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Homologous recombination DNA repair gene alterations identify a subset of pancreatic cancers potentially responding to platinum based therapy

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SOMMARIO

Il sequenziamento massivo dell'intero genoma di un gran numero di cancri del pancreas da parte del consorzio internazionale per il genoma del cancro (ICGC) ha identificato una media di 26 mutazioni per singolo tumore. Le mutazioni di *KRAS* sono l'impronta di questi tumori, seguite dalla inattivazione di *TP53*, *SMAD4* e *CDKN2A*. Accanto a queste alterazioni sono state riscontrate mutazioni in diversi geni che insistono in 10 pathways molecolari, una delle quali è la pathway-BRCA coinvolta nella riparazione del DNA via ricombinazione omologa. Lo scopo di questa tesi è di utilizzare i dati dell'ICGC focalizzandosi su tale pathway, in quanto i geni che a questa partecipano sono coinvolti nella predisposizione ereditaria ai tumori e sono bersaglio di terapie specifiche quali i sali del platino e gli inibitori della poli-ADP-riboso polimerasi.

Lo studio qui presentato ha visto la produzione di 100 xenotrapianti in topo immunodeficiente di cancri del pancreas da pazienti (PDX), per avere a disposizione un modello *in vivo* da utilizzare sia per la caratterizzazione molecolare che per la sperimentazione terapeutica. I 100 PDX e i rispettivi 100 tumori primitivi sono stati oggetto di analisi mutazionale dei geni più comunemente alterati nel cancro del pancreas e dei geni della pathway-BRCA. *KRAS* era mutato nel 96% dei casi; *TP53* nel 66%, *SMAD4* nel 16%, e *CDKN2A* nel 13%. Mutazioni pathogeniche dei geni della pathway-BRCA sono state rilevate nel 13% dei casi: *ATM (1%), BARD1 (1%), BRCA1 (1%), BRCA2 (8%), REV3L (1%),* e *STK11 (1%)*. Tali mutazioni erano mutualmente esclusive. Con l'eccezione di due mutazioni in *STK11* e *REV3L*, tutte le mutazioni erano germinali. Un ulteriore 13% di casi presentava varianti di significato sconosciuto in diversi geni di questa pathway. La concordanza fra i tumori primitivi e gli xenotrapianti è stata riscontrata nel 94% dei casi.

L'esistenza di un sottogruppo significativo (13%) di cancri del pancreas con mutazioni germinali identifica pazienti che possono beneficiare di terapie mirate, e famiglie che possono rientrare in programmi di screening. Inoltre, questo studio ha identificato una serie di varianti di significato patogenico sconosciuto, che possono essere valutate per la potenziale risposta a terapia utilizzando i modelli PDX sviluppati. I PDX, infatti, rappresentano un modello prezioso che rispecchia fedelmente gli assetti genetici della malattia primitiva.

ABSTRACT

Background: The International Cancer Genome Consortium (ICGC) whole genome sequencing effort identified an average of 26 mutations per pancreatic ductal adenocarcinoma (PDAC). *KRAS* mutations are the hallmark, followed by *TP53*, *SMAD4* and *CDKN2A* inactivation. A dominating tail of decreasingly mutated genes follows, but individual pathogenic gene alterations aggregate into ten core molecular pathways, one of which is the homologous recombination (HR) DNA repair genes pathway.

Aim: Within this framework, the aim of this thesis is to avail of ICGC data and focus on the HR DNA damage repair pathway, as genes in this pathway are involved in cancer predisposition and are targets of specific therapies such as platinum salts and innovative PARP inhibitors. The study also envisaged the creation of patient PDAC xenografts (PDX) as a model for primary cancers in molecular stratification and drug validation.

Materials and methods: 100 PDAC and matched PDXs were analysed using targeted next generation sequencing to investigate variants in the genes commonly altered in PDAC and in the homologous recombination (HR) pathway genes.

Results: *KRAS* was mutated in 96% of cases; *TP53* in (66%), *SMAD4* in 16%, and *CDKN2A* in 13%. Pathogenic HR mutations were found in 13% of cases: *ATM (1%), BARD1 (1%), BRCA1 (1%), BRCA2 (8%), REV3L (1%),* and *STK11 (1%)*. These mutations were mutually exclusive. All but those in *STK11* and *REV3L* were germ-line. An additional 13% of cases had variants of unknown significance (VUS) in genes of this pathway. Concordance between PDAC and PDX was found in 94% of cases.

Conclusion: The finding of a significant PDAC subgroup (13%) with germ-line HR gene mutations identifies a group of patients that could profit from existing and novel target therapies as well as screening programs for family members. This study also identifies VUS that may be tested for potential response to therapy availing of the *in vivo* PDX avatars developed herein. PDX in fact, represent a valuable model that faithfully recapitulates the main genetic feature of primary diseases that may be used for novel diagnostics to predict drug responses as well as enable identification of effective therapeutic schemes.

INTRODUCTION

Cancers are uncontrolled growth of cells that have accumulated a number of genetic alterations in multiple cell regulatory systems. The availability of new technologies permits large-scale molecular studies to read the genetic make-up of cancer cells and move towards understanding the biological complexity of health and disease. Deep whole-genome sequencing of cancers shows that structural variation (variation in chromosomal structure) is an important mechanism of DNA damage in carcinogenesis [1].

Recent work exploring the molecular landscape of different cancers has highlighted a high degree of genetic heterogeneity, which necessitates a re-visitation of the classical pathological diagnosis of cancer to take into account tumour heterogeneity at both morphological and molecular level for diagnostics and therapeutics [2,3].

Therefore, personalized medicine requires a significant shift in the clinical routine to include tests that effectively diagnose the disease, determine whether the disease is likely to progress, identify the drug most likely to be effective, whether the patient will suffer side effects from the drug or whether the patient can safely avoid further therapy. In order for this to happen these tests must be created and validated using high quality materials [4,5] complemented by quality information [6] that only organised biobanks may furnish. There is a desperate need of *in vitro* and *in vivo* models derived from the original disease biomaterial to be used through all stages of marker development from discovery through translation, validation and application, as well as for drug/companion diagnostics validation initiatives, selected according to the appropriate target group, before being moved to clinical trials and as a potential for pre-patient tests.

In the present study, Pancreas Cancer was investigated to: 1) identify potential cancer subtypes based on mutational status of genes belonging to the most important pathways altered as part of the International Cancer Genome Consortium [7]; 2) investigate the presence of potentially targetable somatic and germ-line mutations [8]; 3) create clinically validated panels for use in pancreas cancer patient care; 4) confirm the potential of patient tumour xenografts as an adequate representation of the primary tumour and evaluate their use in drug evaluation (**Appendix 1**).

PANCREAS CANCER

Pancreatic Cancer continues to be one of the greatest challenges in oncology of which pancreas ductal adenocarcinoma (PDAC) comprises over 90%. While its incidence globally is low (approximately $1.5x10^5$), PDAC is the 4th leading cause of cancer death in Western societies, and projected to be the 2nd by 2030 [9]. It has a median survival measured in months and a 5-year survival of less than 5%. Despite 50 years of research and therapeutic development this statistic remains largely unchanged [10 11].

Surgery remains the only potentially curative option, but unfortunately less than 20% of patients are eligible for surgical resection [12]. Those who undergo resection and receive adjuvant therapy have a median survival of 12–22 months and a 5-year survival of 20–25% [13]. Neo-adjuvant and adjuvant chemotherapy are only modestly effective with the most recent clinical trial leading to a drug approval extended median overall survival to 8.5 months [14]. There is thus an urgent necessity to better define the molecular pathology of PDAC to improve treatment options for individual patients, to develop novel therapeutic strategies and perhaps re-purpose existing treatment regimens based on molecular diagnostics.

GENOMIC LANDSCAPE OF PANCREATIC CANCER

Activating mutations of the *KRAS* oncogene are the hallmark of PDAC, occuring in 95% of cases. Additional genetic events follow and include the inactivation of *CDKN2A*, *TP53* and *SMAD4* tumour suppressor genes [15].

Previous investigation of the protein encoding genome has suggested that an average of 63 genetic alterations, mainly point mutations, define a core set of 12 cellular signalling pathways and processes that are each genetically altered in 67 to 100% of the tumour samples [16]. Investigation of genomic rearrangement of samples from multiple metastases showed that genomic instability persists after cancer dissemination, that there is continual in heterogeneity among metastases potentially due to clonal evolution, where rearrangements may confer selective advantage on specific clones [17].

Our study of whole exome sequencing on 99 samples within the International Cancer Genome Consortium (ICGC) showed an average number of mutations per samples of 26 but ranges from one to 116 mutations. Activating mutations of *KRAS* are virtually always present, followed by frequent events in *TP53*, *SMAD4* and *CDKN2A*. A dominating tail of ever infrequently mutated genes explains the extreme heterogeneity of these tumours. However, oncogenic point mutations of individual genes aggregate into core molecular pathways including DNA damage repair, cell cycle regulation, TGFβ, chromatin regulation and Axonal Guidance [18] (**Appendix 2**).

A subsequent ICGC study using whole-genome sequencing and copy number variation (CNV) analysis of 100 pancreatic ductal adenocarcinomas (PDACs) highlighted the prevalence of chromosomal rearrangements leading to gene disruption and affecting genes known to be important in pancreatic cancer, including the known ones, the recently discovered *ARID1A* and *ROBO2* [19] and new candidate drivers of pancreatic carcinogenesis, *KDM6A* and *PREX2*. Structural chromosomal variation sub-classified PDAC into 4 subtypes based on frequency and distribution of structural variation. Genomic instability co-segregated with inactivation of DNA maintenance genes (*BRCA1*, *BRCA2* or *PALB2*) and a mutational signature of DNA damage repair deficiency. While a significant number of focal amplifications containing druggable oncogenes were found (*ERBB2, MET, FGFR1, CDK6, PIK3R3 and PIK3CA*), they were at low individual patient prevalence [20] (**Appendix 3**).

Additional ICGC integrated genomic analysis of a larger set of 456 PC, affirmed 32 recurrently mutated genes that aggregate into 10 pathways: KRAS, TGFbeta, WNT, NOTCH, ROBO/SLIT Signalling, G1/S Transition, SWI-SNF, Chromatin Modification, DNA Repair and RNA Processing. Expression profiling defined 4 histopathological subtypes with specific molecular identification: squamous, pancreatic progenitor, immunogenic, and aberrantly differentiated endocrine exocrine (ADEX). Squamous tumours, which have a poor prognosis, are enriched for *TP53* and *KDM6A* mutations, upregulation of the TP63deltaN transcriptional network, and hyper-methylation of pancreatic endodermal cell-fate determining genes such as PDX1, MNX1, GATA6, and HNF1B. Pancreatic progenitor tumours preferentially express genes involved in early

pancreatic development (FOXA2/3, PDX1, MNX1). ADEX tumours display up-regulation of genes that regulate networks involved in *KRAS* activation, exocrine (NR5A2, RBPJL), and endocrine differentiation (NEUROD1, NKX2-2). Immunogenic tumours contained up-regulated immune networks including pathways involved in acquired immune suppression. These data infer that there is molecular evolution in the development of PDAC subtypes that may offer therapeutic opportunities [21] (**Appendix 4**).

AIM OF THE PRESENT THESIS

Within the framework of these developments, the aim of this study is to avail of the information honed from our ICGC studies on pancreatic cancer to create clinically applicable targeted panels that explore DNA damage repair genes to provide a molecular stratification and improve therapeutic strategy of the individual patient cancer. As one of the options for therapeutic strategies, apart from novel strategies, is the re-purposing of therapeutic regimens, the study assesses tumour xenografts as an adequate representation of the primary tumour and their use in the drug validation process.

HOMOLOGOUS RECOMBINATION – DNA DAMAGE REPONSE (HR-DDR)

Homologous recombination repair (HRR) is the process that repairs DNA double strand breaks (DSB) through the alignment of homologous sequences of DNA to maintain genomic stability. HRR acts mainly in the S and G2 phases of the cell cycle. Part of the DNA sequence around the DSB is resected and the DNA sequence on a homologous sister chromatid is used as a template for the synthesis of new DNA at the DSB site. Crucial proteins involved in mediating HRR include those encoded by the *BRCA1*, *BRCA2*, *RAD51* and *PALB2* genes [22] [23]. Given their role in genomic stability these genes suppress tumorigenesis and as such are either confirmed or suspected cancer susceptibility genes[24]. They can be mutated either in the germ-line or somatically in tumours.

Increasing evidence across cancers with mutations in the *BRCA* genes suggests that these tumours have unique vulnerabilities to specific DNA-damaging agents and DNA repair inhibitors [25,26]. Increased risk of PDAC has been associated with pathogenic germ-line mutations in *BRCA1* and *BRCA2*, with estimates of the relative risk of PDAC for mutation carriers between 2.3 and 7 [27,28]. Several previous studies have estimated the prevalence of *BRCA1* and *BRCA2* germ-line mutations in patients with PDAC, which is 4.6%. Other HR-DDR germ-line mutations known to be associated with familial pancreas cancer affect *PALB2* [29,30], *ATM* [31,32], *CHEK1* and *CHEK2* [33].

A recent study, part of the ICGC, investigated 100 PDAC cases by whole genome sequencing and copy number variation analysis [20] (Appendix 3). The study confirmed the prevalence of germ-line BRCA mutations similar to that reported by Holter et al. [27]. Additional germ-line and somatic mutations have been found in six genes in DNA-damage repair pathways (*PALB2, RPA1, REV3L, ATM, FANCM, XRCC4*). Tumours with these mutations were associated with an unstable pattern of genomic structural variation, and comprised 14% of all samples. A significant correlation between the mutation status of the eight identified DNA-damage repair (DDR) genes, the genomically unstable subtype, and BRCA-mutational signature previously described by Alexandrov et al.[34] was also demonstrated.

The study further showed that tumours with these genomic signatures of DDR deficits were associated with response to platinum therapies in patients and patient-derived xenograft models. This suggests the possibility of a subgroup of PDACs defined by compromised DNA repair by homologous recombination [35] that may be used to identify patients that benefit from therapies targeting DDR pathways.

TREATMENT OPTIONS IN HR DEFECTIVE PDAC

Pancreatic cancer has one of the worst outcomes among all solid malignancies [36]. Gemcitabine, the standard for treatment of advanced pancreatic cancer results in extension of the median survival of less than six months [37] . The addition of EGFR inhibitor, erlotinib, to gemcitabine resulted in scarce improvement in median survival [38,39].

Testing for *BRCA1* and *BRCA2* mutations in breast and ovarian cancer has become routine in those considered high risk based on family history. Although PDAC patients with *BRCA* mutations are considered fewer, given the extremely poor prognosis of PDAC, these patients with either, germ-line and somatic *BRCA* mutations or indeed other mutations in the HR-DDR genes may benefit from platinum-based regimens and the newer class of drugs known as poly (ADP-ribose) polymerase (PARP) inhibitors [40].

Recent retrospective reviews suggest that platinum-based regimens (in particular cisplatin, not usually used in patients with PDAC) may increase overall survival in patients with *BRCA*-mutant PDAC [41,42]. Furthermore, early evidence from phase I/II trials of PARP inhibitor monotherapy have shown promising responses in PDAC patients with germ-line *BRCA* mutations [43]. The PARP inhibitor, Olaparib, has recently been approved for the treatment of ovarian cancer in *BRCA*-mutation carriers and thus presents an interesting option also for treatment of PDAC patients harbouring *BRCA* mutations [44,45].

PANCREAS DUCTAL CANCER XENOGRAFT (PDX) AS PATIENT AVATARS

A major challenge in investigating PDAC genomes is the generally low malignant epithelial cell content of this cancer type, which can adversely impact on the sensitivity of mutation detection. One way of enriching for cellular content is by xenografting the primary tumour tissue in immuno-deficient mice. This also permits continual proliferation of tumour tissue for additional analysis [18] (Appendix 2).

One issue regarding the potential of PDX as representative tumour tissue regards the clonal selection pressure when the primary tumour tissue is transplanted in the

murine host [46]. This issue is compounded by the heterogeneity of the primary tumour tissue where the implanted primary tissue may only partially represent the entire composition of the patient malignancy.

Another issue that requires consideration is due to the lack of human stromal components. Cancer-associated fibroblasts are replaced by murine elements and adaptive immune system is missing.

Despite these issues, xenografts are useful models for translational cancer research [47-49]. It has been suggested that the successful xeno-engraftment may be indicators of poor prognosis [46] and representative of patient metastatic cancers [50]. Furthermore, there is developing potential to use xenograft to determine treatment in the personalized therapy of patient treatment based on the observation of xenograft based response to specific drug combinations [51].

STUDY DESIGN

This study has created a collection of PDX from treatment naïve surgically resected PDAC. Using amplicon based sequencing, both primary tumours and matched PDX will be characterised for the 20 genes most frequently involved in pancreas cancer pathogenesis and alterations in HR-DDR.

MATERIALS AND METHODS

Cases

A total of 100 tissue samples from 100 patients, acquired by the ARC-Net biobank at the University and Hospital Trust of Verona - Italy, were selected based on the availability of matched primary (patient) and derived xenograft fresh frozen cancers (**Table 1**). All cases were classified according to WHO 2010 [52] and staged according to AJCC/UICC 7th edition [53].

Table 1. Cancer Type of the 100 patients

Cancer type	subtype	
PDAC	Common type	84
	Clear cell*	3
	Adenosquamous	3
	Focal squamous	2
	IPMN associated**	3
	Colloid	1
	Periampullary	1
Acinar		2
Ampullary		1

* one case was mainly composed of clear cells, one case had focal clear cell areas
** one case had squamous aspects

Ethics

The materials from all patients were collected by the ARC-Net biobank under Program 1885 protocol 52438 23/11/2010 and project approval program 2172 protocol 26773 23/05/2012, approved by the Verona University Hospital Ethics Committee. Protocols for collection included informed consent, approved under this program, from the patient to collect residual tissue samples for molecular research. The program includes approved amendments to address the later regulatory issues of sensitive data in genomic studies and a separate informed consent for access to sensitive data. These informed consents, received from patients, are registered in the biobank database together with samples collected. This approval covers biological material collection for

the ARC-Net coordinated biobank of samples from all cancer patients, including neoplastic and associated local and distant normal tissue.

Tumour xenografts were produced under the ministerial decree no. 107/2012 – B and 108/2012 – B issued by the Ministry of Health based on the legislative decree 106/92 regarding the protection of animals used in scientific research.

Sample Collection

Biological material from patients undergoing surgical resections for cancer is collected for the ARC-Net coordinated research biobank by a parallel pathology process to ensure the quality of the tissue samples collected. The resected organs are immediately vacuum packed using Tissue Vacuum (Kaltek srl) in the operating theatre. The vacuum packed material is then transported to the grossing room and is held in a fridge at +4°C until processing. This procedure reduces cold ischemia time and increases the integrity of the primary tissue samples and the potential viability of cells for implantation in mice to produce pancreatic ductal adenocarcinoma xenografts (**Appendix 5**). The pathologist selects neoplastic and associated local and distant normal tissue. One fresh neoplastic sample is collected and placed in RPMI transport medium for xenografting. Additional samples of neoplastic tissue are snap frozen over LN2 before being conserved at -80°C. A contiguous en-face frozen section is prepared for quality control. The process is detailed in **Figure 1**.

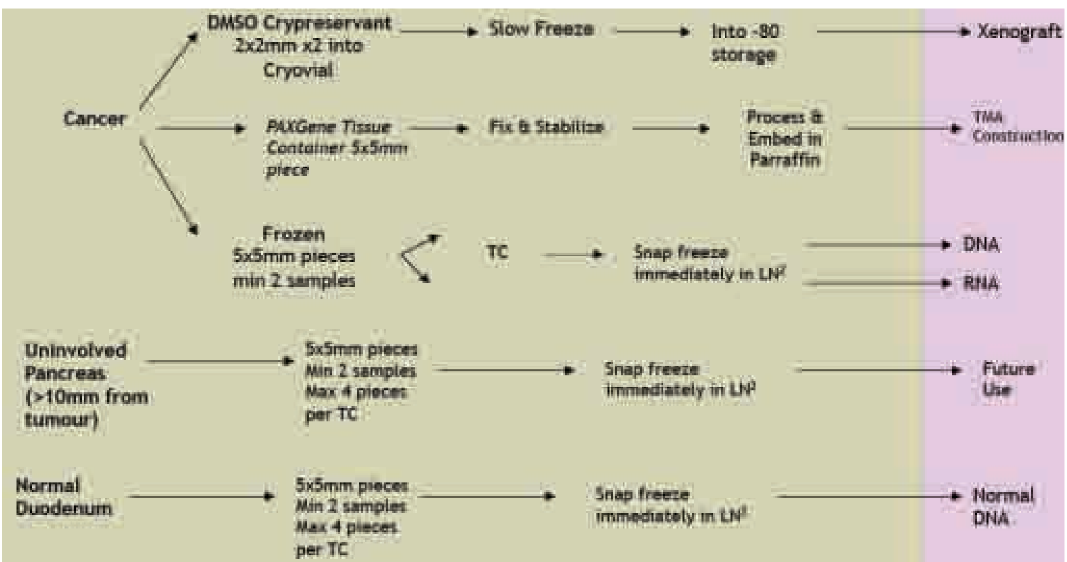


Figure 1: Scheme illustrating the process of biobanking of cancer/normal tissues for the indicated use.

