact with the inferior vena cava (IVC)
Unresectable or locally advanced disease	−Distant metastasis (including non-regional lymph node metastasis) <u>Head/uncinated process</u>: −Solid tumour contact with SMA > 180º −Solid tumour contact with the CA > 180º −Solid tumour contact with the first jejunal SMA branch <u>Body and tail</u>: −Solid tumour contact of > 180º with the SMA or CA −Solid tumour contact with the CA and aortic involvement	<u>Head/uncinated process</u> −Unreconstructible SMV/PV due to tumour involvement or occlusion (can be due to tumour or bland thrombus) −Contact with most proximal draining jejunal branch into SMV <u>Body and tail</u>: Unreconstructible SMV/PV due to tumour involvement or occlusion (can be due to tumour or bland thrombus)

ANNEX 3: <u>Potential Indications for Various Therapies in the Treatment of pancreatic Adenocarcinoma</u>

Regimen	Resectable (adjuvant)	Borderline Resectable (neoadjuvant)	Locally Advanced	Metastatic (good performance status)
Gemcitabine	√ (category 1)		√ (category 1 for poor PS)	√ (category 1 for good and poor PS)
Gemcitabine / Albumin-Bound Paclitaxel		√	√	√ (category 1: preferred)
Gemcitabine/ Erlotinib			√	√ (category 1: survival benefit is small)
Gemcitabine/ Cisplatin			√ (especially if possible hereditary cancer)	√ (especially if possible hereditary cancer)
Gemcitabine/ Capecitabine			√	√
Fixed-dose-rate gemcitabine			√	√ (category 2B)
GTX (Fixed-dose-rate gemcitabine/docetaxel/capecitabine)			√(category2B)	√ (category 2B)
5-FU/ Leucovorin	√ (category 1)			
Folfirinox		√	√	√ (category1: preferred)
Capecitabine	√ (category 2B)		√ (category 2B)	√ (category 2B)
Continuous infusion 5-FU			√ (category 2B)	√ (category 2B)
Fluoropyrimidine/ Oxaliplatin			√ (Category 2B)	√
Radiation	√ (fluoropyrimidine - or gemcitabine-based)	√ (subsequent chemoradiation is sometimes included)	√ (in select patients without systemic metastases; fluoropyrimidine- or gemcitabine-based)	√ (palliative only)

ANNEX 3 (continued)

NCCN Categories of Evidence and Consensus

- Category 1: Based upon high-level evidence, there is uniform NCCN consensus that the intervention is appropriate
- Category 2A: Based upon lower-level evidence, there is uniform NCCN consensus that the intervention is appropriate
- Category 2B: Based upon lower-level evidence, there is NCCN consensus that the intervention is appropriate.
- Category 3: Based upon any level of evidence, there is major NCCN disagreement that the intervention is appropriate.

All recommendations are category 2A unless otherwise noted.

ANNEX 4:

Explanation of the next generation sequencing technique that will be used for the detection of BRCA1/2 somatic mutations

MiSeq System, shown in Figure 5, combine the rapid preparation of genomic libraries and the delivery of results in hours using an accelerated turnaround time. We will prepare the genomic library using the Multiplicom Assay which will allow us to directly go to the regions of interest as the sequencing primers, indexes and complementary regions will be attached near to the mutated BRCA1/2 genes.