Mouse Implantation and Xenograft Harvesting

Each sample harvested for xeno-transplanting was fragmented into nine pieces of 0.2mm^3 and implanted subcutaneously in three immuno-deficient *Nu/Nu* mice, one fragment in the nape and one fragment in the right and left flank of each mouse. Once established, tumours were grown to a size of 1cm^3, at which point they were harvested, divided, and one fragment was re-transplanted into further mice to bank sufficient tissues for experimentation (up to third passage) while one fragment of the same tumour was snap frozen according to the protocol for primary tissue and one fragment was paraffin fixed formalin embedded. Utilization of the *Nu/Nu* mouse model, which is characterized by high immune deficiency, enabled the establishment of a significant biobank of PDXs, with a high rate (79%) of successful engraftment and propagation due to the increased possibility to xenotransplant directly after harvesting of tumour tissue.

A set of PDX, were harvested and propagated to cohorts of mice for treatment with drugs relevant for the treatment of pancreatic carcinomas. Treatments included Gemcitibine, nab-paclitaxel, combinations Gemcitibine and Erlotinib, Gemcitibine and nab-paclitaxel, 5FU and Oxaliplatin..

Investigation of Heterogeneity

For a subset of samples, in order to investigate heterogeneity using specific comparative primary and xenografted tissue, the primary specimen was harvested, by placing a cut at one end to orient the specimen and divide it along the horizontal axis to create two specular samples, one for freezing and one for implanting. A histological image was created from the specular divide. The portion for implanting was divided into central, intermediate and peripheral, then sub-divided into upper and lower and again into three fragments for implantation in *Nu/Nu* mice (**Figure 2**). Orientation was preserved to correlate the portion implanted with respect to the histology and frozen sample.

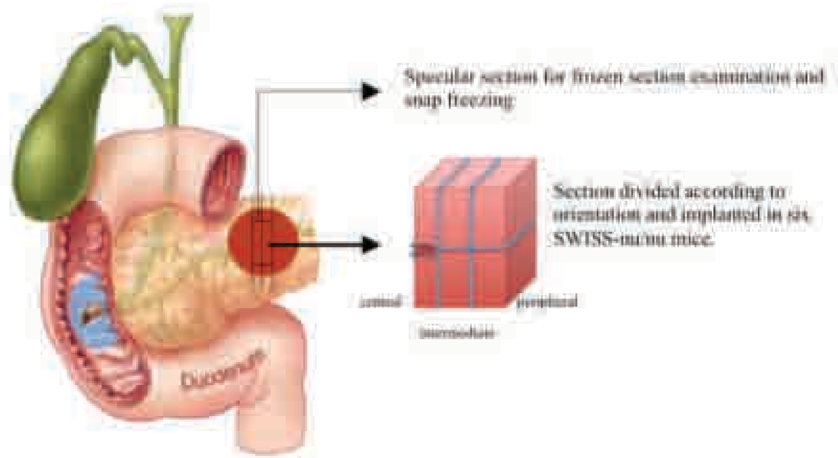


Figure 2: Division of specimen for implantation.

DNA extraction and qualification

Neoplastic cellularity was assessed by microscopic examination and, when below 50%, enriched by manually micro-dissecting four consecutive 10 µm thick sections. Genomic DNA from frozen tissue was extracted using the QiAamp DNA Mini Kit (Qiagen). Purified DNA was quantified and its quality assessed using Nano-Drop (Thermo Fisher Scientific) and Qubit (Thermo Fisher Scientific)) platforms [54] (**Appendix 6**). DNA suitability for PCR downstream applications was further evaluated

through BIOMED 2 PCR multiplex protocol and the PCR products were evaluated by DNA 1000 Assay (Invitrogen Life Technologies) on the Agilent 2100 Bioanalyzer on-chip electrophoresis (Agilent Technologies) [55].

Next-Generation Sequencing of Multiplex PCR Amplicons

Three multi-gene panels were designed based on the pathway specific signatures identified through published whole genome sequencing of a set of PDACs [20] (Appendix 3). The PDAC basic panel explores hotspot regions of 20 cancer genes. The two DNA damage repair panels (BRCA and BRCA+) explore the entire coding sequence of 18 cancer genes. The PDAC basic panel consists of 113 amplicons, the BRCA panel 677 and the BRCA+ 413 amplicons.

The panels have been designed to produce amplicons of an average length of 150 bp (range 100-250) that permits application on partially degraded DNA from FFPE tissues. To ensure complete coverage of the regions of interest, the primers were designed to produce partially overlapping amplicons. In order to avoid primer dimer formation, BRCA and BRCA+ panels each avail of two separate multiplex PCR primer pools. The contents of the custom panels are outlined in **Table 2** and details of the custom panels are detailed in Supplementary Tables S1, S2, and S3.

Table 2. Targeted Next Generation Sequencing Gene

PANEL	PDAC	PDAC	BRCA	BRCA+
GENES	APC	FLT3	BRCA1	BARD1
	ATM	GNAS	BRCA2	BRIP1
	BRAF	HRAS	ATM	CHEK1
	CDH1	KDR	PALB2	CHEK2
	CDKN2A	KRAS	RPA1	FAM175A
	CTNNB1	NRAS	REV3L	MRE11A
	EGFR	PIK3CA	STK11	PTEN
	ERBB2	SMAD4		NBN
	ERBB4	TP53		RAD51B
	FBXW7			RAD51C
	FGFR3			RAD51D

DNA Sequencing

Twenty nanograms of DNA were used for each multiplex PCR amplification. The quality of the obtained libraries was evaluated by the Agilent 2100 Bioanalyzer on-chip electrophoresis (Agilent Technologies). Emulsion PCR was performed with the OneTouch2 system (Life Technologies). Sequencing was run on the Ion Torrent Personal Genome Machine (PGM, Life Technologies) loaded with 316 (50-gene panel) or 318 chips (6-gene panel). Data analysis, including alignment to the hg19 human reference genome and variant calling, was done using the Torrent Suite Software v.3.6 (Life Technologies). Filtered variants were annotated using the SnpEff software v.3.1. Alignments were visually verified with the Integrative Genomics Viewer; IGV v.2.2, Broad Institute.

DNA Sanger Sequencing

Mutations detected by deep sequencing for *KRAS, TP53, BRCA1* and *BRCA2,* were validated by Sanger sequencing. Matched normal DNA samples were also seqeuenced to verify whether the mutations were germ-line or somatic. PCR products were purified using Agencourt AMPure XP magnetic beads (Beckman Coulter) and labelled with BigDye® Terminator v3.1 (Applied Biosystems). Agencourt CleanSEQ magnetic beads (Beckman Coulter) were used for post-labelling DNA fragment purification, and sequence analysis was performed on the Applied Biosystems 3130xl Genetic Analyzer.

Statistical analysis

Data analysis, including alignment to the hg19 human reference genome and variant calling, was done using the Torrent Suite Software v4.6 (Life Technologies). Filtered variants were annotated using a custom pipeline based on vcflib (https://github.com/ekg/vcflib), SnpSift [56], the Variant Effect Predictor (VEP) software [57] and NCBI RefSeq database. Alignments were visually verified with the Integrative Genomics Viewer (IGV) v2.3 [58].

Bioinformatics analysis of PDX to remove murine derived sequences

The presence of mouse DNA in PDX samples is not indifferent. This contamination may cause erroneous mutational calls when aligning to hg19 reference, given the high homology between human and murine for some genes. Some sequence regions of murine origin are recognizable by visual inspection of the sequencing data with the **IGV** software due to a definite pattern of variations between human and murine genomes. However, some regions of high homology are virtually unrecognizable with standard alignment setting against the sole hg19 reference; this may cause erroneous mutation calls or correct mutation calls with erroneous variant allele frequency. Therefore, a specific PDX-oriented reference sequence was created, containing both human hg19 and mouse mm10 reference genomes [59]. PDX sequences were aligned against this reference genome to distinguish DNA originating from murine or human chromosomes and permit the murine component to be subtracted during data analysis. Two regions, one in *PTEN* (exon 1) and one in *REV3L* (exon 2), showed perfect homology between mouse and man and could not be resolved. Therefore, DNA from murine models was sequenced using both PDAC basic and BRCA panels, to confirm the removal of murine sequences from human reference, using this alignment strategy in presence of 100% mouse DNA. Minimal residual murine regions, that remained mapped to the human reference, were filtered by the software due to their low mapping quality.

Clinical Significance Classification of Variants

Variants were ranked using a 5-tiered schema in accordance with the American College of Medical Genetics (ACMG) guidelines for reporting sequence variations [60]: class 5 = pathogenic; class 4 = likely pathogenic; class 3 = uncertain significance; class 2 = probably no pathogenicity; 1 = no pathogenicity. Class 4 and 5 variants are collectively termed pathogenic [61]. Variants with a score of 3 and above were further examined in the published literature and inherited mutation databases including COSMIC, Catalogue of Somatic Mutations in Cancer (http://cancer.sanger.ac.uk/cosmic) [62] ClinVar (http://www.ncbi.nlm.nih.gov/clinvar), and BIC, Breast Cancer Information Core (http://research.nhgri.nih.gov/bic/) to verify pathogenicity.

RESULTS

PDAC xenografted samples (PDX) from 100 patients were sequenced with three multigene panels exploring hotspot mutational regions of 20 genes most frequently altered in PDAC and 18 genes involved in homologous recombination DNA damage repair (HR-DDR). For 79 cases the corresponding matched primary cancer sample was also sequenced to investigate the concordance between primary and xenograft. Matched normal DNA samples for cases with HR-DDR mutations were sequenced to identify whether the mutation was germ-line or somatic.

Cohort demographic

Our study was carried out on 100 patient cases. Criteria selection included PDAC cases resected with curative intent for which both primary and tumour grafted PDX frozen tissue were available. The cohort comprised 50 men and 50 women with a mean age of 65 and median of 67. Based on sex, the mean age for men was 64.6 (median = 66) while the mean age for women was 65.3 (median = 68). The histo-pathological data of the cohort are summarized in **Table 3**.

PDAC basic hotspot gene mutations on PDX Tumours

The results are summarized in **Table 4** and Supplementary Table S4**.** Of the 20 genes investigated in the PDAC basic panel, only 8 were mutated. *KRAS* was mutated in 96 cases (96%); the four cases lacking the *KRAS* mutation were the two acinar carcinomas (#1763, #2693), the single ampullary cancer (#2648) and one of the three IPMN-associated cancers (#2636). *TP53* was mutated in 66 cases (66%), *SMAD4* in 16 (16%), and *CDKN2A* in 13 (13%). *GNAS* was mutated in two cases (2%), (#1524 and #1841) both PDAC. *APC* was mutated in three cases (3%), (#1954, #1885, #1753) two PDAC and the single Colloid. *PIK3CA* was mutated in the single ampullary cancer (#2648) (1%) *FBXW7* was mutated in one PDAC case (1%) (#1542). Two cases had no mutations, one was an IPMN-associated cancer (#2322) and one an acinar cancer (#1763).

Table 3. Clinico-pathological Parameters of Cohort		
Variable	No	%
Sex		
Male	50	50
Female	50	50
Age		
Mean	65	
Median	67	
Range	30 - 83	
Tumour Site		
Head	78	78
Body	12	12
Body-Tail	2	2
Tail	5	5
Istmus	1	1
Peri-ampullary	2	2
Resection Margins		
R0	58	58
R1	42	42
GRADE		
1	5	5
2	59	59
3	36	36
T stage		
T1	1	1
T2	3	3
T3	95	95
T4	1	1
N stage		
N0	16	16
N1	84	84
M stage		
M0	98	98
M1	2	2
Overall Stage		
IA	1	1
IB	2	2
IIA	12	12
IIB	83	83
III	1	1
IV	1	1

Twenty seven cases had one mutation, 49 cases two mutations, 15 three mutations, 6 cases four mutations, 1 case five mutations, while 2 cases had no mutations. Of the 27 cases with a single mutation, 26 had *KRAS*, and one case had a *SMAD4* mutation. Of the 49 cases having 2 mutations, all but one had a *KRAS* mutation in combination with another mutation; 43 with *TP53*, two with *SMAD4,* one with *APC*, one with *CDKN2A*, and one with *GNAS*. Only one case, of ampullary cancer, had a *PIK3CA* and *TP53* mutation (#2648). Cases with three or more mutations favoured, either *KRAS*, *TP53*, *SMAD4*, or *KRAS*, *TP53*, *CDKN2A* combinations. Of the 15 cases with three mutations, 8 were in *KRAS/ TP53/ SMAD4*, 6 were in *KRAS/ TP53/ CDKN2A*, with one in *KRAS/ TP53/ GNAS*. Of the 6 cases with 4 mutations, four cases were in *KRAS/ TP53/*

CDKN2A / *SMAD4,* 1 case was in in *KRAS/ TP53* / *CDKN2A* / *APC* and one case was in *KRAS/ TP53* / *CDKN2A* / *FBXW7*. 1 case had 5 mutations (#1841) in *KRAS, TP53, CDKN2A, SMAD4* and *APC*.

Table 4. Gene Mutations from the PDAC basic panel in 100 PDX			
Gene symbol	Gene name and protein function	No.	%
KRAS	Oncogene; GTPase; activation of MAPK activity	96	96%
TP53	Tumour suppressor p53; DNA damage response	66	66%
SMAD4	Mothers against decapentaplegic homologue 4; BMP signalling pathway	16	16%
CDKN2A	Cyclin-dependent kinase inhibitor 2A; G1/S transition of mitotic cell cycle; tumour suppressor	13	13%
GNAS	GNAS complex locus; signal transduction pathways	2	2%
APC	Adenomatous polyposis coli; tumor suppressor gene	3	3%
PIK3CA	Phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha, metabolic pathways	1	1%
FBXW7	F-box/WD repeat-containing protein 7	1	1%

Homologous Recombination DNA Repair (HR-DDR) genes

The results are summarized in **Table 5** and Supplementary Table S5. 33 cases revealed variants in nine genes in the HR-DDR panels: 13 cases had confirmed pathogenic mutations, which were associated with variants of unknown significance (VUS) in 3 of the cases; a further 13 cases had VUS; 7 cases had only benign variants which were also found in 5 cases having either pathogenic or VUS.

Table 5. Mutated HR-DDR genes in PDX				
Gene symbol	Gene name and protein function	path.	unknown	benign
BRCA1	Tumour suppressor through DNA damage repair	1	0	6
BRCA2	Breast Cancer 2; tumour suppressor through DNA damage repair	8	1	2
ATM	Ataxia telangiectasia mutated; DNA damage repair	1	4	4
BARD1	BRCA1-associated RING domain protein 1; tumour suppressor	1	1	0
CHEK1	Checkpoint kinase 1; DNA damage response	0	2	0
FAM175A	Family With Sequence Similarity 175, Member A; DNA damage response and double-strand break (DSB) repair	0	1	0
PALB2	Partner and localizer of BRCA2; double strand break repair	0	1	0
REV3L	Protein reversionless 3-like; translesion synthesis (TLS)	1	5	0
STK11	Serine/threonine kinase 11 (STK11); liver kinase B1 (LKB1); renal carcinoma antigen NY-REN-19; tumour suppressor; cell metabolism, cell polarity, apoptosis and DNA damage response	1	2	0

Confirmed pathogenic variants

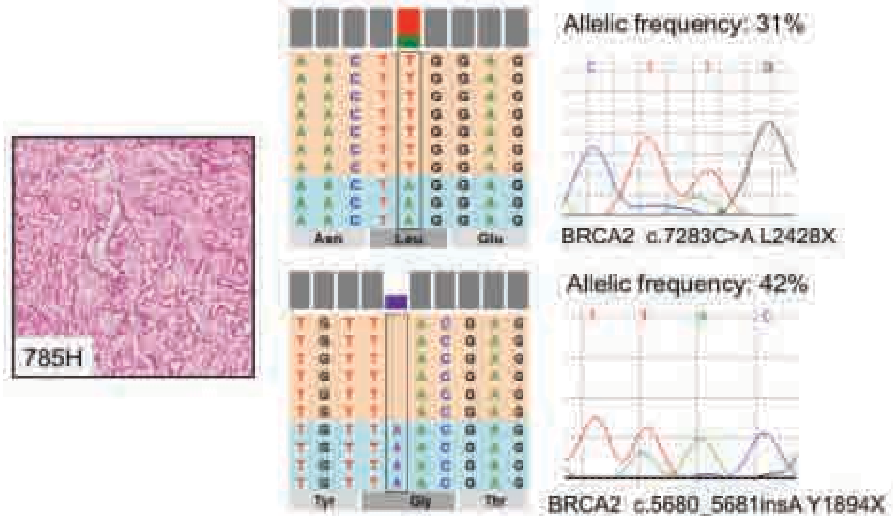
Confirmed pathogenic mutations were found in 13 cases (13%), and affected 6 genes: *ATM, BARD1, BRCA1, BRCA2, REV3L,* and *STK11*. In particular, 9 cases had *BRCA1/2* mutations and 4 cases harboured mutations in HR-DDR genes other than *BRCA1/2*. All cases had single mutually exclusive mutations (**Table 6**).

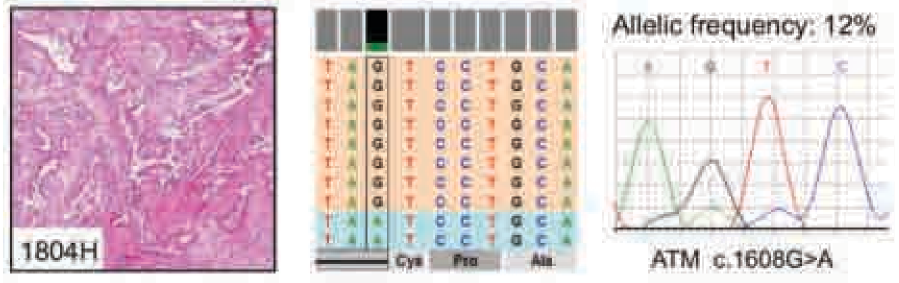
Nine cases had with mutually exclusive *BRCA1/2* mutations: one *BRCA1* germ-line and 8 *BRCA2* germ-line. Four of the *BRCA2* germ-line mutations were stop gains, one an in-frame insertion, all recorded as pathogenic in both the ClinVar and BIC databases. The other four *BRCA2* germ-line mutations were frame-shift variants resulting in a premature stop codon, a feature of pathogenic mutations, and recorded as pathogenic in the BIC database. The one pathogenic *BRCA1* mutation was germ-line a stop gain recorded as pathogenic in both ClinVar and BIC databases.

Two cases had each two *BRCA2* mutations, consisting of a confirmed pathogenic germ-line mutation and a somatic mutation resulting in a premature stop codon. Both germ-line mutations are recorded as pathogenic in the BIC database, and the somatic mutations seemingly cause the biallelic inactivation of *BRCA2* in these cases (**Figure 3**).

Four cases harboured HR-DDR genes other than *BRCA1/2: ATM, BARD1*, *REV3L*, and *STK11*. One case harboured a germ-line *ATM* stop gain mutation recorded as pathogenic in the ClinVar database. One case harboured a germ-line *BARD1* stop gain, one case a somatic *REV3L* frame-shift variant and one case a somatic *STK11* frame-shift variant, each resulting in a premature stop codon (**Figure 4**). As premature stop codons are a feature of pathogenic mutations, these variants were considered potentially pathogenic in nature (Table 6).

Seven of these cases had only *KRAS* mutations, five had *KRAS* and *TP53* mutations and one case had *KRAS*, *TP53* and *CDKN2A* mutations.


785H
Allelic frequency: 31%
Asn
Leu
Glu
BRCA2 c.7283C>A L2428X
Allelic frequency: 42%
Tyr
Gly
Thr
BRCA2 c.5680_5681insA Y1894X


1804H
Cys
Pro
Ala
Allelic frequency: 12%
ATM c.1608G>A

Variants of unknown significance (VUS)

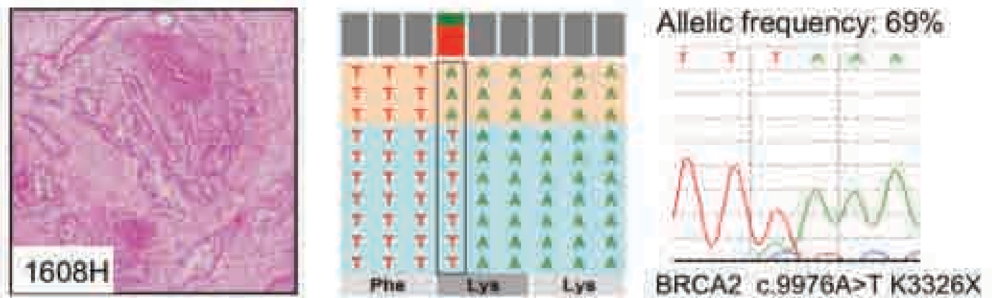
Sixteen cases featured VUS in 8 genes: *ATM, BARD1, BRCA2, CHEK1, FAM175A, PALB2, REV3L,* and *STK11* **(Table 7**). Three cases had both pathogenic and VUS variants; of these, two had pathogenic variants in *BRCA2* and one a pathogenic mutation in *ATM*, all associated with a VUS variant in *REV3L*. 12 cases had a single VUS: three in *ATM*, one in *BRCA2*, two in *REV3L*, two in *CHEK1*, two in *STK11*, one in *FAM175A*, one in *BARD1*. 1 case had two VUS, in *ATM* and *PALB2*.

Considering only VUS, 14 cases had mutually exclusive variants: Variants were confirmed germ-line for one *BARD1*, *CHEK1* mutations, two *STK11, ATM* and three *REV3L*; one *ATM, REV3L* and *CHEK1* were somatic. One case had germ-line missense mutations in both *ATM* and *PALB2*; one case had missense mutations in *BRCA2* (germ-line) and *REV3L* (somatic).

Benign variants and Risk polymorphisms

Twelve cases had **benign** variants: 8 in *BRCA1*, 2 in *BRCA2*, and 4 in *ATM*. Seven of these cases had neither pathogenic mutations nor VUS in the HR-DDR panels.

Furthermore five cases had a *BRCA2* genetic polymorphism (c.9976A>T; p.Lys3326* also called K3326X) that is known to be a cancer risk factor for different cancer types including of breast, lung and upper aero-digestive tract. Of the five cases identified with this polymorphism, one case had a missense variant in *ATM* while one case had a DNA damaging stop codon mutation in *REV3L*. The remaining three cases did not have mutations in the HRDR genes, however all had *KRAS* and *TP53* mutations (**Figure 5**).



[illegible] c.9976A>T; p.Lys3326* [illegible]

Primary and PDX concordance for PDAC basic and HR-DDR panels

79 matched primary tumours were investigated with the 20 gene PDAC basic panel and the HR-DDR panels.

<u>*PDAC basic gene panel mutations in Primary Tumours*</u>

Eight genes were mutated, *KRAS* (73/77, 95%), *TP53* (48/77, 62%), *SMAD4* (16/77, 21%), *CDKN2A* (8/77, 12%), *GNAS* (2/77, 2.5%), *APC* (2/77, 2.5%), PIK3CA (1/77, 1%), FBXW7 (1/77, 1%) **Table 8**.

Table 8. Gene Mutations from the PDAC basic panel in 79 PDAC

Gene symbol	Gene name and protein function	No.	%
KRAS	Oncogene; GTPase; activation of MAPK activity	73	95%
TP53	Tumour suppressor p53; DNA damage response	48	62%
SMAD4	Mothers against decapentaplegic homologue 4; BMP signalling pathway	16	21%
CDKN2A	Cyclin-dependent kinase inhibitor 2A; G1/S transition of mitotic cell cycle; tumour suppressor	8	10%
GNAS	GNAS complex locus; signal transduction pathways	2	3%
APC	Adenomatous polyposis coli; tumor suppressoer gene	2	3%
PIK3CA	phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha, metabolic pathways	1	1%
FBXW7	F-box/WD repeat-containing protein 7;	1	1%

HR-DDR genes in Primary Tumours

25 cases had at least one mutation. Nine genes were mutated: *BRCA1, BRCA2, ATM, PALB2, REV3L, STK11, BRIP1, CHEK1, BARD1*. 15 cases had pathogenic mutations, 10 cases had mutations of unknown significance with 3 cases having both (**Table 9**). 5 cases had missense variants in *BRCA1* or *ATM* registered in ClinVar as benign.

Table 9. Mutated HR-DDR genes in 79 pancreatic ductal adenocarcinoma

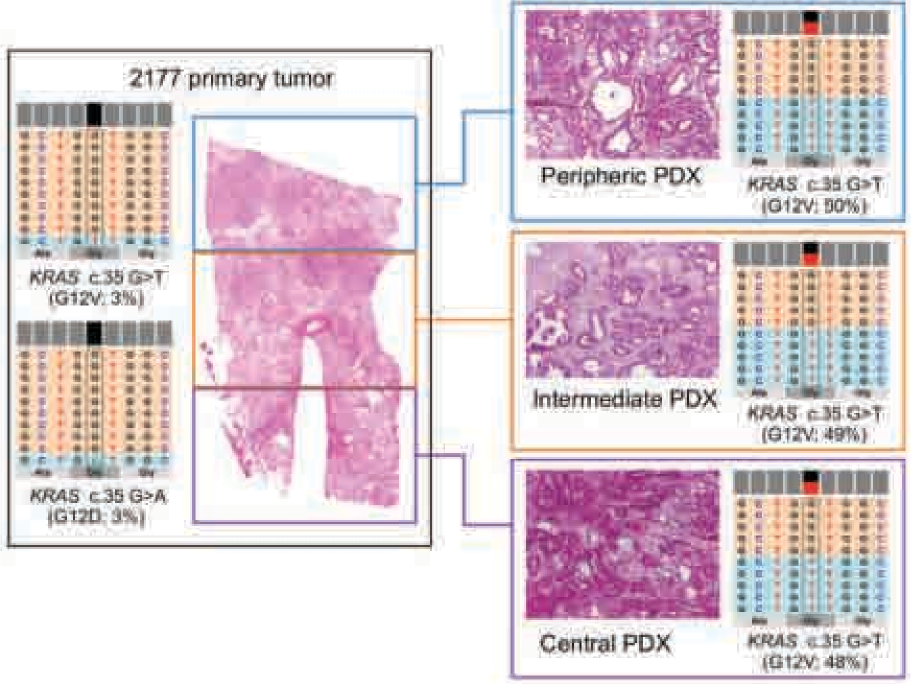
Gene symbol	Gene name and protein function	No.	%
BRCA1	Tumour suppressor through DNA damage repair	6	6%
BRCA2	Breast Cancer 2; tumour suppressor through DNA damage repair	10	10%
ATM	Ataxia telangiectasia mutated; DNA damage repair	9	9%
BARD1	BRCA1-associated RING domain protein 1; tumour suppressor	2	2%
CHEK1	Checkpoint kinase 1; DNA damage response	2	2%
FAM175A	Family With Sequence Similarity 175, Member A; DNA damage response and double-strand break (DSB) repair		
PALB2	Partner and localizer of BRCA2; double strand break repair	1	1%
REV3L	Protein reversionless 3-like; translesion synthesis (TLS)	6	6%
STK11	Serine/threonine kinase 11 (STK11); liver kinase B1 (LKB1); renal carcinoma antigen NY-REN-19; tumour suppressor; cell metabolism, cell polarity, apoptosis and DNA damage response	3	3%

Primary and PDX concordance

Of the 79 cases that were sequenced for both the primary and xenograft samples, discordance was found in one case regarding gene mutations in the *PDAC* basic panel. The discordance occurred in KRAS where the primary sample had two low frequency (3%) mutations, p.Gly12Asp and p.Gly12Val. In the PDX sample, only the p.Gly12Val mutation was amplified. Interestingly this case had low tumour cellularity (3%).

Regarding the *TP53* mutations, while the mutations differed between cases (R249S, R273H, Y234N, D228X, V216M, R175H V157M), the same mutation was detected in both the primary and xenograft tumour of each case. Similarly for the KRAS mutations, all distinct mutations (G12V, G12D, G12R) were detected in both the primary and the xenografted PDX sample.

Three cases, processed according to the heterogeneity protocol, had multiple PDX sequenced, representing the various divisions of the primary tumour. Two cases demonstrated the exact mutations of the primary tissue, even corresponding in the variant frequency. The one exception was the only PDAC that had two KRAS mutations, p.Gly12Val and p.Gly12Asp, both wiht low frequency in the primary sample. In all of the PDX samples that were sequenced only the p.Gly12Val was present (**Figure 6**).



Of the 79 cases that were sequenced for both the primary and xenograft samples, discordance was found in four cases for the HR-DDR panels. One case was missing a somatic *BRIP1* mutation in the PDX and a germ-line *CHEK1* mutation both of unknown significance. This case maintained the germ-line *STK11* mutation. All mutations were missense mutations. This case also lost the *BRCA1* polymorphism (K3326X). All other mutations for this case set corresponded for all panels including all identified polymorphisms. One case lost a *BRCA1* benign mutation, which was the only mutation amplified in the HR-DDR panels for this sample. One case lost a somatic *REV3L* mutation, considered pathogenic, as it was a frame shift variant resulting in a stop codon. This case also had a pathogenic germ-line *BRCA2* mutation and a *REV3L* mutation of unknown significance that were maintained in the PDX. One case lost the BRCA1 K3326X polymorphism. For germ-line variant carrierss, the most likely explanation for its absence of mutated alleles in PDX is the homozygous deletion of the chromosomal region containing these genes.

Table 6. Pathogenic Mutations in HR-DDR panels

Sample	*BRCA2*	*BRCA1*	*ATM*	*STK11*	*REV3L*	*BARD1*	Mutation Type	Germ-line / Somatic	Variant ID	Class
2092	c.657_658delTG Val220IlefsTer4						frameshift_variant&feature_truncation	Germ-line	886delGT *	Pathogenic *
1954	c.4131_4132insTGAGGA Asn1377_Thr1378insTer						stop_gained&inframe_insertion	Germ-line	rs80359429° 1377insXG *	Pathogenic *
1454	c.8878C>T Gln2960Ter						stop_gained	Germ-line	rs80359140° Q2960X *	Pathogenic * °
1185	c.6201_6202insA Ile2068AsnfsTer10						frameshift_variant&feature_elongation	Germ-line	rs397507833 ° 6429delC*	Pathogenic *
785	c.5680_5681insA Tyr1894Ter c.7283T>A Leu2428Ter						frameshift_variant&feature_elongation stop_gained	Germ-line Somatic	5909insA * -	Pathogenic * **
1346	c.7738C>T p.Gln2580Ter c.2905delC p.Gln969LysfsTer3						stop_gained frameshift_variant&feature_truncation	Germ-line Somatic	s80358999° Q2580X * -	Pathogenic * ° **
1060	c.5682C>G Tyr1894Ter						stop_gained	Germ-line	rs41293497° Y1894X *	Pathogenic * °
2434	c.5680_5681insA Tyr1894Ter						frameshift_variant&feature_elongation	Germ-line	5909insA *	Pathogenic *
2515		c.4117G>T Glu1373Ter					stop_gained	Germ-line	rs80357259° E1373X *	Pathogenic * °
2323			c.7456C>T Arg2486Ter				stop_gained	Germ-line	COSM1351002& COSM1351003 °	Pathogenic °
1572				c.223_235delAGGGCCGTCAAGA p.Arg75SerfsTer17			frameshift_variant	Somatic		**
980					c.2890C>T Arg964Ter		stop_gained	Somatic		**
1464						c.2279C>A Ser760Ter	stop_gained	Germ-line		**

*indicates BIC ID and class; ° indicates ClinVar ID and class;
** and indicated in red. These variants are not recorded in either dbSNP or ClinVar, however they cause a premature stop codon, which is a feature of pathogenic mutations;

Table 7. Variants of Unknown Significance in HR-DDR panels

Sample	*ATM*	*PALB2*	*REV3L*	*BRCA2*	*STK11*	*BARD1*	*CHEK1*	*FAM175A*	Mutation Type	Germ-line / Somatic	Variant ID
1804	c.1608-G>A								splice acceptor	somatic	rs755418571&COSM1351001&COSM1351000 °
2666	Arg2453Cys								missense	germ-line	rs755418571&COSM1351001&COSM1351000 °
1846	Leu2211Phe								missense	germ-line	rs80359429 ° 1377insXG *
943	Arg2832His	Arg753Gln							Both missense	Both germ-line	rs80359429 ° 1377insXG *
2323			Ile691Val						missense	germ-line	rs80359429 ° 1377insXG *
1170			Lys2208Glu						missense	germ-line	rs80359140 ° Q2960X *
1462			Ser2422Cys						missense	germ-line	6429delC * rs397507833 °
1954			Gln29Pro						missense	somatic	5909insA *
2092			Ser1045Arg	Leu2085Val					Both missense	*REV3L* somatic and *BRCA2* germ-line	s80358999 ° Q2580X *
1258				Thr1354Met					missense	germ-line	rs41293497 ° Y1894X *
2200					Lys78Arg				germ-line	germ-line	5909insA *
1038					Phe354Leu				germ-line	germ-line	rs80357259 ° E1373X *
1102						Thr54Ala			one missense &splice region	germ-line	COSM1351002& COSM1351003 °
2230							Ile465Val		missense	germ-line	
1152							Lys457Arg		missense	somatic	
1777								Glu276Asp	missense	germ-line	

*indicates BIC ID and clas° indicates ClinVar ID and class;

DISCUSSION

In the last few years, as part of the International Cancer Genome Consortium (ICGC) effort to elucidate the genome of cancer, the once considered single entity PDAC has been dissected into four sub-types based on whole genome characterization of 100 cases defined by the number and type of chromosomal alterations: stable, locally rearranged, scattered and unstable [24]. This genomic analysis extended to 456 PDAC identified anatomical lesions in 32 genes recurrently affecting 10 core pathways: *KRAS*, *TGFbeta*, *WNT, NOTCH, ROBO/SLIT* signalling, *G1/S* transition, *SWI-SNF*, chromatin modification, DNA repair and RNA processing [25]. Expression profiling also identifies four distinct phenotypic PDAC sub-types: Squamous, Pancreatic progenitor, Abnormally differentiated endocrine exocrine (ADEX), Immunogenic [20,21]. The challenge remains to correlate this information to be able to translate these findings into a clinically applicable process.

This thesis commences within the context of ICGC participation and is based on the genomic data produced [18]. These proof of concept data have potential clinical implications but to expound and validate them, it is essential to have PDAC cases that are characterized by pathway and expression accompanied by matched *in vivo* models also characterized morphologically and molecularly as the primary lesions. These models consist in xenografted primary cancers, as they furnish a reproducible source of material to ensure the continual investigation and are actionable, i.e. they can be used for pre-patient therapy trials. This design was presented as part of the Cellular and Animal Models of Pancreatic Cancer (CAM-PaC) project, of which the author of the thesis is Co-PI, supported by the European Union (http://www.cam-pac.eu/).

The present study focuses on the HR-DDR pathway, given its impact for predisposition and therapeutic stratification [20]. Our data suggests that HR-DDR mutations are prevalent in PDAC; that the presence of germ-line *BRCA* and other somatic potentially damaging mutations in HR-DDR genes may be higher than previously reported. These genes also harbour a number of variants that may be possible indicators of risk. Both the pathogenic variants and the risk factor variants underline the correlation of PDAC to other tumour types based on molecular taxonomy for stratified

selection of therapy and a potentially cumulative approach to cancer risk evaluation. The study also validates the use of PDX as a concordant avatar for PDAC may substitute primary tissue in molecular studies and patients in pre-treatment clinical trials.

HR-DDR mutations in PDAC

Our study identified 26 cases with known pathogenic or VUS variants in HR-DDR genes. 13 of these cases were confirmed pathogenic with 9 (9%) were in BRCA1/2, 8 in *BRCA2* and 1 in *BRCA1*. One case had a pathogenic mutation in *ATM.* The other 3 cases had pathogenic mutations in *BARD1*, *REV3L* and *STK11.*

Considering only the 9 patients (5 females and 4 males) with a *BRCA* pathogenic mutation, the mean age was 61 and median was 59. This is younger than the mean and median of the non-*BRCA* mutated cases which is 65 and 67 respectively. This supports suggestions in previous studies that patients with *BRCA* mutations have a younger age of onset [63,64].

The cases in the present study were considered sporadic given the lack of family history. Of note, all our *BRCA2*, *BRCA1* and *ATM* pathogenic mutations were germ-line. In familial pancreatic cancer (FPC), the most recurrent germ-line variant is *BRCA2*, but with disputed levels according to the literature, 6% by Couch *et al.* [65] against 17% by Murphy *et al.* [66]. Other studies have identified *BRCA1*, *PALB2* (partner and localizer of *BRCA2*) and *ATM* as associated with FPC [27,31]. Our recent ICGC studies applying exome and whole genome sequencing to 100 PDAC samples demonstrated that 11% had a germ-line or somatic variant in *BRCA1*, *BRCA2* or *PALB2* and 8% in ATM [18,20] (Appendix 2, Appendix 3). Our PDAC-PDX cohort had 9% and 1% respectively if accounting only for confirmed pathogenic mutations, and 11% and 4% respectively if considering also VUS, which would be in-line with the original set.

Unlike breast cancer that has well defined risk assessment scores, no such definition exists for non-syndrome familial pancreatic cancer and indeed different working definitions result in different degrees of risk. Klein *et al.* defines it as a pair of

first-degree relatives with PDAC and results in a six fold risk factor [67]; with the definition of Hruban *et al.* as three or more first-degree relatives, the risk increases significantly [68]. Given the lack of family history, our cases would not fall within any of the working definitions for familial pancreatic cancer and therefore these patients would not be considered for *BRCA* risk assessment. The one exception was a patient who had a prior breast cancer suggesting the presence of a *BRCA* syndrome.

Low penetrance polymorphisms as the basic of associated cancer pre-disposition

Germ-line variants in *BRCA2* have been verified as strong indicators of predisposition to breast and ovarian cancer, but also to prostate, stomach and pancreas. In particular, many pathogenic mutations have been confirmed as increasing risk of breast cancer, but many variants are still of unknown clinical significance. One such variant is the variant *BRCA2* K3326X, located in exon 27 of the gene that results in loss of the final 93 amino acids of the BRCA2 protein. This variant has had a varied history in its consideration as a cancer predisposition gene from pathogenic to non pathogenic, supported by its prevalence in between 1% and 2% of Caucasian populations. However, more recent studies have reported its association with breast cancer risk (Michailidou et al. 2013; Thompson et al., 2015; Meeks et al. 2016) [69-71], with lung (Wang et al. 2014) [72] and with oesophageal cancer (Delahaye-Sourdeix et al. 2015) [73]. Our study identified six (6%) cases with this variant. These cases had *KRAS* and *TP53* mutations but no other *BRCA* or HR-DDR variants. This is in line with a study of K3326X in pancreatic cancer (Martin et al. 2005) [74], which identified the variant in 5.5% (8/144) and demonstrated that it had statistical relevance in individuals with familial pancreatic cancer, compared to healthy controls (OR=4.84, 95%CI 1.27–18.55, $p<0.01$) but not for sporadic pancreatic cancer patients (OR=2.37, 95% CI 0.61–9.27, $p=0.22$). All of our cases were presumed sporadic due to the lack of personal or family history of cancer. Although neither our study nor the Martin study had the case size of genetic epidemiological studies, our results do suggest that K3326X may be associated with low to moderate risk of pancreatic cancer and should not be excluded from consideration as a low penetrance pre-disposition SNP, particularly in its potential to identify cancer risk

in a set of cancers (breast, ovary, pancreas) that have already been identified as potentially linked in familial cancers, but also to correlate with cancers of the lung and oesophagus.

DNA damage repair impairment as a potential for stratified therapeutics

The four most commonly mutated genes were *KRAS, TP53, CDKN2A* and *SMAD4.* Of the 26 cases with variants in HR-DDR genes, 12 cases had a single *KRAS* mutation, 11 cases had *KRAS* and *TP53* mutations and three cases had *KRAS*, *TP53* plus either a *SMAD4*, *CDKN2A* or *APC* mutation. As targeting KRAS has yet to be successful, and attempting to correct the loss of a tumour suppression gene, such as *TP53*, currently remains unattainable, other options are required to stratify therapy for these cases.

BRCA mutated PDACs represent a unique subtype as they manifest enhanced susceptibility to DNA damaging agents and PARP-inhibition [41,43]. In our study we find that 9% of PDAC occurs in patients with germ-line *BRCA* mutation, higher than previously published [20,27,75].

BRCA1 and *BRCA2* mutated PDACs have a distinct clinical outcome [41] and are responsive to DNA damaging therapies, including platinum salts, anthracyclines and radiation, as these treatments are selectively lethal to HR-defective cells in diverse tumour types [20,76-78]. Oxaliplatin, a platinum compound, has proved efficient as second line therapy in PDAC [79], and the platinum-containing FOLFIRINOX combination therapy shows promise as a treatment option for advanced PDAC although toxicity remains an issue [80].

Somatic biallelic inactivation of the *BRCA1* or *BRCA2* genes confers sensitivity to inhibition of poly (ADP-ribose)-polymerase (PARP) an enzyme involved in base excision repair of single strand DNA breaks [81]. Loss of both HR and base excision repair pathways leads to synthetic lethality during DNA replication. The Food and Drug Administration (FDA) has approved the PARP inhibitor (PARPi) Olaparib for the treatment of advanced ovarian cancer patients who have a germ-line *BRCA* mutation and have been previously treated at least three lines of chemotherapy [44,45]. PARP

inhibitors are also being investigated in different tumour types, either alone or in combination with chemo-radiotherapy. PARP inhibitors increase chemo-radiotherapy sensitivity in *BRCA2*-deficient pancreatic cancer cells [82]. Clinical trials of PARPi in germ-line *BRCA* mutated PDAC are underway with promising preliminary findings [43,83,84].

This idea of DNA damage agents and PARP-inhibition extends beyond germ-line *BRCA* mutations. Fogelman *et al.*, recently demonstrated that metastatic PDAC cases with family history or pedigree of breast, ovarian or pancreatic cancers, in the absence of a known germ-line *BRCA* mutations, had improved overall survival with first line platinum therapy, similar to *BRCA* mutant cases, compared with those without the family history who had poor survival [85]. This concept is referred to as 'BRCAness' and, although the underlying molecular variants of BRCAness are not clearly defined, it potentially extends to other non *BRCA* HR repair defect such as *ATM, BARD1*, *PALB2, REV3L, STK11*; the recently described unstable subtype (> 200 structural variation events) [20]; and a mutational signature of DNA damage repair deficiency [20]. Applying the concept of BRCAness to our cohort by including the other HR-DDR known pathogenic mutations our potential sub-group increases to 13 (13%) of our cohort. In particular, *ATM* loss may represent a specific target for PARPi. Deleterious *ATM* mutations have been recognized in the germ-line of families with familial PDAC [86]. *ATM* loss also occurs in sporadic cases, with a higher incidence in familial cases as compared with sporadic cases (24% vs. 11%, p = 0.0.19). ATM loss is associated with poor survival in surgically-resected PDAC [87], and responsiveness to the PARPi olaparib in gastric cancer [88-90]. In our study, while we only found one case with a confirmed pathogenic germ-line ATM mutation, we also found four mutations (3 germ-line and one somatic) VUS indicating additional variants for further studies also as risk factor variants. Waddell *et al.* showed that this subgroup were platinum-sensitive thus indicating a possible treatment option also for DNA repair-targeting agents, such as PARPi. In fact, pre-clinical studies have demonstrated that PARPi are synthetically lethal in pancreatic sporadic cancers with somatic or epigenetic silencing of HR-DDR genes [91].