Figure 5: MiSeq System *extracted from MiSeq System by Illumina®*

─The first step to do is to prepare the sample. We will use custom oligo capture probes that come from the Multiplicom Assay. They flank each region of interest that we are sure to find in the tumour sample. These probes hybridize in unfragmented DNA flanking the region of interest. Through a polymerase, extension and ligation between custom probes will be done across the regions of interest. After this, PCR will add sequencing primers, indexes and complementary regions to the flow cell oligoes; this is represented in Figure 10 and it is called an amplicon.
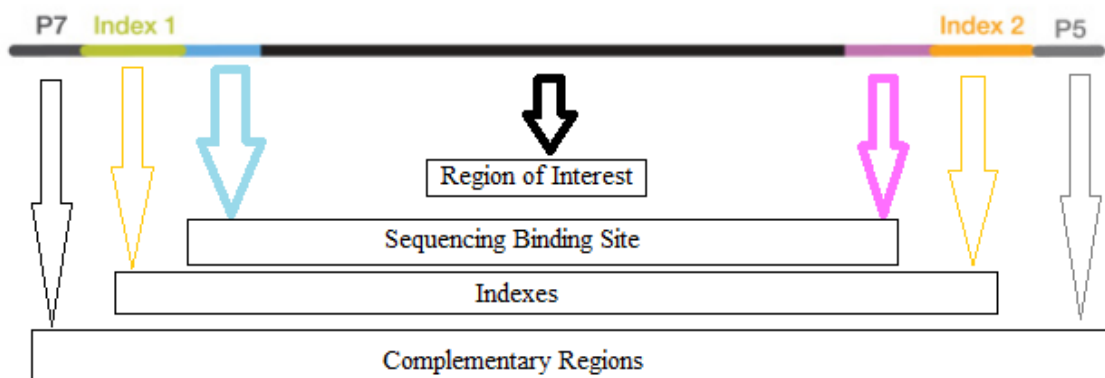

Figure 10: Amplicon ready for cluster, adapted from *Illumina®.*

─The second step is to generate a cluster. Clustering is a process where each fragment molecule is isothermally amplified. The flow cell is a glass slide with lanes represented in Figure 11. Each lane is a channel coated with a lawn composed of two types of oligoes. Hybridization is enabled by the first of the two types of oligoes on the surface of the sample.
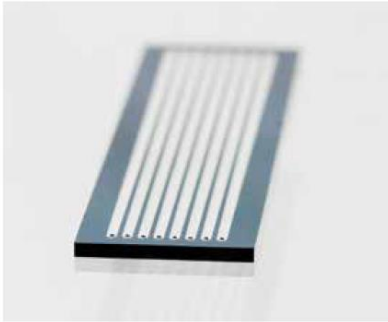
Figure 11: Flow cell, *Illumina®*

This oligo is complementary to the adapter region on one of the fragment strands. A polymerase creates a complement of the hybridized fragment.

The double-strand molecule is denatured and the original template is washed away. The strands are clonally amplified through bridge amplification. In this process, the strand folds over and the adapter region hybridizes to the second type of oligo on the flow cell. Polymerase generate the complementary strand, forming a double-stranded bridge. This bridge is denatured resulting in two single-stranded copies of the molecule that are tethered to the flow cell. The process is repeated over and over, and occurs simultaneously for millions of clusters resulting in clonal amplification of all the fragments. After bridge amplification, the reverse strands are cleaved and washed off, leaving only the forward strands. The 3' ends are blocked to prevent unwanted priming.
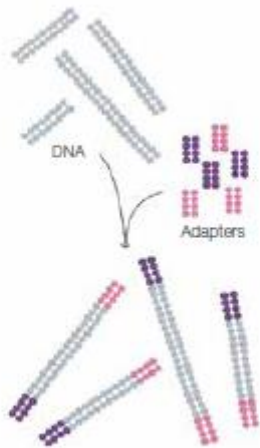
Randomly fragment genomic DNA and ligate adapters to both ends of the fragments.
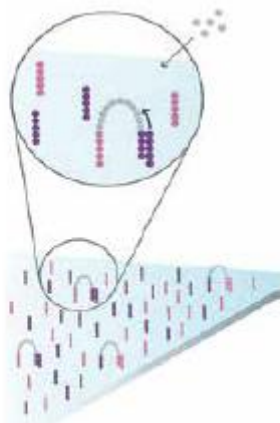
Figure 12a: Preparation of Genomic DNA sample
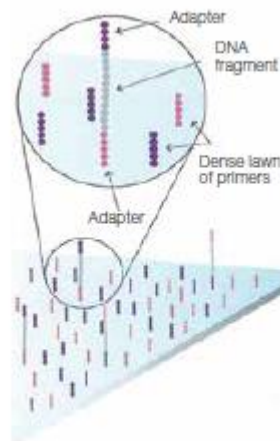
Bind single-stranded fragments randomly to the inside surface of the flow cell channels.
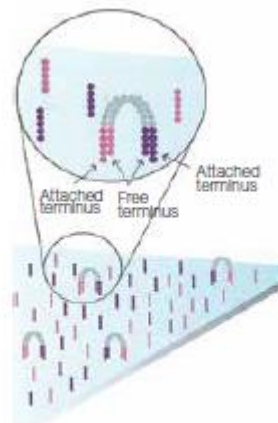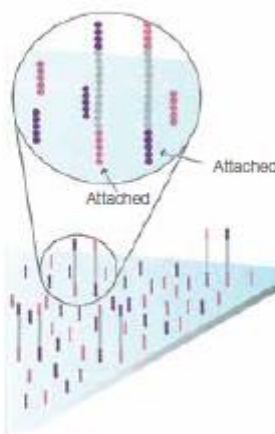
Figure 12b: Attach DNA to surface