Companion Diagnostics to identify this sub-group in a clinical setting

Challenges remain in the application of companion diagnostics for BRCAness in a clinical setting. To date, *BRCA* was investigated only in a germ-line context on blood but as somatic mutations must also be considered to indicate all patients that could potentially avail of these therapies, there is a requirement to be able to carry out these test on small diagnostic samples such a formalin fixed paraffin embedded tissue (FFPE). The panels designed within this study (Supplementary Table S1, S2, S3) have been designed to work with FFPE samples and as they apply targeted sequencing technologies, they can return results in a clinically relevant timeframe. To address the requirement for a tool to perform in histopathological diagnostics, in parallel to this study, we also validated a CE-IVD panel that identifies all BRCA mutations on FFPE on a set of ovarian cancers [92] (**Appendix 8**).

Use of PDX

Our study analysed 79 cases for genes known to be mutated in PDAC, and genes involved in homologous recombination and DNA damage repair in both primary tissue and PDX. All cases correctly replicated all variants from the primary in the PDX samples with the exception of 5 cases (6.5%), one in *KRAS* and the other four in HR-DDR panels. Three of these were missing a benign or polymorphic *BRCA* variant. The fact that these mutations were not found in the PDX is potentially due to homozygous deletion as they were germ-line variants. The fourth case lost one of its two *REV3L* variants, which could potentially be due to clonal selection pressure.

Three cases were sequenced on multiple PDX samples representing different areas of the primary tumour, developed as part of a study on heterogeneity. The PDX samples displayed the same variants in similar frequencies for all PDX primary tissue areas.

Regarding the case that lost one of the two low frequency *KRAS* primary tumour mutations (p.Gly12Val was retained while p.Gly12Asp was lost), PDX representing all areas of the primary tumour were concordant with each other and did not display the

second *KRAS* mutation. This is potentially an issue of clonal selection pressure when the primary tumour tissue is transplanted in the foreign murine host [46]. Interestingly, this was the only case to have multiple *KRAS* mutations.

Despite the issues of clonal selection and tumour heterogeneity, our study highlights prevalent concordance of PDX to primary PDAC in molecular characterization terms. Current standard of care treatment options have poor or modest results [14,46] and only single sets of genes and therapies can be tested in a clinical trial setting. Therefore, the use of PDX avatars provide more options for simultaneous testing, personalized medicine applications and drug resistance.

CONCLUSION

Here, we develop clinically applicable sequencing panels that might improve management of PDAC patients. The verification of the existence of a BRCAness subgroup is the dawn of the revolution towards prevention and stratification of PDAC. This BRCAness subgroup includes 9% of cases harbouring pathogenic mutations of *BRCA1* and *BRCA2* genes. As more HR gene variants are confirmed pathogenic, this subgroup will potentially increase.

Furthermore, we show that PDXs might represent a valuable model that faithfully recapitulates the main genetic feature of primary diseases. The availability of molecularly characterized primary cancer and matched *in vivo* models paves the way for novel diagnostics and therapeutics based on molecular phenotype of individual tumours, as they have the potential of being used to predict drug responses as well as to enable identification of effective therapeutic schemes.

FUTURE PERSPECTIVES

Investigation of gene variants is underway to search for anatomical DNA lesions in other pathways: Chromatin Remodelling [93], SWI/SNF [94], TGFß and complete the genetic taxonomy of PDAC. The molecular framework will be completed with expression profiles of primary and PDX to provide a factual identification of PDAC subgroups by aggregating morphological genomic, transcriptomic, and immuno-histochemical characterization.

Preliminary data on the Chromatin Remodelling pathway are reported in **Table 10** below. These data show pathogenic mutations and VUS from this pathway in our cohort. Mutations in chromatin remodelling pathways are indicative of poor prognosis and indicate a sub-group for particular attention[95]. Interestingly, cases with these mutations were mutually exclusive from cases that had HR mutations.

Gene	Pathogenic	Unknown
ARID1A	4	5
ARID1B	-	1
ARID2	-	1
DPF1	-	1
DPF3	-	-
HLTF	-	1
KDM5C	1	-
KDM6A	2	-
KMT2C	3	4
KMT2D	3	11
SETD2	1	3
SMARCA2	-	2
SMARCA4	1	4
PBRM1	1	-
BAP1	1	2
Total	17 (21%)	35 (45%)

Furthermore, we report preliminary data on the use of PDX avatars to correlate pathway alterations and therapy response. 11 PDX were used as avatars to directly monitor response to drugs relevant for the treatment of pancreatic carcinomas. Preliminary results are outlined in **Table 11**. Five of these (T2460, T2316, T2347, T2346, T2367) had only *KRAS* mutations but showed varying response to therapy. Two cases (T2330, T2355) had mutations in *KRAS*, *TP53*, *SMAD4* and *CDKN2A.* While the former showed exceptional response to all therapies, the latter only responded to nab-paclitaxel and its combined use. This case harboured the K3326X variant.

Table 11. Response of 9 PDAC avatars to drug treatments

DRUG	T2346	T2316	T2460	T2347	T2367	T2149	T2373	T2567	T2570	T2330	T2355
Gemcitibine	17	25	50	77	68	20	62	41	45	32	91
Gemcitibine Erlotinib	30	25	48	97	70	47	65	32	30	20	63
Gemcitibine Abraxane	22	18	33	20	64	16	4	10	31	6	25
5FU Oxaliplatin	n/a	41	38	93	63	n/a	9	20	22	32	75
Abraxane	25	19	2	9	68	7	4	12	23	8	18

Legend: Numbers indicate tumour growth inhibition in comparison to control (T/C) red – progression (T/C <= 25); yellow – stable disease; (T/C 26 – 50); green – partial/ complete response (T/C > 51).

Post treatment avatar tumour was harvested and molecularly characterized in three of these cases. One PDX prior to treatment had a *BRCA2* pathogenic mutation. This avatar (T2373) responded to nab-paclitaxel and nab-paclitaxel / gemicitbine combination as well as to the Oxaliplatin / 5FU combination but not to gemicitabine or gemcitabine – Erlotinib combination (**Figure 7**). The post treatment avatar tumour material from the nab-paclitaxel treated group was sequenced with the PDAC basic and HR-DDR panels. No new mutations were identified but the *BRCA2* was not longer present.

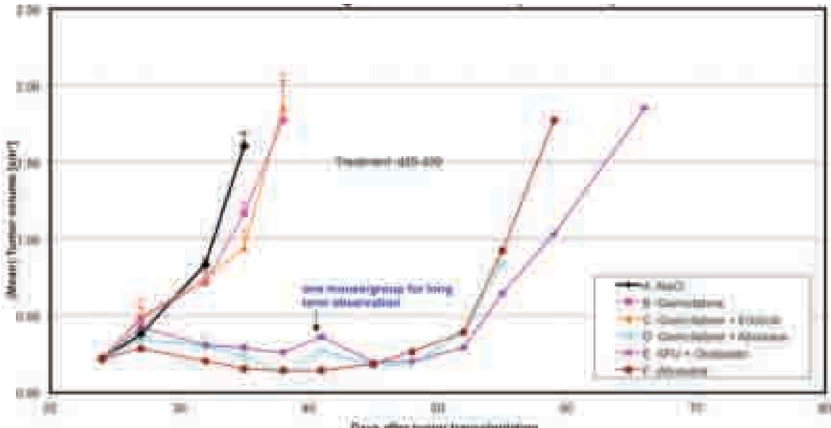


Figure 7. Aggregated data of one control group and five treatment groups of mice per one PDX. Treatment began upon advanced tumour volume (>0.2cm) Treatment regimens were Gemcitibine (pink), Gemcitibine with Erlotinib (orange), Gemcitibine with nab-paclitaxel (blue), 5FU with Oxaliplatin (purple), nab-paclitaxel (brown). Treat progress is measured in days and tumour volume.

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SUPPLEMENTARY TABLES

Supplementary Table S1. Targeted regions of the PDAC basic panel

Gene	Chromosome	Chr Start	Chr End
APC	chr5	112173871	112173962
APC	chr5	112174557	112174666
APC	chr5	112175143	112175268
APC	chr5	112175315	112175443
APC	chr5	112175567	112175703
APC	chr5	112175740	112175862
APC	chr5	112175920	112176035
ATM	chr11	108117765	108117865
ATM	chr11	108200915	108200993
ATM	chr11	108204634	108204684
ATM	chr11	108205731	108205816
ATM	chr11	108206523	108206628
ATM	chr11	108218015	108218144
ATM	chr11	108225549	108225632
ATM	chr11	108236042	108236140
ATM	chr11	108236186	108236285
ATM	chr11	108119815	108119891
ATM	chr11	108123515	108123618
ATM	chr11	108137931	108138025
ATM	chr11	108155083	108155180
ATM	chr11	108170456	108170556
ATM	chr11	108172362	108172467
ATM	chr11	108173630	108173703
ATM	chr11	108180902	108180960
BRAF	chr7	140481391	140481515
BRAF	chr7	140453102	140453221
CDH1	chr16	68835602	68835697
CDH1	chr16	68846024	68846151
CDH1	chr16	68847199	68847302
CDKN2A	chr9	21971090	21971219
CDKN2A	chr9	21970940	21971066
CTNNB1	chr3	41266029	41266147
EGFR	chr7	55211044	55211126
EGFR	chr7	55221792	55221919
EGFR	chr7	55260430	55260552
EGFR	chr7	55232962	55233053
EGFR	chr7	55241635	55241729
EGFR	chr7	55242411	55242540
EGFR	chr7	55248965	55249090
EGFR	chr7	55249122	55249245
EGFR	chr7	55259507	55259628
ERBB2	chr17	37880212	37880340
ERBB2	chr17	37880953	37881061
ERBB2	chr17	37881324	37881453
ERBB4	chr2	212812075	212812169

ERBB4	chr2	212652719	212652806
ERBB4	chr2	212589764	212589867
ERBB4	chr2	212587133	212587239
ERBB4	chr2	212578288	212578415
ERBB4	chr2	212576799	212576910
ERBB4	chr2	212530051	212530180
ERBB4	chr2	212288904	212288990
FBXW7	chr4	153258901	153259023
FBXW7	chr4	153250852	153250926
FBXW7	chr4	153249355	153249477
FBXW7	chr4	153247277	153247369
FBXW7	chr4	153245410	153245492
FGFR3	chr4	1803551	1803653
FGFR3	chr4	1806081	1806187
FGFR3	chr4	1807833	1807930
FGFR3	chr4	1808311	1808399
FGFR3	chr4	1808881	1809006
FLT3	chr13	28610093	28610184
FLT3	chr13	28608227	28608348
FLT3	chr13	28602275	28602379
FLT3	chr13	28592579	28592663
GNAS	chr20	57484396	57484504
GNAS	chr20	57484562	57484672
HRAS	chr11	534220	534308
HRAS	chr11	533812	533930
KDR	chr4	55980238	55980359
KDR	chr4	55979574	55979655
KDR	chr4	55972952	55973071
KDR	chr4	55962444	55962548
KDR	chr4	55960976	55961059
KDR	chr4	55955078	55955168
KDR	chr4	55953775	55953860
KDR	chr4	55946250	55946371
KDR	chr4	55946088	55946208
KRAS	chr12	25398186	25398304
KRAS	chr12	25380260	25380364
KRAS	chr12	25378549	25378658
NRAS	chr1	115258689	115258774
NRAS	chr1	115256504	115256584
NRAS	chr1	115252185	115252269
PIK3CA	chr3	178916775	178916881
PIK3CA	chr3	178951996	178952097
PIK3CA	chr3	178952140	178952237
PIK3CA	chr3	178916931	178917035
PIK3CA	chr3	178921464	178921570
PIK3CA	chr3	178927405	178927525
PIK3CA	chr3	178927901	178927986
PIK3CA	chr3	178928069	178928160
PIK3CA	chr3	178936023	178936105
PIK3CA	chr3	178938787	178938918
PIK3CA	chr3	178947818	178947896
SMAD4	chr18	48575099	48575213
SMAD4	chr18	48575556	48575677

SMAD4	chr18	48581190	48581302
SMAD4	chr18	48584551	48584678
SMAD4	chr18	48586251	48586361
SMAD4	chr18	48591814	48591931
SMAD4	chr18	48593399	48593519
SMAD4	chr18	48603028	48603119
SMAD4	chr18	48604658	48604774
TP53	chr17	7579853	7579960
TP53	chr17	7579350	7579485
TP53	chr17	7578516	7578601
TP53	chr17	7578352	7578483
TP53	chr17	7578180	7578298
TP53	chr17	7577508	7577612
TP53	chr17	7577015	7577151
TP53	chr17	7573923	7574035

Supplementary Table S2. Targeted regions of the BRCA panel

Gene	Chromosome	Chr Start	Chr End
ATM	chr11	108098276	108098392
ATM	chr11	108098384	108098493
ATM	chr11	108098517	108098588
ATM	chr11	108098577	108098691
ATM	chr11	108099835	108099963
ATM	chr11	108099952	108100025
ATM	chr11	108100014	108100131
ATM	chr11	108106276	108106377
ATM	chr11	108106361	108106449
ATM	chr11	108106429	108106546
ATM	chr11	108106535	108106652
ATM	chr11	108114593	108114709
ATM	chr11	108114707	108114788
ATM	chr11	108114777	108114895
ATM	chr11	108115453	108115524
ATM	chr11	108115513	108115622
ATM	chr11	108115611	108115733
ATM	chr11	108115730	108115821
ATM	chr11	108117557	108117676
ATM	chr11	108117661	108117766
ATM	chr11	108117811	108117904
ATM	chr11	108119574	108119687
ATM	chr11	108119686	108119776
ATM	chr11	108119765	108119882
ATM	chr11	108121340	108121455
ATM	chr11	108121444	108121571
ATM	chr11	108121562	108121651
ATM	chr11	108121632	108121735
ATM	chr11	108121724	108121850
ATM	chr11	108122507	108122625
ATM	chr11	108122621	108122734
ATM	chr11	108122722	108122794
ATM	chr11	108122789	108122905
ATM	chr11	108123477	108123590
ATM	chr11	108123579	108123691
ATM	chr11	108124450	108124563
ATM	chr11	108124553	108124634
ATM	chr11	108124623	108124737
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ATM	chr11	108126827	108126955
ATM	chr11	108126956	108127017
ATM	chr11	108127006	108127118
ATM	chr11	108128148	108128252
ATM	chr11	108128241	108128315
ATM	chr11	108128304	108128401
ATM	chr11	108129599	108129712
ATM	chr11	108129701	108129794
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ATM	chr11	108137835	108137916
ATM	chr11	108137905	108138006

ATM	chr11	108137995	108138119
ATM	chr11	108139073	108139186
ATM	chr11	108139175	108139281
ATM	chr11	108139270	108139392
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ATM	chr11	108141861	108141965
ATM	chr11	108141990	108142075
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ATM	chr11	108143114	108143230
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ATM	chr11	108143285	108143378
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ATM	chr11	108150139	108150253
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ATM	chr11	108150336	108150427
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ATM	chr11	108163997	108164082
ATM	chr11	108164067	108164165
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ATM	chr11	108180917	108181023
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ATM	chr11	108183090	108183194
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ATM	chr11	108186606	108186688
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ATM	chr11	108236177	108236285
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BRCA1	chr17	41246564	41246641
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PALB2	chr16	23619038	23619161
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PALB2	chr16	23625358	23625477
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PALB2	chr16	23632681	23632773
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PALB2	chr16	23634211	23634307
PALB2	chr16	23634296	23634376
PALB2	chr16	23634365	23634475
PALB2	chr16	23635183	23635293
PALB2	chr16	23635282	23635407
PALB2	chr16	23635396	23635514
PALB2	chr16	23637420	23637543
PALB2	chr16	23637532	23637657
PALB2	chr16	23637646	23637768
PALB2	chr16	23640414	23640536
PALB2	chr16	23640527	23640648
PALB2	chr16	23640866	23640988
PALB2	chr16	23640977	23641082
PALB2	chr16	23641081	23641210
PALB2	chr16	23641199	23641321
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PALB2	chr16	23641407	23641523
PALB2	chr16	23641512	23641630
PALB2	chr16	23641619	23641735
PALB2	chr16	23641724	23641840
PALB2	chr16	23646127	23646252
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PALB2	chr16	23646775	23646891
PALB2	chr16	23646880	23646952
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PALB2	chr16	23647192	23647308
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PALB2	chr16	23647384	23647512
PALB2	chr16	23647501	23647608
PALB2	chr16	23649090	23649160
PALB2	chr16	23649149	23649238
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PALB2	chr16	23652338	23652427

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REV3L	chr6	111621248	111621325
REV3L	chr6	111621314	111621431
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REV3L	chr6	111628541	111628665
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REV3L	chr6	111630929	111631041
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REV3L	chr6	111634699	111634821
REV3L	chr6	111636415	111636529
REV3L	chr6	111636518	111636637
REV3L	chr6	111643635	111643754
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REV3L	chr6	111650662	111650779
REV3L	chr6	111650768	111650887
REV3L	chr6	111650877	111650998
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REV3L	chr6	111654236	111654355
REV3L	chr6	111654344	111654429
REV3L	chr6	111654418	111654529
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REV3L	chr6	111656577	111656681
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REV3L	chr6	111656773	111656877
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REV3L	chr6	111678109	111678226
REV3L	chr6	111678203	111678291
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REV3L	chr6	111688804	111688930
REV3L	chr6	111688919	111689023
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REV3L	chr6	111689116	111689198
REV3L	chr6	111689187	111689290
REV3L	chr6	111693699	111693822
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REV3L	chr6	111693897	111693980
REV3L	chr6	111693969	111694092
REV3L	chr6	111694081	111694190
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REV3L	chr6	111694279	111694379
REV3L	chr6	111694368	111694479
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REV3L	chr6	111694565	111694689
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REV3L	chr6	111695001	111695126
REV3L	chr6	111695105	111695172
REV3L	chr6	111695232	111695341
REV3L	chr6	111695330	111695454
REV3L	chr6	111695443	111695558
REV3L	chr6	111695547	111695632
REV3L	chr6	111695621	111695729
REV3L	chr6	111695718	111695839
REV3L	chr6	111695830	111695945
REV3L	chr6	111695973	111696043
REV3L	chr6	111696032	111696134
REV3L	chr6	111696123	111696205
REV3L	chr6	111696195	111696296
REV3L	chr6	111696285	111696392
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REV3L	chr6	111696801	111696917
REV3L	chr6	111696907	111697030
REV3L	chr6	111697019	111697137
REV3L	chr6	111697128	111697241
REV3L	chr6	111697223	111697339

REV3L	chr6	111697328	111697456
REV3L	chr6	111697453	111697535
REV3L	chr6	111697522	111697593
REV3L	chr6	111697632	111697736
REV3L	chr6	111697725	111697813
REV3L	chr6	111697802	111697902
REV3L	chr6	111697891	111698011
REV3L	chr6	111698850	111698933
REV3L	chr6	111698922	111698993
REV3L	chr6	111698982	111699098
REV3L	chr6	111701058	111701175
REV3L	chr6	111701202	111701275
REV3L	chr6	111701264	111701358
REV3L	chr6	111701347	111701430
REV3L	chr6	111702387	111702505
REV3L	chr6	111702494	111702607
REV3L	chr6	111702596	111702706
REV3L	chr6	111708877	111708990
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REV3L	chr6	111709074	111709193
REV3L	chr6	111709224	111709338
REV3L	chr6	111710249	111710328
REV3L	chr6	111710317	111710432
REV3L	chr6	111710421	111710524
REV3L	chr6	111711298	111711363
REV3L	chr6	111711352	111711459
REV3L	chr6	111713961	111714070
REV3L	chr6	111714033	111714101
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REV3L	chr6	111726685	111726802
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REV3L	chr6	111803983	111804077
REV3L	chr6	111804085	111804213
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RPA1	chr17	1746104	1746197
RPA1	chr17	1747161	1747281
RPA1	chr17	1747270	1747352
RPA1	chr17	1747810	1747930
RPA1	chr17	1747919	1748040
RPA1	chr17	1756344	1756425
RPA1	chr17	1756414	1756533
RPA1	chr17	1775667	1775798
RPA1	chr17	1775731	1775855
RPA1	chr17	1778872	1778988
RPA1	chr17	1778979	1779094
RPA1	chr17	1779087	1779168
RPA1	chr17	1780406	1780510
RPA1	chr17	1780499	1780608

RPA1	chr17	1780608	1780737
RPA1	chr17	1782214	1782338
RPA1	chr17	1782327	1782453
RPA1	chr17	1782379	1782505
RPA1	chr17	1782494	1782578
RPA1	chr17	1782567	1782691
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RPA1	chr17	1782859	1782988
RPA1	chr17	1782980	1783108
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RPA1	chr17	1783885	1783999
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RPA1	chr17	1787053	1787129
RPA1	chr17	1787118	1787199
RPA1	chr17	1787188	1787288
RPA1	chr17	1791876	1791996
RPA1	chr17	1791985	1792110
RPA1	chr17	1792099	1792208
RPA1	chr17	1795028	1795129
RPA1	chr17	1795091	1795181
RPA1	chr17	1798231	1798357
RPA1	chr17	1798348	1798472
RPA1	chr17	1800298	1800407
RPA1	chr17	1800396	1800520
STK11	chr19	1206847	1206975
STK11	chr19	1206965	1207081
STK11	chr19	1207065	1207190
STK11	chr19	1207175	1207288
STK11	chr19	1218296	1218403
STK11	chr19	1218379	1218487
STK11	chr19	1218476	1218592
STK11	chr19	1219339	1219473
STK11	chr19	1220262	1220387
STK11	chr19	1220383	1220502
STK11	chr19	1221073	1221200
STK11	chr19	1221189	1221321
STK11	chr19	1221283	1221381
STK11	chr19	1221849	1221976
STK11	chr19	1222933	1223038
STK11	chr19	1223006	1223133
STK11	chr19	1223124	1223246
STK11	chr19	1226368	1226505
STK11	chr19	1226617	1226738

Supplementary Table S3. Targeted regions of the BRCA + panel

Gene	Chromosome	Chr Start	Chr End
BARD1	chr2	215593263	215593363
BARD1	chr2	215593358	215593435
BARD1	chr2	215593424	215593513
BARD1	chr2	215593502	215593614
BARD1	chr2	215593603	215593721
BARD1	chr2	215593718	215593816
BARD1	chr2	215595055	215595172
BARD1	chr2	215595161	215595252
BARD1	chr2	215595232	215595301
BARD1	chr2	215609726	215609833
BARD1	chr2	215609822	215609895
BARD1	chr2	215609884	215609971
BARD1	chr2	215610348	215610468
BARD1	chr2	215610457	215610530
BARD1	chr2	215610519	215610598
BARD1	chr2	215610587	215610699
BARD1	chr2	215617068	215617188
BARD1	chr2	215617177	215617292
BARD1	chr2	215617283	215617392
BARD1	chr2	215632154	215632260
BARD1	chr2	215632249	215632358
BARD1	chr2	215632350	215632468
BARD1	chr2	215633889	215634004
BARD1	chr2	215633993	215634102
BARD1	chr2	215645175	215645296
BARD1	chr2	215645285	215645387
BARD1	chr2	215645376	215645502
BARD1	chr2	215645491	215645601
BARD1	chr2	215645590	215645712
BARD1	chr2	215645701	215645773
BARD1	chr2	215645762	215645876
BARD1	chr2	215645865	215645984
BARD1	chr2	215645972	215646086
BARD1	chr2	215646073	215646175
BARD1	chr2	215646164	215646283
BARD1	chr2	215656934	215657034
BARD1	chr2	215657023	215657088
BARD1	chr2	215657175	215657242
BARD1	chr2	215661774	215661846
BARD1	chr2	215661835	215661922
BARD1	chr2	215674074	215674202
BARD1	chr2	215674135	215674266
BRIP1	chr17	59760624	59760711
BRIP1	chr17	59760690	59760756
BRIP1	chr17	59760838	59760939
BRIP1	chr17	59760928	59761019
BRIP1	chr17	59761082	59761198
BRIP1	chr17	59761187	59761307
BRIP1	chr17	59761295	59761414

BRIP1	chr17	59761403	59761519
BRIP1	chr17	59761508	59761622
BRIP1	chr17	59763083	59763193
BRIP1	chr17	59763182	59763289
BRIP1	chr17	59763278	59763387
BRIP1	chr17	59763376	59763498
BRIP1	chr17	59763488	59763583
BRIP1	chr17	59770717	59770805
BRIP1	chr17	59770794	59770923
BRIP1	chr17	59793205	59793328
BRIP1	chr17	59793320	59793396
BRIP1	chr17	59820312	59820435
BRIP1	chr17	59820424	59820537
BRIP1	chr17	59821679	59821795
BRIP1	chr17	59821787	59821894
BRIP1	chr17	59821883	59822005
BRIP1	chr17	59853671	59853796
BRIP1	chr17	59853785	59853904
BRIP1	chr17	59853893	59853984
BRIP1	chr17	59857542	59857663
BRIP1	chr17	59857652	59857749
BRIP1	chr17	59857738	59857862
BRIP1	chr17	59858137	59858244
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BRIP1	chr17	59861548	59861613
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BRIP1	chr17	59861738	59861848
BRIP1	chr17	59870892	59870998
BRIP1	chr17	59870987	59871059
BRIP1	chr17	59876335	59876460
BRIP1	chr17	59876449	59876563
BRIP1	chr17	59876552	59876629
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BRIP1	chr17	59878484	59878596
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BRIP1	chr17	59878794	59878894
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BRIP1	chr17	59885858	59885983
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BRIP1	chr17	59934407	59934482
BRIP1	chr17	59934471	59934545
BRIP1	chr17	59934534	59934642
BRIP1	chr17	59937101	59937223
BRIP1	chr17	59937212	59937321

BRIP1	chr17	59938731	59938837
BRIP1	chr17	59938831	59938950
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CHEK1	chr11	125496730	125496804
CHEK1	chr11	125497435	125497557
CHEK1	chr11	125497553	125497677
CHEK1	chr11	125497663	125497775
CHEK1	chr11	125499031	125499153
CHEK1	chr11	125499142	125499243
CHEK1	chr11	125499213	125499327
CHEK1	chr11	125499316	125499431
CHEK1	chr11	125502972	125503087
CHEK1	chr11	125503084	125503159
CHEK1	chr11	125503148	125503263
CHEK1	chr11	125503252	125503376
CHEK1	chr11	125505222	125505343
CHEK1	chr11	125505332	125505405
CHEK1	chr11	125505468	125505582
CHEK1	chr11	125507189	125507311
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CHEK1	chr11	125507375	125507491
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CHEK1	chr11	125513670	125513781
CHEK1	chr11	125513770	125513886
CHEK1	chr11	125513917	125513995
CHEK1	chr11	125513984	125514056
CHEK1	chr11	125514045	125514155
CHEK1	chr11	125514166	125514276
CHEK1	chr11	125514302	125514421
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CHEK1	chr11	125523513	125523631
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CHEK1	chr11	125523706	125523799
CHEK1	chr11	125525044	125525164
CHEK1	chr11	125525153	125525270
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CHEK2	chr22	29084986	29085116
CHEK2	chr22	29085160	29085280
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CHEK2	chr22	29090068	29090155
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CHEK2	chr22	29091079	29091178
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CHEK2	chr22	29091643	29091759
CHEK2	chr22	29091748	29091830
CHEK2	chr22	29091819	29091911
CHEK2	chr22	29092800	29092926
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CHEK2	chr22	29095879	29096001
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CHEK2	chr22	29099456	29099571

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CHEK2	chr22	29107892	29108001
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CHEK2	chr22	29130584	29130669
CHEK2	chr22	29130722	29130821
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FAM175A	chr4	84383597	84383691
FAM175A	chr4	84383732	84383813
FAM175A	chr4	84383802	84383915
FAM175A	chr4	84383915	84384000
FAM175A	chr4	84383989	84384072
FAM175A	chr4	84384061	84384153
FAM175A	chr4	84384482	84384606
FAM175A	chr4	84384637	84384701
FAM175A	chr4	84384690	84384801
FAM175A	chr4	84384790	84384887
FAM175A	chr4	84388504	84388602
FAM175A	chr4	84388591	84388696
FAM175A	chr4	84390054	84390180
FAM175A	chr4	84390169	84390283
FAM175A	chr4	84390272	84390354
FAM175A	chr4	84391244	84391360
FAM175A	chr4	84391342	84391439
FAM175A	chr4	84391428	84391539
FAM175A	chr4	84393324	84393436
FAM175A	chr4	84393429	84393511
FAM175A	chr4	84397693	84397780
FAM175A	chr4	84397769	84397882
FAM175A	chr4	84403212	84403337
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FAM175A	chr4	84406149	84406276
MRE11A	chr11	94153158	94153259
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MRE11A	chr11	94163099	94163202
MRE11A	chr11	94168936	94169004
MRE11A	chr11	94168992	94169084

MRE11A	chr11	94169077	94169175
MRE11A	chr11	94170288	94170393
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MRE11A	chr11	94178911	94179037
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MRE11A	chr11	94180476	94180594
MRE11A	chr11	94180583	94180694
MRE11A	chr11	94189369	94189474
MRE11A	chr11	94189459	94189526
MRE11A	chr11	94192485	94192547
MRE11A	chr11	94192536	94192644
MRE11A	chr11	94192634	94192752
MRE11A	chr11	94192748	94192849
MRE11A	chr11	94193972	94194092
MRE11A	chr11	94194092	94194181
MRE11A	chr11	94194170	94194252
MRE11A	chr11	94197169	94197286
MRE11A	chr11	94197308	94197385
MRE11A	chr11	94197374	94197459
MRE11A	chr11	94200885	94201006
MRE11A	chr11	94200995	94201109
MRE11A	chr11	94203560	94203675
MRE11A	chr11	94203661	94203753
MRE11A	chr11	94203739	94203845
MRE11A	chr11	94203840	94203957
MRE11A	chr11	94204686	94204803
MRE11A	chr11	94204798	94204914
MRE11A	chr11	94204914	94204979
MRE11A	chr11	94209373	94209478
MRE11A	chr11	94209467	94209558
MRE11A	chr11	94209553	94209650
MRE11A	chr11	94211839	94211947
MRE11A	chr11	94211932	94212006
MRE11A	chr11	94211995	94212092
MRE11A	chr11	94212788	94212905
MRE11A	chr11	94212876	94212989
MRE11A	chr11	94219038	94219154
MRE11A	chr11	94219118	94219230
MRE11A	chr11	94219219	94219332
MRE11A	chr11	94223893	94223992
MRE11A	chr11	94223981	94224077
MRE11A	chr11	94224066	94224174
MRE11A	chr11	94225858	94225931
MRE11A	chr11	94225912	94226021
NBN	chr8	90947720	90947828
NBN	chr8	90947812	90947897
NBN	chr8	90949149	90949249
NBN	chr8	90949238	90949355
NBN	chr8	90955430	90955558
NBN	chr8	90955548	90955665
NBN	chr8	90958311	90958425

NBN	chr8	90958369	90958488
NBN	chr8	90958479	90958570
NBN	chr8	90958561	90958645
NBN	chr8	90959945	90960073
NBN	chr8	90960073	90960173
NBN	chr8	90965398	90965510
NBN	chr8	90965499	90965574
NBN	chr8	90965563	90965680
NBN	chr8	90965669	90965795
NBN	chr8	90965786	90965868
NBN	chr8	90965857	90965973
NBN	chr8	90967408	90967526
NBN	chr8	90967513	90967626
NBN	chr8	90967618	90967711
NBN	chr8	90967700	90967802
NBN	chr8	90967798	90967917
NBN	chr8	90970856	90970979
NBN	chr8	90970968	90971089
NBN	chr8	90971078	90971176
NBN	chr8	90976544	90976622
NBN	chr8	90976611	90976714
NBN	chr8	90976724	90976842
NBN	chr8	90982531	90982647
NBN	chr8	90982639	90982755
NBN	chr8	90982744	90982860
NBN	chr8	90983312	90983429
NBN	chr8	90983427	90983495
NBN	chr8	90983484	90983570
NBN	chr8	90990365	90990482
NBN	chr8	90990466	90990539
NBN	chr8	90992821	90992940
NBN	chr8	90992916	90993004
NBN	chr8	90992993	90993062
NBN	chr8	90993135	90993237
NBN	chr8	90993483	90993593
NBN	chr8	90993572	90993649
NBN	chr8	90993680	90993776
NBN	chr8	90993765	90993856
NBN	chr8	90994862	90994984
NBN	chr8	90994973	90995059
NBN	chr8	90995048	90995133
NBN	chr8	90996606	90996730
NBN	chr8	90996723	90996815
NBN	chr8	90996804	90996907
PTEN	chr10	89624161	89624283
PTEN	chr10	89624272	89624362
PTEN	chr10	89653674	89653793
PTEN	chr10	89653782	89653849
PTEN	chr10	89653838	89653916
PTEN	chr10	89685186	89685260
PTEN	chr10	89685250	89685367
PTEN	chr10	89690728	89690836
PTEN	chr10	89690825	89690912

PTEN	chr10	89692713	89692826
PTEN	chr10	89692815	89692942
PTEN	chr10	89692931	89693024
PTEN	chr10	89693013	89693089
PTEN	chr10	89711801	89711921
PTEN	chr10	89711894	89712007
PTEN	chr10	89712027	89712103
PTEN	chr10	89717489	89717607
PTEN	chr10	89717596	89717712
PTEN	chr10	89717701	89717799
PTEN	chr10	89717799	89717863
PTEN	chr10	89720563	89720684
PTEN	chr10	89720673	89720773
PTEN	chr10	89720762	89720868
PTEN	chr10	89720857	89720926
PTEN	chr10	89724920	89725033
PTEN	chr10	89725022	89725096
PTEN	chr10	89725085	89725191
PTEN	chr10	89725180	89725299
RAD51B	chr14	68290161	68290228
RAD51B	chr14	68290217	68290324
RAD51B	chr14	68290346	68290464
RAD51B	chr14	68292119	68292232
RAD51B	chr14	68292229	68292354
RAD51B	chr14	68301706	68301825
RAD51B	chr14	68301814	68301927
RAD51B	chr14	68301916	68302026
RAD51B	chr14	68331630	68331749
RAD51B	chr14	68331738	68331817
RAD51B	chr14	68331806	68331916
RAD51B	chr14	68352483	68352603
RAD51B	chr14	68352594	68352696
RAD51B	chr14	68352688	68352809
RAD51B	chr14	68353681	68353808
RAD51B	chr14	68353800	68353886
RAD51B	chr14	68758498	68758615
RAD51B	chr14	68758604	68758693
RAD51B	chr14	68758682	68758764
RAD51B	chr14	68878087	68878209
RAD51B	chr14	68878198	68878297
RAD51B	chr14	68934798	68934917
RAD51B	chr14	68934905	68935017
RAD51B	chr14	68944292	68944414
RAD51B	chr14	68944403	68944479
RAD51B	chr14	68963684	68963803
RAD51B	chr14	68963792	68963913
RAD51B	chr14	69061151	69061287
RAD51B	chr14	69061303	69061407
RAD51C	chr17	56769935	56770046
RAD51C	chr17	56770034	56770137
RAD51C	chr17	56770126	56770235
RAD51C	chr17	56772204	56772327
RAD51C	chr17	56772319	56772441

RAD51C	chr17	56772431	56772554
RAD51C	chr17	56772543	56772644
RAD51C	chr17	56773904	56774017
RAD51C	chr17	56774006	56774080
RAD51C	chr17	56774069	56774176
RAD51C	chr17	56774165	56774270
RAD51C	chr17	56780495	56780595
RAD51C	chr17	56780584	56780662
RAD51C	chr17	56780651	56780759
RAD51C	chr17	56787160	56787249
RAD51C	chr17	56787238	56787312
RAD51C	chr17	56787301	56787408
RAD51C	chr17	56798059	56798162
RAD51C	chr17	56798156	56798232
RAD51C	chr17	56801345	56801448
RAD51C	chr17	56801437	56801511
RAD51C	chr17	56809791	56809868
RAD51C	chr17	56809857	56809959
RAD51C	chr17	56811389	56811510
RAD51C	chr17	56811499	56811620
RAD51C	chr17	56811609	56811675
RAD51D	chr17	33427876	33427988
RAD51D	chr17	33427977	33428105
RAD51D	chr17	33428134	33428261
RAD51D	chr17	33428235	33428359
RAD51D	chr17	33428298	33428388
RAD51D	chr17	33430213	33430327
RAD51D	chr17	33430317	33430402
RAD51D	chr17	33430445	33430579
RAD51D	chr17	33430568	33430665
RAD51D	chr17	33433325	33433455
RAD51D	chr17	33433455	33433552
RAD51D	chr17	33433881	33434007
RAD51D	chr17	33433995	33434113
RAD51D	chr17	33434102	33434215
RAD51D	chr17	33434303	33434430
RAD51D	chr17	33434419	33434517
RAD51D	chr17	33443833	33443956
RAD51D	chr17	33443944	33444067
RAD51D	chr17	33444067	33444141
RAD51D	chr17	33445450	33445550
RAD51D	chr17	33445528	33445653
RAD51D	chr17	33446003	33446126
RAD51D	chr17	33446115	33446241
RAD51D	chr17	33446449	33446586
RAD51D	chr17	33446586	33446720

Supplementary Table S4. Mutation in the PDAC Basic panel

ID	*APC*	*CDKN2A*	*FBXW7*	*GNAS*	*KRAS*	*PIK3CA*	*SMAD4*	*TP53*
785					**p.Gly12Arg (49%)**			
803					**p.Gly12Val (60%)**			
812		**p.Val51SerfsTer2 (100%)**			**p.Gly12Arg (67%)**		**p.Trp524Cys (100%)**	**p.Arg249Ser (99%)**
817					**p.Gly12Val (49%)**			
834		**p.Leu130Gln (100%)**			**p.Gly12Val (64%)**			**p.Val157Phe (99%)**
867					**p.Gly12Asp (67%)**			**p.Cys135Trp (95%)**
883					**p.Gly12Asp (33%)**			
897					**p.Gly12Arg (80%)**			**p.Cys176Phe (99%)**
934		**p.Leu63ArgfsTer78 (100%)**			**p.Gly12Asp (65%)**		**p.Asp415GlufsTer20 (100%)**	**p.Tyr234Cys (99%)**
943					**p.Gly12Arg (59%)**		**p.Arg361His (99%)**	**p.Arg273His (99%)**
963					**p.Gly12Asp (50%)**			**p.Tyr234Asn (95%)**
980					**p.Gly12Val (78%)**			**p.Arg175His (99%)**
985					**p.Gly12Asp (48%)**			**p.Lys132Glu (99%)**
1009					**p.Gly12Val (60%)**			**p.Phe109Ser (99%)**
1020					**p.Gly12Val (50%)**		**p.Gln248Ter (99%)**	
1038					**p.Gly12Asp (49%)**			**p.Arg181Cys (99%)**
1060		**p.Arg58Ter (99%)**			**p.Gly12Asp (99%)**			**p.Cys242AlafsTer5 (100%)**
1061					**p.Gly12Asp (50%)**			**p.Gly245Ser (99%)**
1102					**p.Gly12Asp (49%)**			
1116					**p.Gln61Leu (38%)**			**p.Pro152AlafsTer14 (19%)**
1128					**p.Gly12Asp (70%)**			**p.Gln167Ter (98%)**
1152					**p.Gly12Val (52%)**			
1170					**p.Gly12Asp (49%)**		**p.Asp360Val (98%)**	**p.Val272Leu (98%)**
1185					**p.Gly12Val (49%)**			**p.Arg248Trp (99%)**
1258					**p.Gly12Val (48%)**			**p.Phe270Ile (98%)**
1269					**p.Gly12Asp (65%)**			**p.Thr155Pro (99%)**

Supplementary Table S4. Mutations in PDAC Basic Panel cont'd

ID	*APC*	*CDKN2A*	*FBXW7*	*GNAS*	*KRAS*	*PIK3CA*	*SMAD4*	*TP53*
1284					**p.Gly12Cys (33%)**		**p.Gln334Ter (98%)**	**p.Pro219Leufs Ter2 (99%)**
1290					**p.Gln61His (47%)**			
1335		**p.Leu78Hisfs Ter41 (99%)**			**p.Gly12Val (46%)**			**p.Pro177_Cys182del (92%)**
1346					**p.Gly12Val (47%)**			
1350					**p.Gly12Val (64%)**			**p.Leu93CysfsTer30 (99%)**
1364					**p.Gly12Asp (48%)**			**p.Val272Leu (98%)**
1378					**p.Gly12Val (76%)**			**p.Asp228Ter (98%)**
1392					**p.Gly12Val (45%)**			**p.Arg342Glnfs Ter3 (99%)**
1433					**p.Gly12Asp (51%)**			
1454					**p.Gly12Val (50%)**			**p.Tyr234Ter (99%)**
1462					**p.Gly12Asp (50%)**			**p.Pro153AlafsTer28 (81%)**
1464					**p.Gly12Asp (50%)**			
1504					**p.Gly12Arg (50%)**		**p.Ala118Val (97%)**	**p.Val216Met (97%)**
1524				***p.Arg201His (54%)***	**p.Gly12Asp (36%)**			
1542		**p.His83Tyr (55%)**	***p.Arg505Cys (48%)***		**p.Gly12Asp (49%)**			**p.Gln165Ter (98%)**
1572					**p.Gly12Asp (44%)**			
1579					**p.Gly12Asp (47%)**			**p.Arg175His (90%)**
1585					**p.Gly12Val (48%)**		**p.His132Asp (95%)**	**p.Val157Phe (99%)**
1590					**p.Gly12Val (49%)**			**p.Tyr163Asn (98%)**
1608					**p.Gly12Val (48%)**			**p.Thr81AsnfsTer42 (99%)**
1609					**p.Gly12Arg (49%)**			**p.Tyr220Cys (100%)**
1628					**p.Gly12Asp (67%)**			
1630					**p.Gly12Val (50%)**			
1753	***p.Glu1317Gln (52%)***				**p.Gly12Arg (99%)**			
1762					**p.Gly12Arg (55%)**			**p.Arg273Cys (99%)**
1763								
1764					**p.Gly12Asp (58%)**			**p.Gly244Cys (99%)**