ANNEX 4 (Continued)

Add unlabeled nucleotides and enzyme to initiate solid-phase bridge amplification.

Figure 12c: Bridge amplification
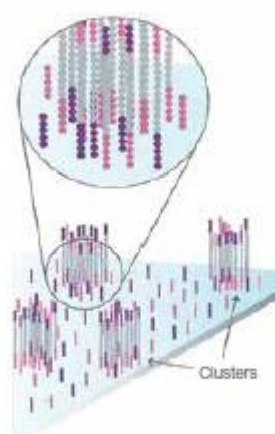

The enzyme incorporates nucleotides to build double-stranded bridges on the solid-phase substrate.

Figure 12d: Fragments become double stranded

Denaturation leaves single-stranded templates anchored to the substrate.

Figure 12e: Denature the double-stranded

Several million dense clusters of double-stranded DNA are generated in each channel of the flow cell.

Figure 12f: Complete amplification

Figures 12 a-f summarizes the clustering process that has been explained above.

─The third step is sequencing. NGS have been introduced in the past decade that allow for massively parallel sequencing reactions. These systems are capable of analysing millions or even billions of sequencing reactions at the same time.

Figure 12 g-l reflects the sequencing and data analysis steps that are done in MySeq System. All this figure 8 is extracted from *Illumina*®.
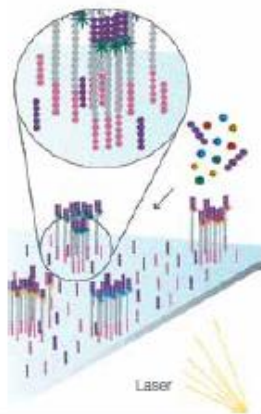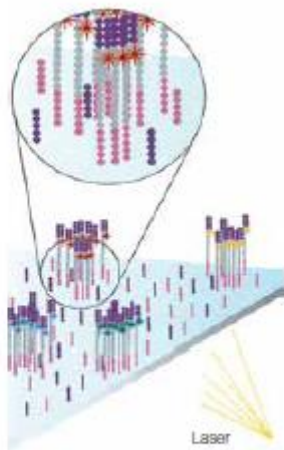
The first sequencing cycle begins by adding four labeled reversible terminators, primers, and DNA polymerase.

Figure 12g: Determine first base

After laser excitation, the emitted fluorescence from each cluster is captured and the first base is identified.

Figure 12h: Image first base

The next cycle repeats the incorporation of four labeled reversible terminators, primers, and DNA polymerase.

Figure 12i: Determine second base

After laser excitation, the image is captured as before, and the identity of the second base is recorded.

Figure12j: Image second chemistry cycle
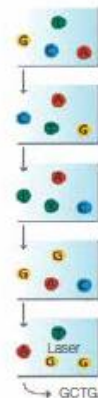
The sequencing cycles are repeated to determine the sequence of bases in a fragment, one base at a time.

Figure 12k: Sequence reads over multiple chemistry cycles

The data are aligned and compared to a reference, and sequencing differences are identified.