Supplementary Table S4. Mutations in PDAC Basic Panel cont'd

ID	*APC*	*CDKN2A*	*FBXW7*	*GNAS*	*KRAS*	*PIK3CA*	*SMAD4*	*TP53*
1768					**p.Gly12Val (68%)**		**p.Arg361Cys (99%)**	**p.Leu194Arg (99%)**
1771					**p.Gly12Arg (51%)**			
1777		**p.Arg80Ter (99%)**			**p.Gly12Asp (65%)**			**p.His178AlafsTer71 (97%)**
1778					**p.Gly12Asp (99%)**			**p.Val157Phe (100%)**
1786					**p.Gly12Val (47%)**			**p.Cys135Ser (99%)**
1789					**p.Gln61His (50%)**			**p.Tyr205IlefsTer42 (99%)**
1790					**p.Gly12Arg (48%)**			
1804					**p.Gly12Val (47%)**			
1827		**p.Ala68Leu (99%)**			**p.Gly12Asp (49%)**			**p.Val157_Met160del (99%)**
1841				***p.Arg201Leu (50%)***	**p.Gly12Asp (48%)**			**p.Pro278Ser (99%)**
1846					**p.Gly12Asp (49%)**			**p.Val157Gly (100%)**
1855					**p.Gly12Val (51%)**			**p.Gly245Asp (99%)**
1885	***p.Glu1317Gln (47%)***	**p.Val82CysfsTer64 (55%)**			**p.Gly12Arg (45%)**		**p.Arg361His (98%)**	**p.Leu194Pro (99%)**
1953					**p.Gly12Asp (50%)**			
1954	***p.Asn862Lys (100%)***				**p.Gly12Arg (57%)**			
1957		**p.Val82AlafsTer74 (29%)**			**p.Gly12Asp (48%)**			**p.Arg248Trp (99%)**
2069		**p.Arg58Ter (98%)**			**p.Gly12Asp (47%)**		**p.Ala319LeufsTer17 (99%)**	**p.Arg282Trp (25%)**
2092					**p.Gly12Asp (99%)**			**p.Arg248Gln (99%)**
2143					**p.Gly12Val (96%)**		**p.Asp351Val (94%)**	**p.Leu265Pro (93%)**
2145					**p.Gly12Val (51%)**		**p.Arg361His (97%)**	
2150					**p.Gly12Asp (39%)**			**p.Arg175His (74%)**
2177					**p.Gly12Val (50%)**			
2187					**p.Gly12Val (49%)**		**p.Arg361Cys (98%)**	**p.Val157Asp (99%)**
2191					**p.Gly12Val (50%)**			**p.Arg273Cys (100%)**

Supplementary Table S4. Mutations in PDAC Basic Panel cont'd

ID	***APC***	***CDKN2A***	***FBXW7***	***GNAS***	***KRAS***	***PIK3CA***	***SMAD4***	***TP53***
2192		**p.Asp84Asn (97%)**			**p.Gly12Val (97%)**		**p.Arg361His (98%)**	**p.Arg282Trp (98%)**
2200					**p.Gly12Arg (47%)**			
2230					**p.Gly12Arg (50%)**			
2243					**p.Gly12Val (49%)**			
2322								
2323					**p.Gly12Arg (48%)**			
2342					**p.Gly12Arg (50%)**			**p.Arg175His (98%)**
2434					**p.Gly12Asp (49%)**			**p.Ala276Asp (99%)**
2453					**p.Gln61Arg (51%)**			
2460					**p.Gly12Asp (50%)**			**p.His179Arg (99%)**
2491					**p.Gly12Val (99%)**			**p.Arg445Ter (99%)**
2496					**p.Gly12Asp (49%)**			**p.Ser241Ala (99%)**
2515					**p.Gly12Arg (50%)**			
2524		**p.Arg80Ter (99%)**			**p.Gly12Val (48%)**			
2561	***p.Ile1287Thr (55%)***	**p.Glu119ValfsTer28 (99%)**			**p.Gly12Asp (50%)**			**p.Pro151Arg (99%)**
2632					**p.Gly12Val (51%)**			**p.Ser403ValfsTer12 (100%)**
2636					**p.Gly12Asp (66%)**			
2637					**p.Gly12Asp (62%)**			**p.Arg175His (99%)**
2648						**p.Lys111Glu (55%)**		**p.Arg248Gln (49%); p.Arg158Cys (48%)**
2661					**p.Gly12Val (50%)**			**p.Glu271Ter (99%)**
2666					**p.Gly12Asp (50%)**			**p.Val218Glu (99%)**
2693							**p.Asp351Tyr (99%)**	
2816					**p.Gly12Val (49%)**			**Gly266Val 99%**

Supplementary Table S5. HR-DDR Variants in 100 PDX.

CASE	GENE	MUTATION TYPE	NUCLEOTIDE CHANGE	AMINO ACID CHANGE	GERMLINE/SOMATIC	VARIANT ID	ClinVar CLASS	BIC* DESIGNATION	BIC* CLASS
785x	BRCA2	stop_gained	c.7283T>A	p.Leu2428Ter	somatic	-	-	-	-
	BRCA2	frameshift_variant&feature_elongation	c.5680_5681insA	**p.Tyr1894Ter**	germline	-	-	5909insA	pathogenic
943x	PALB2	missense_variant	c.2258G>A	p.Arg753Gln	germline	COSM174350	uncertain_significance	-	-
	ATM	missense_variant	c.8495G>A	p.Arg2832His	germline	-	uncertain_significance	-	-
963x	BRCA1	missense_variant	c.3119G>A	p.Ser1040Asn	-	rs4986852&COSM1166811	benign	S1040N	unknown
908x	REV3L	stop_gained	c.2890C>T	p.Arg964Ter		-	-	-	-
	BRCA2			p.Lys3326Ter					
1038X	STK11	missense_variant	c.1062C>G	p.Phe354Leu	germline	rs59912467, COSM21360	uncertain_significance	-	-
	BRIP1			Pro210His (39%)	homoz del?		unknown		
	CHEK1			Lys457Arg (23%)	Homoz del?		unknown		
	BRCA2			p.Lys3326Ter	Homoz del?		SNP		
1060x	BRCA2	stop_gained	c.5682C>G	**p.Tyr1894Ter**	germline	rs41293497	pathogenic	Y1894X	pathogenic
1061x	BRCA2			p.Lys3326Ter	germline				
1102x	BARD1	missense_variant&splice_region_variant	c.160A>G	p.Thr54Ala	germline	rs200254470	uncertain_significance	-	-
1152x	CHEK1	missense_variant	c.1370A>G	p.Lys457Arg	somatic	-	-	-	-
1170x	REV3L	missense_variant	c.6622A>G	p.Lys2208Glu	germline	-	-	-	-

Supplementary Table S5. HR-DDR Varinats in 100 PDX cont'd.

CASE	GENE	MUTATION TYPE	NUCLEOTIDE CHANGE	AMINO ACID CHANGE	GERMLINE/ SOMATIC	VARIANT ID	ClinVar CLASS	BIC* DESIGNATION	BIC* CLASS
1185x	BRCA2	frameshift_variant&feature_elongation	c.6201_6202insA	**p.Ile2068AsnfsTer10**	germline	rs397507833	-	6429delC	pathogenic
1346x	BRCA2	frameshift_variant&feature_truncation	c.2905delC	p.Gln969LysfsTer3	somatic	-	-	-	-
	BRCA2	stop_gained	c.7738C>T	**p.Gln2580Ter**	germline	rs80358999	pathogenic	Q2580X	pathogenic
1454x	BRCA2	stop_gained	c.8878C>T	**p.Gln2960Ter**	germline	rs80359140	pathogenic	Q2960X	pathogenic
1464x	BARD1	stop_gained	c.2279C>A	p.Ser760Ter	germline	-	-	-	-
1608x	BRCA2			p.Lys3326Ter					
1804x	ATM	splice_acceptor_variant	c.1608-1G>A	-	somatic	-	-	-	-
1841x	BRCA1	missense_variant	c.1456T>C	p.Phe486Leu	germline	rs55906931	benign	F486L	unknown
1846x	ATM	missense_variant	c.6631C>T	p.Leu2211Phe	germline	-	-	-	-
	BRCA2			p.Lys3326Ter	homozygous del?		SNP		
1954x	BRCA2	stop_gained&inframe_insertion	c.4131_4132insTGAGGA	**p.Asn1377_Thr1378insTer**	germline	rs80359429	pathogenic	1377insXG	unknown
	REV3L	frameshift variant		**Trp1744Cys (20%)**	somatic		pthogenic		
	REV3L	missense_variant	c.86A>C	p.Gln29Pro	-	-	-	-	-

Supplementary Table S5. HR-DDR Varinats in 100 PDX cont'd.

CASE	GENE	MUTATION TYPE	NUCLEOTIDE CHANGE	AMINO ACID CHANGE	GERMLINE/SOMATIC	VARIANT ID	ClinVar CLASS	BIC* DESIGNATION	BIC* CLASS
2092x	ATM	missense_variant	c.146C>G	p.Ser49Cys	germline	rs1800054	benign, risk factor	-	-
	BRCA2	frameshift_variant &feature_truncation	c.657_658delTG	**p.Val220IlefsTer4**	germline	-	-	886delGT	pathogenic
	BRCA2	missense_variant	c.6253T>G	p.Leu2085Val	germline	-	-	-	-
	REV3L	missense_variant	c.3133A>C	p.Ser1045Arg	somatic	-	-	-	-
2187x	ATM	missense_variant	c.146C>G	p.Ser49Cys	germline	rs1800054	benign, risk factor	-	-
2192x	BRCA2			p.Lys3326Ter	germline				
	BRCA1	missense_variant	c.3119G>A	p.Ser1040Asn	germline	rs4986852&COSM1166811	benign	S1040N	unknown
2200x	STK11	missense_variant	c.233A>G	p.Lys78Arg	somatic	COSM1390407	-	-	-
	BRCA1	missense_variant	c.3119G>A	p.Ser1040Asn	germline	rs4986852&COSM1166811	benign	S1040N	unknown
2230x	CHEK1	missense_variant	c.1393A>G	p.Ile465Val	germline	-	-	-	-
2243x	BRCA1			Ser1040Asn (34%)	homozygous del?		benign		
2323x	ATM	stop_gained	c.7456C>T	**p.Arg2486Ter**	germline	COSM1351002&COSM1351003	pathogenic	-	-
	REV3L	missense_variant	c.2071A>G	p.Ile691Val	germline	-	-	-	-
2342x	BRCA1	missense_variant	c.314A>G	p.Tyr105Cys	germline	rs28897673	Benign	Y105C	unknown

Supplementary Table S5. HR-DDR Varinats in 100 PDX cont'd.

CASE	GENE	MUTATION TYPE	NUCLEOTIDE CHANGE	AMINO ACID CHANGE	GERMLINE/SOMATIC	VARIANT ID	ClinVar CLASS	BIC* DESIGNATION	BIC* CLASS
2434x	BRCA2	frameshift_variant&feature_elongation	c.5680_5681insA	**p.Tyr1894Ter**	germline	-	-	5909insA	pathogenic
	BRCA1	missense_variant	c.3119G>A	p.Ser1040Asn	germline	rs4986852&COSM1166811	benign	S1040N	unknown
2515x	BRCA1	stop_gained	c.4117G>T	**p.Glu1373Ter**	germline	rs80357259	pathogenic	E1373X	pathogenic
	BRCA1	missense_variant	c.3119G>A	p.Ser1040Asn	germline	rs4986852&COSM1166811	benign	S1040N	unknown
1258x	BRCA2	missense_variant	c.4061C>T	p.Thr1354Met	germline	rs80358656, COSM69844, COSM1366436	uncertain_significance&ikely_benign	T1354M	unknown
1462x	BRCA2	missense_variant	c.5744C>T	p.Thr1915Met	germline	-	benign	T1915M	not pathogenic/low clinical significance
	REV3L	missense_variant	c.7264A>T	p.Ser2422Cys	germline	-	-	-	-
1572x	STK11	frameshift_variant	c.223_235delAGGGCCGTCAAGA	p.Arg75SerfsTer17	somatic	-	-	-	-
1777x	FAM175A	missense_variant	c.828G>C	p.Glu276Asp	Somatic	-	-	-	-
1778x	BRCA1	missense_variant	c.3119G>A	p.Ser1040Asn	germline	rs4986852&COSM1166811	benign	S1040N	unknown
2460x	ATM	missense_variant	c.146C>G	p.Ser49Cys	germline	rs1800054	benign, risk factor	-	-
2491x	ATM	missense_variant	c.146C>G	p.Ser49Cys	germline	rs1800054	benign, risk factor	-	-
2666x	ATM	missense_variant	c.7357C>T	p.Arg2453Cys	germline	rs755418571&COSM1351001&COSM1351000	Uncertain significance	-	-

APPENDIX 1

Information presented in the introduction are summarized in the following articles co-authored by the candidate.

A. Exome sequencing identifies frequent inactivating mutations in BAP1, ARID1A and PBRM1 in intrahepatic cholangiocarcinomas.

B. Histomolecular phenotypes and outcome in adenocarcinoma of the ampulla of Vater.

C. Reporting Tumor Molecular Heterogeneity in Histopathological Diagnosis.

D. Building capacity for sustainable research programmes for cancer in Africa.

E. Research capacity. Enabling the genomic revolution in Africa.

F. 'Life in Data'-Outcome of a Multi-Disciplinary, Interactive Biobanking Conference Session on Sample Data.

G. International network of cancer genome projects.

H. Multigene mutational profiling of cholangiocarcinomas identifies actionable molecular subgroups.

APPENDIX 1.A

Exome sequencing identifies frequent inactivating mutations in *BAP1*, *ARID1A* and *PBRM1* in intrahepatic cholangiocarcinomas

Yuchen Jiao[1–3,20], Timothy M Pawlik[3,4,20], Robert A Anders[3,5,20], Florin M Selaru[6], Mirte M Streppel[5], Donald J Lucas[7], Noushin Niknafs[8], Violeta Beleva Guthrie[8], Anirban Maitra[3,5], Pedram Argani[3,5], G Johan A Offerhaus[9], Juan Carlos Roa[10], Lewis R Roberts[11], Gregory J Gores[11], Irinel Popescu[12], Sorin T Alexandrescu[12], Simona Dima[12], Matteo Fassan[13,14], Michele Simbolo[13,14], Andrea Mafficini[13], Paola Capelli[14], Rita T Lawlor[13,14], Andrea Ruzzenente[15], Alfredo Guglielmi[15], Giampaolo Tortora[16], Filippo de Braud[17], Aldo Scarpa[13,14], William Jarnagin[18], David Klimstra[19], Rachel Karchin[8], Victor E Velculescu[1–3], Ralph H Hruban[3,5], Bert Vogelstein[1–3], Kenneth W Kinzler[1–3], Nickolas Papadopoulos[1–3] & Laura D Wood[5]

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Through exomic sequencing of 32 intrahepatic cholangiocarcinomas, we discovered frequent inactivating mutations in multiple chromatin-remodeling genes (including *BAP1*, *ARID1A* and *PBRM1*), and mutation in one of these genes occurred in almost half of the carcinomas sequenced. We also identified frequent mutations at previously reported hotspots in the *IDH1* and *IDH2* genes encoding metabolic enzymes in intrahepatic cholangiocarcinomas. In contrast, *TP53* was the most frequently altered gene in a series of nine gallbladder carcinomas. These discoveries highlight the key role of dysregulated chromatin remodeling in intrahepatic cholangiocarcinomas.

Carcinomas of the biliary tract are aggressive malignancies, with 5-year survival of less than 10% (ref. 1). These carcinomas arise throughout the biliary tree and are anatomically classified as either intrahepatic or extrahepatic cholangiocarcinomas[2]. In addition to cholangiocarcinomas, gallbladder carcinomas also arise from the biliary tree. Although often grouped with cholangiocarcinomas owing to the relative rarity of both diseases, gallbladder carcinomas have distinct natural histories compared to cholangiocarcinomas, suggesting different underlying tumor biology. Although a subset of individuals with biliary tract cancers has identifiable risk factors such as primary sclerosing cholangitis or liver fluke infestation, the majority lack such risk factors[2]. There is currently no way to screen effectively for early disease, and, other than surgery, there are no effective therapies.

Previous studies of the molecular alterations in biliary tract cancers have focused on small sets of selected genes, usually those known to be altered in pancreatic ductal adenocarcinoma. Somatic alterations in the *KRAS*, *TP53*, *CDKN2A* and *SMAD4* (*DPC4*) genes have been reported in cholangiocarcinoma[3–7]. The prevalence of these alterations varies widely among studies, perhaps in part owing to an inability to analyze the anatomical subtypes of cholangiocarcinoma separately. Mutations in genes coding for components of the phosphatidylinositide 3-kinase (PI3K) cell signaling pathway, including *PIK3CA*, *PTEN* and *AKT1*, have also been reported in cholangiocarcinoma, as have mutations in previously identified hotspots in *IDH1* and *IDH2* (encoding isocitrate dehydrogenase 1 and 2, respectively)[3–5]. Interestingly, mutations in these latter genes encoding metabolic enzymes occur frequently in tumors of the central nervous system and in leukemias but have not been identified in any other gastrointestinal malignancy studied so far[8]. The molecular alterations in gallbladder

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APPENDIX 1.B

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ORIGINAL REPORT

Histomolecular Phenotypes and Outcome in Adenocarcinoma of the Ampulla of Vater

David K. Chang, Nigel B. Jamieson, Amber L. Johns, Christopher J. Scarlett, Marina Pajic, Angela Chou, Mark Pinese, Jeremy L. Humphris, Marc D. Jones, Christopher Toon, Adnan M. Nagrial, Lorraine A. Chantrill, Venessa T. Chin, Andreia V. Pinho, Ilse Rooman, Mark J. Cowley, Jianmin Wu, R. Scott Mead, Emily K. Colvin, Jaswinder S. Samra, Vincenzo Corbo, Claudio Bassi, Massimo Falconi, Rita T. Lawlor, Stefano Crippa, Nicola Sperandio, Samantha Bersani, Euan J. Dickson, Mohamed A.A. Mohamed, Karin A. Oien, Alan K. Foulis, Elizabeth A. Musgrove, Robert L. Sutherland, James G. Kench, C. Ross Carter, Anthony J. Gill, Aldo Scarpa, Colin J. McKay, and Andrew V. Biankin

Author affiliations appear at the end of this article.

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D.K.C., N.B.J., A.S., C.J.M., and A.V.B. contributed equally to this work.

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Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

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ABSTRACT

Purpose
Individuals with adenocarcinoma of the ampulla of Vater demonstrate a broad range of outcomes, presumably because these cancers may arise from any one of the three epithelia that converge at that location. This variability poses challenges for clinical decision making and the development of novel therapeutic strategies.

Patients and Methods
We assessed the potential clinical utility of histomolecular phenotypes defined using a combination of histopathology and protein expression (CDX2 and MUC1) in 208 patients from three independent cohorts who underwent surgical resection for adenocarcinoma of the ampulla of Vater.

Results
Histologic subtype and CDX2 and MUC1 expression were significant prognostic variables. Patients with a histomolecular pancreaticobiliary phenotype (CDX2 negative, MUC1 positive) segregated into a poor prognostic group in the training (hazard ratio [HR], 3.34; 95% CI, 1.69 to 6.62; $P < .001$) and both validation cohorts (HR, 5.65; 95% CI, 2.77 to 11.5; $P < .001$ and HR, 2.78; 95% CI, 1.25 to 7.17; $P = .0119$) compared with histomolecular nonpancreaticobiliary carcinomas. Further stratification by lymph node (LN) status defined three clinically relevant subgroups: one, patients with histomolecular nonpancreaticobiliary (intestinal) carcinoma without LN metastases who had an excellent prognosis; two, those with histomolecular pancreaticobiliary carcinoma with LN metastases who had a poor outcome; and three, the remainder of patients (nonpancreaticobiliary, LN positive or pancreaticobiliary, LN negative) who had an intermediate outcome.

Conclusion
Histopathologic and molecular criteria combine to define clinically relevant histomolecular phenotypes of adenocarcinoma of the ampulla of Vater and potentially represent distinct diseases with significant implications for current therapeutic strategies, the ability to interpret past clinical trials, and future trial design.

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INTRODUCTION

Adenocarcinoma of the ampulla of Vater is the second most common malignancy of the periampullary region and accounts for up to 30% of all pancreaticoduodenectomies.[1,2] The broad range of outcomes for patients with adenocarcinoma of the ampulla of Vater[3-8] impairs the interpretation of clinical trials and hampers clinical decision making. This is perhaps not surprising, because they may arise from any one of the three epithelia (duodenal, biliary, or pancreatic) that converge at this location.

The inability to predict individual outcomes for cancers in this anatomic location has made aspects of clinical decision making difficult with regard to the aggressiveness of therapy and the choice of appropriate chemotherapeutic strategies. Randomized, controlled trials[9-11] and single-institution cohorts[12-18] grouping all adenocarcinomas together have failed to definitively demonstrate a survival benefit for adjuvant chemotherapy. Some studies have suggested that adenocarcinoma of the ampulla of Vater may be subdivided based on histologic appearances[19,20] and GI markers such as caudal-type

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APPENDIX 1.C

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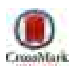
Reporting Tumor Molecular Heterogeneity in Histopathological Diagnosis



Andrea Mafficini[1¶], Eliana Amato[1¶], Matteo Fassan[1¶], Michele Simbolo[1], Davide Antonello[1,2], Caterina Vicentini[1], Maria Scardoni[1], Samantha Bersani[1], Marisa Gottardi[1], Borislav Rusev[1], Giorgio Malpeli[1,2], Vincenzo Corbo[1], Stefano Barbi[1], Katarzyna O. Sikora[1], Rita T. Lawlor[1], Giampaolo Tortora[3], Aldo Scarpa[1]*

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Abstract

Background: Detection of molecular tumor heterogeneity has become of paramount importance with the advent of targeted therapies. Analysis for detection should be comprehensive, timely and based on routinely available tumor samples.

Aim: To evaluate the diagnostic potential of targeted multigene next-generation sequencing (TM-NGS) in characterizing gastrointestinal cancer molecular heterogeneity.

Methods: 35 gastrointestinal tract tumors, five of each intestinal type gastric carcinomas, pancreatic ductal adenocarcinomas, pancreatic intraductal papillary mucinous neoplasms, ampulla of Vater carcinomas, hepatocellular carcinomas, cholangiocarcinomas, pancreatic solid pseudopapillary tumors were assessed for mutations in 46 cancer-associated genes, using Ion Torrent semiconductor-based TM-NGS. One ampulla of Vater carcinoma cell line and one hepatic carcinosarcoma served to assess assay sensitivity. *TP53*, *PIK3CA*, *KRAS*, and *BRAF* mutations were validated by conventional Sanger sequencing.

Results: TM-NGS yielded overlapping results on matched fresh-frozen and formalin-fixed paraffin-embedded (FFPE) tissues, with a mutation detection limit of 1% for fresh-frozen high molecular weight DNA and 2% for FFPE partially degraded DNA. At least one somatic mutation was observed in all tumors tested; multiple alterations were detected in 20/35 (57%) tumors. Seven cancers displayed significant differences in allelic frequencies for distinct mutations, indicating the presence of intratumor molecular heterogeneity; this was confirmed on selected samples by immunohistochemistry of p53 and Smad4, showing concordance with mutational analysis.

Conclusions: TM-NGS is able to detect and quantitate multiple gene alterations from limited amounts of DNA, moving one step closer to a next-generation histopathologic diagnosis that integrates morphologic, immunophenotypic, and multigene mutational analysis on routinely processed tissues, essential for personalized cancer therapy.

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Data Availability: The authors confirm that all data underlying the findings are fully available without restriction. Patients/tumors data are in Table S1 of the paper. Sequences used to produce all the data have been uploaded to Dryad and are available under the DOI: doi:10.5061/dryad.hf93m.

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¶ AM, EA, MF are joint senior authors on this work.

Introduction

Cancer inter-tumor and intra-tumor heterogeneity, a well-known fact described by pathologists in the classification of tumors over the last two centuries, has finally risen to the forefront of clinical interest. Cancer genomics and transcriptomics studies have shown that tumors belonging to the same histotype display remarkable differences in their genetic assets; such inter-tumor heterogeneity is the basis of molecular subclassification with clinical impact for targeted therapeutic approaches. It has also become clear that phenotypically and genetically diverse clones of neoplastic cells may be juxtaposed within the same tumor[1,2]. These clones are thought to be players in a branching clonal evolution scenario leading to the formation of metastases that are more aggressive and resistant to treatments than the primary tumor [1].

APPENDIX 1.D

Building capacity for sustainable research programmes for cancer in Africa

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Abstract | Cancer research in Africa will have a pivotal role in cancer control planning in this continent. However, environments (such as those in academic or clinical settings) with limited research infrastructure (laboratories, biorespositories, databases) coupled with inadequate funding and other resources have hampered African scientists from carrying out rigorous research. In September 2012, over 100 scientists with expertise in cancer research in Africa met in London to discuss the challenges in performing high-quality research, and to formulate the next steps for building sustainable, comprehensive and multi-disciplinary programmes relevant to Africa. This was the first meeting among five major organizations: the African Organisation for Research and Training in Africa (AORTIC), the Africa Oxford Cancer Foundation (AfrOx), and the National Cancer Institutes (NCI) of Brazil, France and the USA. This article summarizes the discussions and recommendations of this meeting, including the next steps required to create sustainable and impactful research programmes that will enable evidenced-based cancer control approaches and planning at the local, regional and national levels.

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Correspondence to: I.A. [illegible]

Introduction

Africa is facing an unprecedented growth in cancer burden and is inadequately prepared to meet this public health challenge. By 2030, the projected new cancer cases per year are 1.27 million and 0.97 million deaths.[1,2] This increasing number of cancer cases takes into account the predicted increase in the African population from 1.02 billion to 1.56 billion.[1,3] Given the inadequate surveillance of cancer in the African population and the dearth of high quality cancer registries, projections for cancer incidence and mortality may be underestimates. These projections might be even higher owing to the increasing exposure to established cancer risk factors, such as infections with viruses (including HIV, Epstein–Barr virus [EBV], human herpesvirus 8 [HHV8], hepatitis B and C, human papilloma virus [HPV]),[2–4] tobacco, diet, obesity and physical inactivity, and altered reproductive patterns.[4] Available data from few cancer registries have also shown that a significant proportion of cancers are diagnosed in children, including HIV-related paediatric malignancies.[5–9] The cancer burden in Africa is further exacerbated by the low survival, which is among the worse in the world because of advanced-stage disease at diagnosis and extremely limited human resources and treatment options.[1]

Given the limited preventive and health-care resources in Africa, it is imperative that cancer control policies are evidence-based and target those cancers associated with the highest burden (cancer incidence, morbidity and mortality) in this continent.[10] Comprehensive cancer control planning evaluates a variety of ways to enforce the most cost-effective and beneficial ways to reduce cancer incidence, mortality and morbidity, and to improve the quality of life of cancer survivors through the implementation of evidence-based strategies across the cancer continuum (that is, prevention, early detection, diagnosis, treatment, and palliation).[11] Research will have a pivotal role in cancer control in Africa because it will address the aetiology of cancers unique to Africa, which will lead to developing locally-appropriate strategies to prevent and treat cancer. Research will also contribute to effective, affordable and evidence-based interventions (practices that have been proven effective through research and outcome evaluations) that can be integrated into existing clinical and public health. Cancer research can become an opportunity for development and poverty alleviation through the creation of sustainable cancer control programmes because cancer is a common cause of loss of social status and bankruptcy in low-and middle-income countries.[10–12] Through these programmes, African scientists are trained to conduct, lead and formulate new directions for cancer research, thus establishing

Competing interests
The authors declare no competing interests.

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APPENDIX 1.E

Speciation battleground. On either side of the narrow hybridization zone (dark brown), the carrion crow (*Corvus corone*) (dark area) and hooded crow (*Corvus cornix*) (pale area) (*2*) maintain their marked phenotypic differentiation, despite apparent lack of genetic differentiation. Genome-wide admixture analyses (inset at bottom) show that German carrion crows most closely resemble (80%) hooded crows, and are quite distinct from Spanish carrion crows. Sampling sites for the present study (*6*) are shown as circles. Sp, Spain; Ge, Germany; Po, Poland; Sw, Sweden.

Yet, roughly a decade ago, newly proposed DNA-based taxonomy (*11*) promised to solve the species debate. A Barcode of Life Data Systems (BOLD) (*12*) quickly emerged, seeking to provide a reliable, cost-effective solution to the problem of species identification (*12*) and a standard screening threshold of sequence difference (10× average intraspecific difference) to speed the discovery of new animal species (*13*). Sometimes considered a "caricature of real taxonomy" (*14*), this approach failed to identify, perhaps not surprisingly, two American crow species and a number of members of the herring gull *Larus argentatus* species assemblage above the set threshold (*13*). Furthermore, despite past (*3*) and present (*6*) sequencing projects, carrion crows and hooded crows can also not be differentiated from one another by means of DNA-barcode approaches. By contrast, Poelstra *et al.* show that much more DNA sequencing data are needed, combined with RNA expression data, to reconstruct the evolution of a reproductive barrier that culminated in the speciation of these two crow taxa. Armed with this new very detailed genetic information, it is clear that none of the currently formulated species concepts fully apply to these two crow taxa (unless one is willing relax some stringency in the various definitions). Indeed, the genomes of German carrion crows are much more similar to those of hooded crows than to Spanish carrion crows. Put simply, apart from the few carrion crow type "speciation islands," German carrion crows could be considered to represent hooded crows with a black (carrion crow) phenotype.

There is a clear need for additional population genomic studies using a more dense sampling, especially among the fully black carrion crows, before the complexity of reproductive isolation and speciation among these two taxa can be fully understood. The speciation genomics strategy already proved itself in unraveling the complexities of mimicry among many *Heliconius* butterfly taxa (*7*) and, as in the study of Poelstra *et al.*, stresses the importance of using RNA-based information in addition to DNA. Only time will tell if, and when, German carrion crows will adopt the "hooded phenotype," a fate that seems unavoidable. Until then, we can only applaud these crows for defeating Linnaeus's curse. ■

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By **The H3Africa Consortium*†**

POLICY

Our understanding of genome biology, genomics, and disease, and even human history, has advanced tremendously with the completion of the Human Genome Project. Technological advances coupled with significant cost reductions in genomic research have yielded novel insights into disease etiology, diagnosis, and therapy for some of the world's most intractable and devastating diseases—including malaria, HIV/AIDS, tuberculosis, cancer, and diabetes. Yet, despite the burden of infectious diseases and, more recently, noncommunicable diseases (NCDs) in Africa, Africans have only participated minimally in genomics research. Of the thousands of genome-wide association studies (GWASs) that have been conducted globally, only seven (for HIV susceptibility, malaria, tuberculosis, and podoconiosis) have been conducted exclusively on African participants; four others (for prostate cancer, obsessive compulsive disorder, and anthropometry) included some African participants (www.genome.gov/gwastudies/). As discussed in 2011 (www.h3africa.org), if the dearth of genomics research involving Africans persists, the potential health and economic benefits emanating from genomic science may elude an entire continent.

The lack of large-scale genomics studies in Africa is the result of many deep-seated issues, including a shortage of African scientists with genomic research expertise, lack of biomedical research infrastructure, limited computational expertise and resources, lack of adequate support for biomedical research by African governments, and the participation of many African scientists in collaborative research at no more than the level of sample collection. Overcoming these limitations will, in part, depend on African

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APPENDIX 1.F

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RESEARCH ARTICLE

'*Life in Data*'—Outcome of a Multi-Disciplinary, Interactive Biobanking Conference Session on Sample Data

Sara Y. Nussbeck,[1] Muriel Rabone,[2] Erica E. Benson,[3] Gabriele Droege,[4]
Jackie Mackenzie-Dodds,[2] and Rita T. Lawlor[5]

Introduction: Clinical, biodiversity, and environmental biobanks share many data standards, but there is a lack of harmonization on how data are defined and used among biobank fields. This article reports the outcome of an interactive, multidisciplinary session at a meeting of the European, Middle Eastern, and African Society for Biopreservation and Biobanking (ESBB) designed to encourage a 'learning-from-each-other' approach to achieve consensus on data needs and data management across biobank communities.
Materials, Methods, and Results: The Enviro-Bio and ESBBperanto Working Groups of the ESBB co-organized an interactive session at the 2013 conference (Verona, Italy), presenting data associated with biobanking processes, using examples from across different fields. One-hundred-sixty (160) diverse biobank participants were provided electronic voting devices with real-time screen display of responses to questions posed during the session. The importance of data standards and robust data management was recognized across the conference cohort, along with the need to raise awareness about these issues within and across different biobank sectors.
Discussion and Conclusion: While interactive sessions require a commitment of time and resources, and must be carefully coordinated for consistency and continuity, they stimulate the audience to be pro-active and direct the course of the session. This effective method was used to gauge opinions about significant topics across different biobanking communities. The votes revealed the need to: (a) educate biobanks in the use of data management tools and standards, and (b) encourage a more cohesive approach for how data and samples are tracked, exchanged, and standardized across biobanking communities. Recommendations for future interactive sessions are presented based on lessons learned.

Introduction

The biobanking landscape comprises a diverse and expanding collection of institutions, researchers, and practitioners who, regardless of their different functions, share a common need for best practices to implement data standards, ethical regulations, and risk management.[1,2] These regulatory, ethical, and operational standards must continually evolve to keep biobanks in step with technical and scientific advancements, and the present and future demands of their stakeholders and clients. However, procedures, policies, and standards are designed with limited consideration given to the potential advantage of adapting those created by other thematic biobanks. Encouraging and sustaining cooperation and knowledge-sharing across globally dispersed and diverse biobanks is challenging, and scaling-up interactions is a limiting factor in terms of resources, costs, and coordination.

Data constitute a 'universal language' across biobank disciplines as they are the result of sample collection, management, and use. Additionally, genomics research technologies that apply increasingly sensitive biomolecular analyses are rapidly evolving, increasing the intrinsic value of all associated data. In any biobank the value and utility of a biospecimen or biological resource is determined by a) its fitness-for-purpose (assurance that the quality of the biospecimen meets the standard(s) of its end use; and b) the quality of the associated and attributed information (information used to describe, annotate, and authenticate the biospecimen as well as the data that provide a record of the processing and pre-analytical

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APPENDIX 1.G

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PERSPECTIVES

International network of cancer genome projects

The International Cancer Genome Consortium*

The International Cancer Genome Consortium (ICGC) was launched to coordinate large-scale cancer genome studies in tumours from 50 different cancer types and/or subtypes that are of clinical and societal importance across the globe. Systematic studies of more than 25,000 cancer genomes at the genomic, epigenomic and transcriptomic levels will reveal the repertoire of oncogenic mutations, uncover traces of the mutagenic influences, define clinically relevant subtypes for prognosis and therapeutic management, and enable the development of new cancer therapies.

The genomes of all cancers accumulate somatic mutations[1]. These include nucleotide substitutions, small insertions and deletions, chromosomal rearrangements and copy number changes that can affect protein-coding or regulatory components of genes. In addition, cancer genomes usually acquire somatic epigenetic 'marks' compared to non-neoplastic tissues from the same organ, notably changes in the methylation status of cytosines at CpG dinucleotides.

A subset of the somatic mutations in cancer cells confers oncogenic properties such as growth advantage, tissue invasion and metastasis, angiogenesis, and evasion of apoptosis[2]. These are termed 'driver' mutations. The identification of driver mutations will provide insights into cancer biology and highlight new drug targets and diagnostic tests. Knowledge of cancer mutations has already led to the development of specific therapies, such as trastuzumab for *HER2* (also known as *NEU* or *ERBB2*)-positive breast cancers[3] and imatinib, which targets BCR-ABL tyrosine kinase for the treatment of chronic myeloid leukaemia[4,5]. The remaining somatic mutations in cancer genomes that do not contribute to cancer development are called 'passengers'. These mutations provide insights into the DNA damage and repair processes that have been operative during cancer development, including exogenous environmental exposures[6,7]. In most cancer genomes, it is anticipated that passenger mutations, as well as germline variants not yet catalogued in polymorphism databases, will substantially outnumber drivers.

Large-scale analyses of genes in tumours have shown that the mutation load in cancer is abundant and heterogeneous[8–13]. Preliminary surveys of cancer genomes have already demonstrated their relevance in identifying new cancer genes that constitute potential therapeutic targets for several types of cancer, including *PIK3CA*[14], *BRAF*[15], *NF1* (ref. 10), *KDR*[10], *PIK3R1* (ref. 9), and histone methyltransferases and demethylases[16,17]. These projects have also yielded correlations between cancer mutations and prognosis, such as *IDH1* and *IDH2* mutations in several types of gliomas[13,18]. Advances in massively parallel sequencing technology have enabled sequencing of entire cancer genomes[19–22].

Following the launch of comprehensive cancer genome projects in the United Kingdom (Cancer Genome Project)[23] and the United States (The Cancer Genome Atlas)[24], cancer genome scientists and funding agencies met in Toronto (Canada) in October 2007 to discuss the opportunity to launch an international consortium. Key reasons for its formation were: (1) the scope is huge; (2) independent cancer genome initiatives could lead to duplication of effort or incomplete studies; (3) lack of standardization across studies could diminish the opportunities to merge and compare data sets; (4) the spectrum of many cancers is known to vary across the world; and (5) an international consortium will accelerate the dissemination of data sets and analytical methods into the user community.

Working groups were created to develop strategies and policies that would form the basis for participation in the ICGC. The goals of the consortium (Box 1) were released in April 2008 (http://www.icgc.org/files/ICGC_April_29_2008.pdf). Since then, working groups and initial member projects have further refined the policies and plans for international collaboration.

Bioethical framework

ICGC members agreed to a core set of bioethical elements for consent as a precondition of membership (Box 2). The Ethics and Policy

Box 1 | Goals of the ICGC

The goals of the ICGC are:

- To coordinate the generation of comprehensive catalogues of genomic abnormalities (somatic mutations) in tumours in 50 different cancer types and/or subtypes that are of clinical and societal importance across the globe.
- To ensure high quality by defining the catalogue for each tumour type or subtype to include the full range of somatic mutations, such as single-nucleotide variants, insertions, deletions, copy number changes, translocations and other chromosomal rearrangements, and to have the following features. (1) Comprehensiveness, such that most cancer genes with somatic abnormalities occurring at a frequency of greater than 3% are discovered. (2) High resolution, ideally at a single nucleotide level. (3) High quality, using common standards for pathology and technology. (4) Data from matched non-tumour tissue, to distinguish somatic from inherited sequence variants and aberrations. (5) Generate complementary catalogues of transcriptomic and epigenomic data sets from the same tumours.
- Make the data available to the entire research community as rapidly as possible, and with minimal restrictions, to accelerate research into the causes and control of cancer.
- Coordinate research efforts so that the interests and priorities of individual participants, self-organizing consortia, funding agencies and nations are addressed, including use of the burden of disease and the minimization of unnecessary redundancy in tumour analysis efforts.
- Support the dissemination of knowledge and standards related to new technologies, software, and methods to facilitate data integration and sharing with cancer researchers around the globe.

*A list of participants and their affiliations appears at the end of the paper.

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APPENDIX 1.H

[illegible]

Multigene mutational profiling of cholangiocarcinomas identifies [illegible]

Michele Simbolo[illegible], Andrea Ruzzenente[illegible], Andrea Mafficini[illegible], Laura D. Wood[illegible], Vincenzo Corbo[illegible], Davide Melisi[illegible], Caterina [illegible], Davide Antonello[illegible], Nicola Sperandio[illegible], Caterina Capelli[illegible], Anna Tomezzoli[illegible], Calogero Iacono[illegible], Rita T. Lawlor[illegible], Claudio Bassi[illegible], Ralph H. Hruban[illegible], Alfredo Guglielmi[illegible], Filippo de Braud[illegible], Aldo Scarpa[illegible]

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shared first authorship

[illegible]

[illegible]: cholangiocarcinoma; next-generation sequencing; molecular subclassification; target therapy; multigene mutational panels

[illegible]

[illegible]

[illegible]

One-hundred-fifty-three biliary cancers, including 70 intrahepatic cholangiocarcinomas (ICC), 57 extrahepatic cholangiocarcinomas (ECC) and 26 gallbladder carcinomas (GBC) were assessed for mutations in 56 genes using multigene next-generation sequencing. Expression of EGFR and mTOR pathway genes was investigated by immunohistochemistry. At least one mutated gene was observed in 118/153 (77%) cancers. The genes most frequently involved were [illegible] (28%), [illegible] (18%), [illegible] (12%), [illegible] (9%), [illegible] (9%), [illegible] (7%), and [illegible] (7%). [illegible] (p=0.0005) and [illegible] (p=0.0097) mutations were characteristic of ICC, while [illegible] (p=0.0019) and [illegible] (p=0.0019) were more frequent in ECC and GBC. Multivariate analysis identified tumour stage and [illegible] mutations as independent predictors of survival. Alterations in chromatin remodeling genes ([illegible]) were seen in 31% of cases. Potentially actionable mutations were seen in 104/153 (68%) cancers: i) [illegible] mutations were found in 34% of cancers; ii) mTOR pathway activation was documented by immunohistochemistry in 51% of cases and by mutations in mTOR pathway genes in 19% of cancers; iii) TGF-ß/Smad signaling was altered in 10.5% cancers; iv) mutations in tyrosine kinase receptors were found in 9% cases. Our study identified molecular subgroups of cholangiocarcinomas that can be explored for specific drug targeting in clinical trials.

[illegible]

[illegible] classified according to the World Health Organization (WHO) as intrahepatic (ICC) or extrahepatic cholangiocarcinomas (ECC) [1, 2]. The former arise in the substance of the liver, the latter in large extrahepatic ducts, i.e. hepatic ducts and common bile duct. Gallbladder carcinomas (GBC) also have biliary epithelial

APPENDIX 2

Data on Pancreatic cancer genomes reveal aberrations in axon guidance pathway genes are summarized in the following article co-authored by the candidate in Nature doi:10.1038/nature11547.

ARTICLE

doi:10.1038/nature11547

Pancreatic cancer genomes reveal aberrations in axon guidance pathway genes

A list of authors and their affiliations appears at the end of the paper

Pancreatic cancer is a highly lethal malignancy with few effective therapies. We performed exome sequencing and copy number analysis to define genomic aberrations in a prospectively accrued clinical cohort ($n = 142$) of early (stage I and II) sporadic pancreatic ductal adenocarcinoma. Detailed analysis of 99 informative tumours identified substantial heterogeneity with 2,016 non-silent mutations and 1,628 copy-number variations. We define 16 significantly mutated genes, reaffirming known mutations (*KRAS*, *TP53*, *CDKN2A*, *SMAD4*, *MLL3*, *TGFBR2*, *ARID1A* and *SF3B1*), and uncover novel mutated genes including additional genes involved in chromatin modification (*EPC1* and *ARID2*), DNA damage repair (*ATM*) and other mechanisms (*ZIM2*, *MAP2K4*, *NALCN*, *SLC16A4* and *MAGEA6*). Integrative analysis with *in vitro* functional data and animal models provided supportive evidence for potential roles for these genetic aberrations in carcinogenesis. Pathway-based analysis of recurrently mutated genes recapitulated clustering in core signalling pathways in pancreatic ductal adenocarcinoma, and identified new mutated genes in each pathway. We also identified frequent and diverse somatic aberrations in genes described traditionally as embryonic regulators of axon guidance, particularly SLIT/ROBO signalling, which was also evident in murine Sleeping Beauty transposon-mediated somatic mutagenesis models of pancreatic cancer, providing further supportive evidence for the potential involvement of axon guidance genes in pancreatic carcinogenesis.