Figure 12l: Align Data

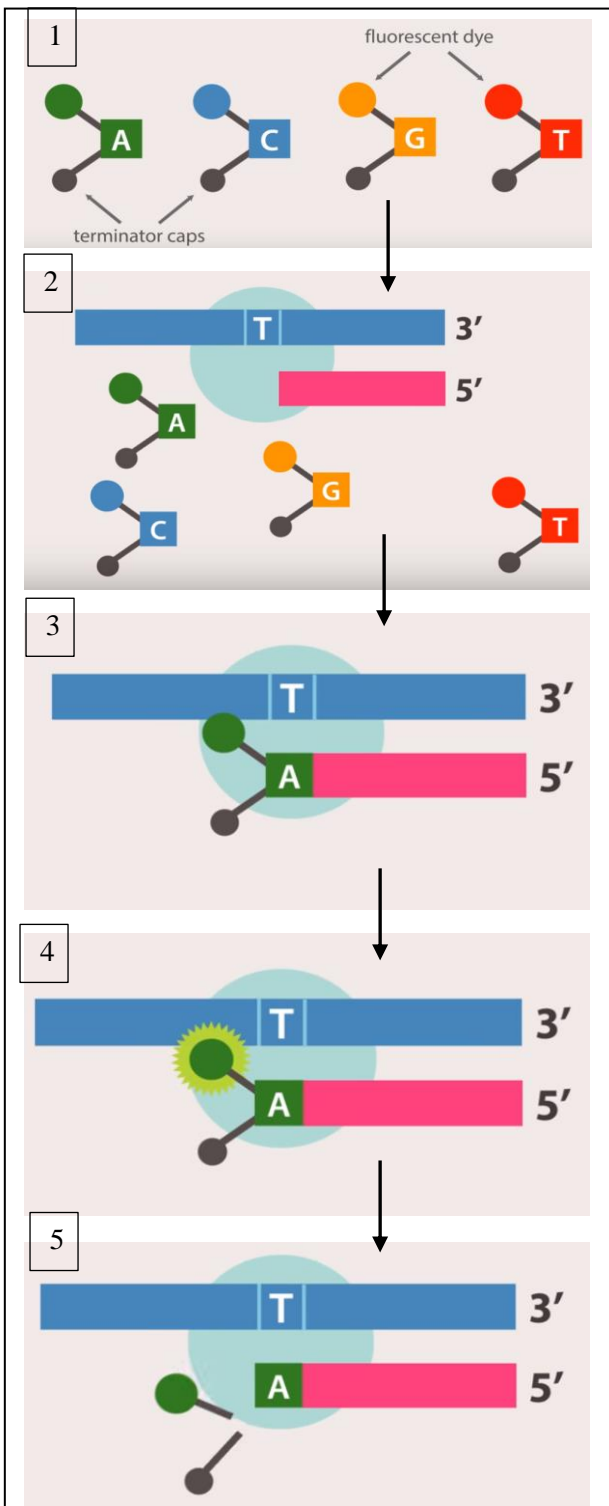Figure 13: SBS technique (1) Nucleotides incorporate reversibly fluorescent dyes (2) the four nucleotides are incorporated for DNA sequencing (3) after incorporation, the non-incorporated nucleotides are washed away (4) machine emits light that actives the fluorescence of the specific nucleotide (5) fluorescent molecule and terminator group are cleaved and washed away. Then, the process will be repeated until the sequencing is completed.

ANNEX 4 (Continued)

In our case, we will use the **Sequencing by synthesis** which is represented in Figure 13. It uses the step-by-step incorporation of reversibly fluorescent and terminated nucleotides for DNA sequencing and is used by the Illumina NGS platforms. All four nucleotides are added to the sequencing chip at the same time and after nucleotide incorporation, the remaining DNA bases are washed away. The fluorescent signal is read at each cluster and recorded; both the fluorescent molecule and the terminator group are then cleaved and washed away. This process is repeated until sequencing reaction is completed. This system, however, has its limitations. As the sequencing reaction proceeds, the error rate of the machine also increases. This is due to incomplete removal of the fluorescent signal which leads to higher background noise levels.

─The final step is data analysis (Figure 12l). This entire process generates millions of reads representing all the fragments. Sequences from pooled sample libraries are separated based on the unique indexes introduced during the sample preparation. For each sample, reads with similar stretches of base calls are locally clustered. Forward and reverse reads are paired creating contiguous sequences. These contiguous sequences are aligned back to the reference genome for variant identification. The paired and information is used to resolve ambiguous alignments.

ANNEX 5:

<u>Informed Consent form for the Utilisation of Clinical Data and Biological Samples surplus of the medical process for Biomedical Investigation</u>

A copy of the full Statement Consent will be given to you

This Informed Consent Form has two parts:

1. Information Sheet (to share information about the research with you)
2. Certificate of Consent (for signature if **you agree** to take part)

Part 1. INFORMATION SHEET

This Inform Consent Form concerns men and women who attended Dr. Josep Trueta Hospital and affiliated centres. In Dr Josep Trueta Hospital as well as the others affiliated centres, biomedical investigation is currently done.

─ **Why do we do biomedical investigation?**

Biomedical investigation is done in order to improve our knowledge about different illnesses. Researches will allow us to find out other ways to diagnose, treat and even prevent specific illnesses. That is why it is such an important part of the medical process. Biomedical investigation requires to recollect clinical data and biological samples of patients and healthy donors. This allow the research team to analyse and get important conclusions about how to deal with illnesses and to benefit directly the patient with news techniques to take care of him.

We are requesting your authorisation for using your clinical data and surplus biological samples that had been made and stored during your current research in order to proceed with our investigation.

Read thoroughly the information contained in this informed consent and the certificate consent that you will find at the end of the document. This document follows the stablished "Ley 14/2007 de Investigación Biomédica" and "Ley Orgánica 15/1999, de Protección de Datos Personales".

─ **Investigation purpose**

We are inviting you to participate in a research project on pancreatic cancer's histological samples containing BRCA1/2 somatic mutation. The title of our research project is "*Impact of BRCA1/2 somatic mutations in patients with pancreatic cancer in Girona, a population-based study*".

ANNEX 5 (Continued)

This project will constitute the beginning of an advanced knowledge of the behaviour of pancreatic cancer. Our research project is dedicated to genetic research in tumour samples that

had been once extracted and stored in our Hospital. We want to use a specific molecular technique in order to find out a specific mutation that only can be seen in pancreatic cancer samples. In our research project, we will study BRCA1/2 somatic mutation. Somatic mutation means a mutation that only can be found in the tumour, not through blood.

> Do not hesitate to tell us if there is something that you do not want your sample to be used for, or if you don't want your sample to be used at all.

─ Biological samples and recollected data

Biological samples, once analyse it, will be returned to the pathology department of origin. We will keep and dispose of these surplus of biological samples that had been once extracted from you during your previous study (in our case, biopsies or cytological samples of Pancreatic Cancer) in order to perform the project research. Furthermore, you and your family will be able to dispose of these biological samples, when available.

─ Confidentiality

Confidentiality will be respected according to the "Ley Orgánica 15/1999, de 13 de diciembre, de Protección de Datos de Carácter Personal" of Spain and approved by the "Real Decreto 1720/2007, de 21 de diciembre", providing the anonymity of the involved patients and allowing the access, modification or destruction of the collected data.

We will act in conformity with the "Real Decreto 411/1996, de 1 de marzo" that regulates all activity related to human tissues, and also with the "Real Decreto 2070/1999, de 30 de diciembre" that controls the extraction and use of human tissues besides organ transplantation.

Compiled data will be used by the investigational team and returned to the pathology department of origin. Codification of the samples will be used in order to identify them by the research team. Only the investigation team will be able to correlate the code with the identity of the patient. Through this process your identity will be protected. Your identity will not be revealed. even if results are published in a scientific journal. Your sample will not be sold for profit and any research which uses your sample will have been approved before you notify it.

– Voluntary participation

Your participation is completely voluntary. If informed consent is signed, you will confirm your participation on the cessation of your biological samples to this project research. If you do not agree the terms, consultation would be pursuit without any change on the follow-up.

– Withdrawal of Consent

If in the future you would like to decline your consent, your biological samples would be destroyed and the data associated to them will be removed from the data-managing department of the ICO. You would be able to apply for the anonymization of the samples which will eliminate the relation with your identity. Cancelation or anonymization would not be extended to the research that had been performed. Current research study will not be affected in any way. In order to do so, you must contact to our investigation team.

– Investigation Results Information

You could ask for the global results of the investigation to the research team. Information will be provided about the actual phase of the investigation and about how your samples has been used <u>except</u> if you have cancelled the consent or if your sample had been anonymized.