APPENDIX 3

Data on Whole genomes redefine the mutational landscape of pancreatic cancer are summarized in the following article co-authored by the candidate in Nature. Doi: 10.1038/nature14169

doi:10.1038/nature14169

Whole genomes redefine the mutational landscape of pancreatic cancer

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Pancreatic cancer remains one of the most lethal of malignancies and a major health burden. We performed whole-genome sequencing and copy number variation (CNV) analysis of 100 pancreatic ductal adenocarcinomas (PDACs). Chromosomal rearrangements leading to gene disruption were prevalent, affecting genes known to be important in pancreatic cancer (*TP53, SMAD4, CDKN2A, ARID1A* and *ROBO2*) and new candidate drivers of pancreatic carcinogenesis (*KDM6A* and *PREX2*). Patterns of structural variation (variation in chromosomal structure) classified PDACs into 4 subtypes with potential clinical utility: the subtypes were termed stable, locally rearranged, scattered and unstable. A significant proportion harboured focal amplifications, many of which contained druggable oncogenes (*ERBB2, MET, FGFR1, CDK6, PIK3R3* and *PIK3CA*), but at low individual patient prevalence. Genomic instability co-segregated with inactivation of DNA maintenance genes (*BRCA1, BRCA2* or *PALB2*) and a mutational signature of DNA damage repair deficiency. Of 8 patients who received platinum therapy, 4 of 5 individuals with these measures of defective DNA maintenance responded.

APPENDIX 4

Data on Integrative genomic analysis of pancreatic cancer identifies subtypes with distinct histopathological characteristics are summarized in the following article co-authored by the candidate in Nature. doi:10.1038/nature16965

ARTICLE

doi:10.1038/nature16965

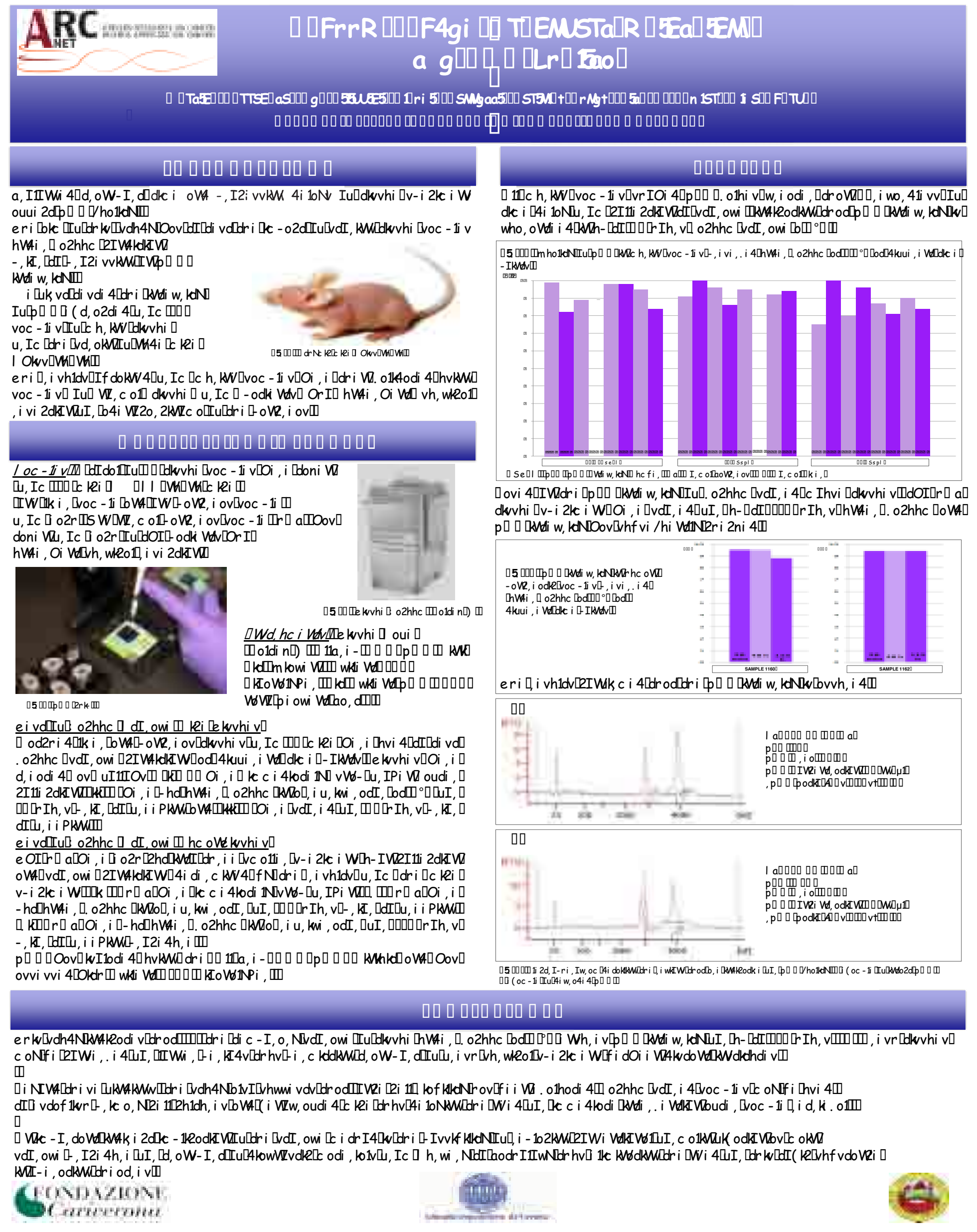
Genomic analyses identify molecular subtypes of pancreatic cancer

Peter Bailey[1,2], David K. Chang[2,3,4,5], Katia Nones[1,6], Amber L. Johns[3], Ann-Marie Patch[1,6], Marie-Claude Gingras[7,8,9], David K. Miller[1,3], Angelika N. Christ[1], Tim J. C. Bruxner[1], Michael C. Quinn[1,6], Craig Nourse[1,2], L. Charles Murtaugh[10], Ivon Harliwong[1], Senel Idrisoglu[1], Suzanne Manning[1], Ehsan Nourbakhsh[1], Shivangi Wani[1,6], Lynn Fink[1], Oliver Holmes[1,6], Venessa Chin[3], Matthew J. Anderson[1], Stephen Kazakoff[1,6], Conrad Leonard[1,6], Felicity Newell[1], Nick Waddell[1], Scott Wood[1,6], Qinying Xu[1,6], Peter J. Wilson[1], Nicole Cloonan[1,6], Karin S. Kassahn[1,11,12], Darrin Taylor[1], Kelly Quek[1], Alan Robertson[1], Lorena Pantano[13], Laura Mincarelli[2], Luis N. Sanchez[2], Lisa Evers[2], Jianmin Wu[3], Mark Pinese[3], Mark J. Cowley[3], Marc D. Jones[2,3], Emily K. Colvin[3], Adnan M. Nagrial[3], Emily S. Humphrey[3], Lorraine A. Chantrill[3,14], Amanda Mawson[3], Jeremy Humphris[3], Angela Chou[3,15], Marina Pajic[3,16], Christopher J. Scarlett[3,17], Andreia V. Pinho[3], Marc Giry-Laterriere[3], Ilse Rooman[3], Jaswinder S. Samra[18,19], James G. Kench[3,19,20], Jessica A. Lovell[3], Neil D. Merrett[5,21], Christopher W. Toon[3], Krishna Epari[22], Nam Q. Nguyen[23], Andrew Barbour[24], Nikolajs Zeps[25], Kim Moran-Jones[2], Nigel B. Jamieson[2,26,27], Janet S. Graham[2,28], Fraser Duthie[29], Karin Oien[3,29], Jane Hair[30], Robert Grützmann[31], Anirban Maitra[32], Christine A. Iacobuzio-Donahue[33], Christopher L. Wolfgang[34,35], Richard A. Morgan[34], Rita T. Lawlor[36,37], Vincenzo Corbo[36], Claudio Bassi[38], Borislav Rusev[36], Paola Capelli[37], Roberto Salvia[38], Giampaolo Tortora[39], Debabrata Mukhopadhyay[40], Gloria M. Petersen[40], Australian Pancreatic Cancer Genome Initiative*, Donna M. Munzy[7,8], William E. Fisher[41], Saadia A. Karim[42], James R. Eshleman[34], Ralph H. Hruban[34], Christian Pilarsky[31], Jennifer P. Morton[42], Owen J. Sansom[42,43], Aldo Scarpa[36,37], Elizabeth A. Musgrove[2], Ulla-Maja Hagbo Bailey[2], Oliver Hofmann[2,13], Robert L. Sutherland[3] , David A. Wheeler[7,8], Anthony J. Gill[3,19], Richard A. Gibbs[7,8], John V. Pearson[1,6], Nicola Waddell[1,6], Andrew V. Biankin[2,3,4,5,27] & Sean M. Grimmond[1,2,44]

Integrated genomic analysis of 456 pancreatic ductal adenocarcinomas identified 32 recurrently mutated genes that aggregate into 10 pathways: *KRAS, TGF-β, WNT, NOTCH, ROBO/SLIT* signalling, *G1/S* transition, *SWI-SNF*, chromatin modification, DNA repair and RNA processing. Expression analysis defined 4 subtypes: (1) squamous; (2) pancreatic progenitor; (3) immunogenic; and (4) aberrantly differentiated endocrine exocrine (ADEX) that correlate with histopathological characteristics. Squamous tumours are enriched for *TP53* and *KDM6A* mutations, upregulation of the *TP63ΔN* transcriptional network, hypermethylation of pancreatic endodermal cell-fate determining genes and have a poor prognosis. Pancreatic progenitor tumours preferentially express genes involved in early pancreatic development (*FOXA2/3*, *PDX1* and *MNX1*). ADEX tumours displayed upregulation of genes that regulate networks involved in *KRAS* activation, exocrine (*NR5A2* and *RBPJL*), and endocrine differentiation (*NEUROD1* and *NKX2-2*). Immunogenic tumours contained upregulated immune networks including pathways involved in acquired immune suppression. These data infer differences in the molecular evolution of pancreatic cancer subtypes and identify opportunities for therapeutic development.

APPENDIX 5

Data on the use of vaccum packing to maintain tissue quality and cell viability are summarized in the following 2 posters co-authored by the candidate presented at the ESBB European, Middle Eastern and African Society for Biobanking Annual Conference in 2012 and 2013 respectively.



Prolonged Cell Viability for Mouse Implantation of Human Tumor Tissues

Rita T. Lawlor, Dea Filippini, Nicola Sperandio, Nadia Mori, Vincenza Favuzzi, Irene Dalai, Aldo Scarpa
ARC-NET APPLIED RESEARCH ON CANCER, VERONA-ITALY

INTRODUCTION

Prolonged transport time and processing delays of tissue specimens are know to affect cell vitality.
The aim of this study was to test the impact of storing tissue samples under vacuum condition prior to use for mouse implantation.

MATERIALS AND METHODS

Samples were obtained from patients who underwent surgical resection for pancreas ductal adenocarcinoma (PDAC). Samples were used from a total of 10 cases of PDAC. 80 SWISS-nu/nu mice were used for tumor implantation.

5 cases of fresh pancreas tumor tissue were cut in 3 samples: one was processed immediately (T0) and the other two were placed in a vacuum packed using Tissue Vacuum (Kaltek)® (Fig. 1) and stored refrigerated at 4°C for 24 hours (T24) and 48 hours (T48).

Fig.1: Tissue Vacuum (Kaltek)®

Fig.2: Athymic mice Swiss-nu/nu with tumor in the nape and right flank

Each sample was then fragmented into four pieces which were implanted in two immunodecifient SWISS-nu/nu mice, one fragment in each of the nape and right flank of each mouse (Fig. 2).

Based on results of 48 hours we then successfully tested other 5 cases up to 96 hours using the same methods. Cases with larger tumor size were selected to permit 5 samples from each case to be used for the study for implanting immediately (T0), at 24 hours (T24), at 48 hours (T48), at 72 hours (T72) and at 96 hours (T96).

RESULTS

Tumor fragments implanted in the right flank of each mouse grew within 17 days of implantation (Fig 3) showing the viability of tumor tissue stored vaccum refrigerated for up 48 hours for 3 of the first 5 cases (Table 1).

Fig.3: tumorgraft

Table 1: Growth of tumor implant in mice up to 48 hours

SAMPLE NO.	T0	T24	T48
1590	growth	no growth	no growth
1592	growth	growth	growth
1608	growth	growth	growth
1609	growth	growth	growth
1610	growth	no growth	no growth

Table 2: Growth of tumor implant in mice up to 96 hours

SAMPLE NO.	TO	T24	T48	T72	T96
1613	growth	growth	no growth	growth	growth
1626	growth	growth	growth	growth	growth
1630	growth	growth	growth	growth	growth
1635	growth	growth	growth	no growth	growth
1638	growth	growth	growth	growth	growth

growth
no growth

In the second set of 5 cases, tumor fragments grew within 20 days of implantation showing the viability of tumor tissue stored vaccum refrigerated for up 96 hours (Table 2 and Fig. 4).

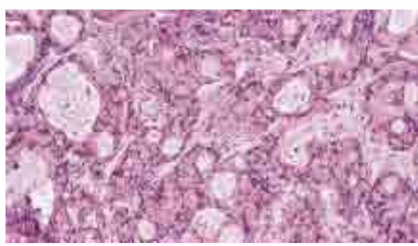


Fig.4: H&E of PDAC xeno-graft from human tissue vacuum refrigerated for 96 hours

CONCLUSIONS

Samples can be maintained fresh for up to 96 hours and still guarantee cellular vitality. This permits the possibilty to produce cell cultures even after prolonged delays from tissue sampling. Furthermore it facilitiates xenograft production by maintaining cellular viability for implantation and growth. Perhaps most important of all, it provides options for long distance transport of fresh tissue with less stringent transport conditions.

APPENDIX 6

Data on DNA qualification workflow for next generation sequencing of histopathological samples are summarized in the following article co-authored by the candidate in PLoS One. Doi: 10.1371/journal.pone.0062692.

OPEN ACCESS Freely available online

DNA Qualification Workflow for Next Generation Sequencing of Histopathological Samples

Michele Simbolo[1,2], Marisa Gottardi[1], Vincenzo Corbo[1], Matteo Fassan[1], Andrea Mafficini[1], Giorgio Malpeli[3], Rita T. Lawlor[1], Aldo Scarpa[1,2,3]*

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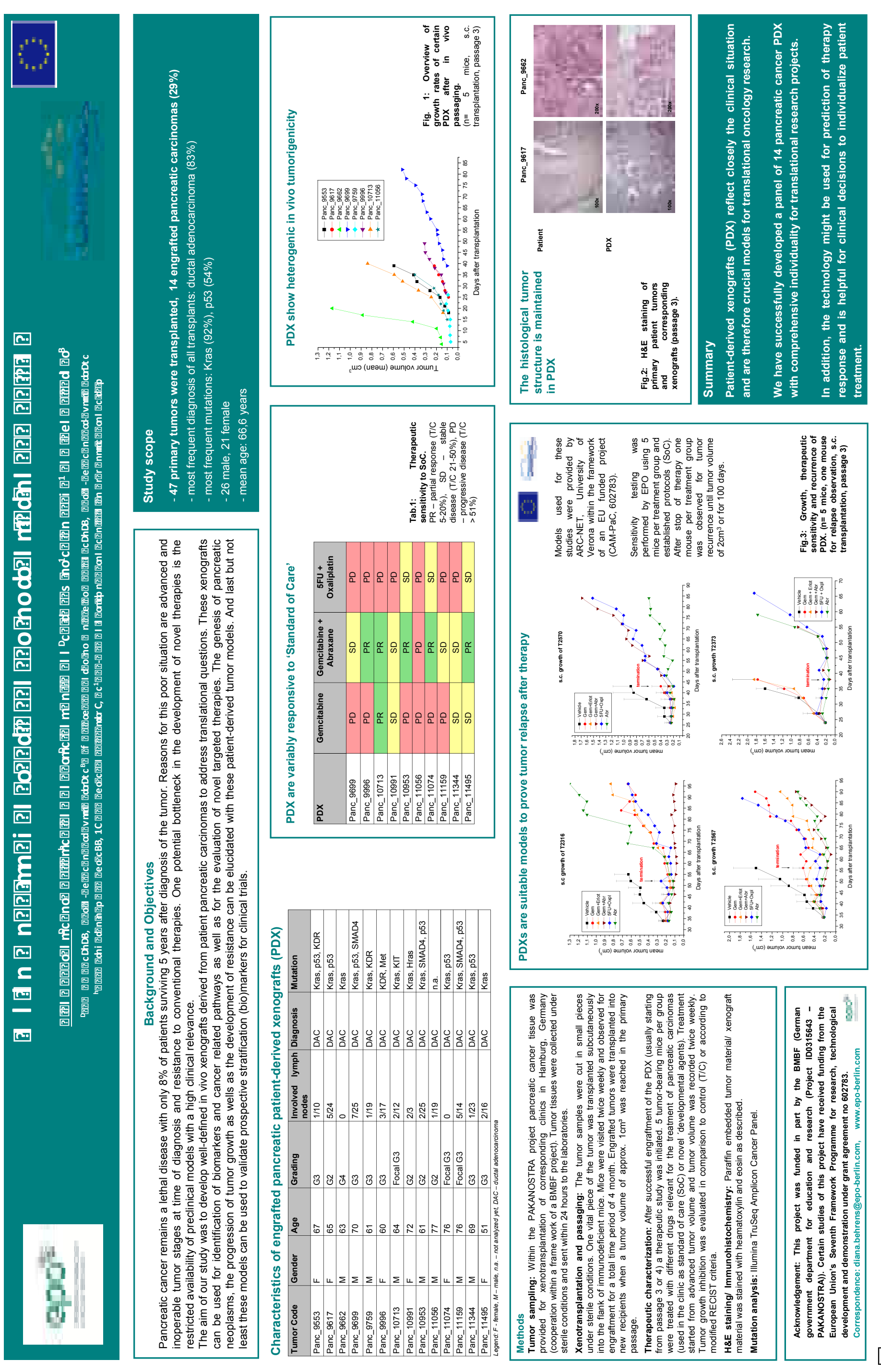
Abstract

Histopathological samples are a treasure-trove of DNA for clinical research. However, the quality of DNA can vary depending on the source or extraction method applied. Thus a standardized and cost-effective workflow for the qualification of DNA preparations is essential to guarantee interlaboratory reproducible results. The qualification process consists of the quantification of double strand DNA (dsDNA) and the assessment of its suitability for downstream applications, such as high-throughput next-generation sequencing. We tested the two most frequently used instrumentations to define their role in this process: NanoDrop, based on UV spectroscopy, and Qubit 2.0, which uses fluorochromes specifically binding dsDNA. Quantitative PCR (qPCR) was used as the reference technique as it simultaneously assesses DNA concentration and suitability for PCR amplification. We used 17 genomic DNAs from 6 fresh-frozen (FF) tissues, 6 formalin-fixed paraffin-embedded (FFPE) tissues, 3 cell lines, and 2 commercial preparations. Intra- and inter-operator variability was negligible, and intra-methodology variability was minimal, while consistent inter-methodology divergences were observed. In fact, NanoDrop measured DNA concentrations higher than Qubit and its consistency with dsDNA quantification by qPCR was limited to high molecular weight DNA from FF samples and cell lines, where total DNA and dsDNA quantity virtually coincide. In partially degraded DNA from FFPE samples, only Qubit proved highly reproducible and consistent with qPCR measurements. Multiplex PCR amplifying 191 regions of 46 cancer-related genes was designated the downstream application, using 40 ng dsDNA from FFPE samples calculated by Qubit. All but one sample produced amplicon libraries suitable for next-generation sequencing. NanoDrop UV-spectrum verified contamination of the unsuccessful sample. In conclusion, as qPCR has high costs and is labor intensive, an alternative effective standard workflow for qualification of DNA preparations should include the sequential combination of NanoDrop and Qubit to assess the purity and quantity of dsDNA, respectively.

APPENDIX 7

Data on In vivo models of pancreatic cancer for translational medicine are summarized in the following poster co-authored by the candidate.



APPENDIX 8

Data on BRCA somatic and germ-line mutation detection in paraffin embedded ovarian cancers by next-generation sequencing are summarized in the following article for which the candidate is corresponding author.

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BRCA somatic and germline mutation detection in paraffin embedded ovarian cancers by next-generation sequencing

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ABSTRACT

BRCA mutated ovarian cancers respond better to platinum-based therapy and to the recently approved PARP-inhibitors. There is the need for efficient and timely methods to detect both somatic and germline mutations using formalin-fixed paraffin-embedded (FFPE) tissues and commercially available technology. We used a commercial kit exploring all exons and 50bp exon-intron junctions of _BRCA1_ and _BRCA2_ genes, and semiconductor next-generation sequencing (NGS) on DNA from 47 FFPE samples of high-grade serous ovarian cancers. Pathogenic mutations were found in 13/47 (28%) cancers: eight in _BRCA1_ and five in _BRCA2_. All _BRCA1_ and two _BRCA2_ mutations were germline; three _BRCA2_ mutations were somatic. All mutations were confirmed by Sanger sequencing. To evaluate the performance of the NGS panel, we assessed its capability to detect the 6,953 variants described for _BRCA1_ and _BRCA2_ in ClinVar and COSMIC databases using callability analysis. 6,059 (87.1%) variants were identified automatically by the software; 829 (12.0%) required visual verification. The remaining 65 (0.9%) variants were uncallable, and would require 15 Sanger reactions to be resolved. Thus, the sensitivity of the NGS-panel was 99.1%. In conclusion, NGS performed with a commercial kit is highly efficient for detection of germline and somatic mutations in _BRCA_ genes using routine FFPE tissue.