The methods that can be used in biomedical investigation could be different from the current clinical practice, which means that you do not have to consider clinical value to the results for you or your acquainted. Even though, if the investigation can provide interesting and relevant clinical or genetical results that may interest your health or your family, the will be communicated if you deem it necessary. In this case, you will have to decide if you want or not to know the results of the investigation. If so, you have to tick the box that you will find on the end of the document.

If you do not wat to be informed, you have to consider that the law states that, when this information is necessary to avoid damage to third parties, an expert Committee will study your case and will decide if it is convenient to tell the information to those affected.

> There may be some words that you do not understand. Please **<u>do not hesitate</u>** to ask questions to us as we will take time to explain it. If later questions come to you, do not hesitate to contact and ask directly to your study doctor or all the members of the crew.

Thank you for your collaboration,

The Investigation Team.

Part 2. CERTIFICATE OF CONSENT

If you have thoroughly understood the information that has been provided to you in the **Information Sheet**, doubts has been resolved and you have decided to collaborate in our research project, please read carefully and sign this sheet:

To anyone that sign this document, you will authorize to the research team to store and use scientific information, including assistance and clinical information's as well as imaging tests and the leftover biological samples that had been done as part of your assistance process in Dr. Josep Trueta Hospital and/or other affiliated centres in order to carry out the biomedical investigation. You will authorize so as long as the Clinical Research Ethics Committee (CEIC) has approved the research project. You grant this authorization after you have been orally informed and after you have read the above information. You confirm that:

1. You authorize that the biomedical investigation uses the leftover biological material used for the diagnosis of your illness:

   National patient ☐ YES ☐ NO   International patients ☐ YES ☐ NO

2. I wish the health relevant information that would be derived from the investigation to be communicated to me or my family:

   ☐ YES   ☐ NO   Email or phone contact:_____

3. I authorize to be contacted in case of information need or additional biological sample:

   ☐ YES   ☐ NO   Email or phone contact:_____

4. I want to the investigation team to respect my wish of the following exceptions regarding the methods and objectives of the investigation:

   _____
   _____
   _____
   _____

ANNEX 5 (Continued)

PARTICIPANT	PERSON TAKING CONSENT	■ WITNESS / ■ TUTOR
Name	Name	Name
First Name	First Name	First Name
DNI	DNI	DNI
Age	Age	Age
Signature	Signature	Signature

Date (Day/Month/Year) and Location: _____

ANNEX 6: ABBREVIATIONS

AUS	Abdominal UltraSound
CA	Celiac Axis
CEIC	Clinical Research Ethics Committee
CHA	Common Hepatic Artery
DM	Diabetes Mellitus
ECOG	Eastern Cooperative Oncology Group
ERCP	Endoscopic retrograde cholangiopancreatography
ESMO	European Society of Medical Oncology
EUS	Endoscopic Ultrasound
EUS-FNA	Endoscopic Ultrasound guided Fine-Needle Aspiration
FOLFIRINOX	Folinic acid (leucovorin) /5-FU / Irinotecan / Oxaliplatin
GGT	Gamma Glutamyl Transferase
GTX	Gemcitabine Docetaxel and Capecitabine
HR	Homologous Recombination
ICH	International Conference on Harmonisation
ICO	Institut Català d'Oncologia
IdibGi	Institut D'Investigació Biomèdica de Girona
IPMN	Intraductal Papillary Mucinous Neoplasm
IVC	Inferior Vena Cava
MDCT	Contrast-Enhanced multidetector
MRCP	Magnetic Resonance Cholangiopancreatography
MRI	Magnetic Resonance Imaging
NCCN	National Comprehensive Cancer Network
NGS	Next Generation Sequencing
PanIN	Pancreatic intraepithelial Neoplasia
PARPi	Poly (ADP-Ribose) Polymerase 1 inhibitors
PC	Pancreatic Cancer
PDAC	Pancreatic Ductal Adenocarcinoma
PET	Positron emission tomography
PS	Performance Status

ANNEX 6 (Continued)

PTC   Percutaneous Transhepatic Cholangiography

PV   Portal Vein

QALY   Quality-adjusted life year

RF   Risk Factors

SBS   Sequencing by synthesis

SMA   Superior Mesenteric Artery

SMV   Superior Mesenteric Vein