Dissecting the germline background of Pancreatic Cancer: preliminary of NGS screening for hereditary variants in DNA Damage Repair genes

56

Matteo Pallocca*[1], Frauke Goeman*[2], Francesca De Nicola[1], Stefano Scalera[1], Mariangela Panetta[3], Paola Nisticò[3], Vanja Vaccaro[4], Francesco Cognetti[4], Gennaro Ciliberto[5], Maurizio Fanciulli[1], Michele Milella[4]

[1]UOSD SAFU [2]UOSD Oncogenomic and Epigenetic Unit [3]UOSD Immunology and Immunotherapy Unit [4]Oncology Unit 1 [5]Scientific Directorate
IRCCS Regina Elena National Cancer Institute, Rome

PDAC

Pancreatic Adenocarcinoma (PDAC) is a rare disease with an incidence that varies with region: from 1 in 100.000 in Middle Africa/South Central Asia to 7.3-7.4 in 100.000 in Northern America and Western Europe. In the UE, annual case number sits around 79.000 (GLOBOCAN) and 5-years survival is less than 10% due to late diagnosis and still few tratment options. The causes of PDAC linked to lifestyle are: cigarette smoking, diabetes mellitus, obesity, dietary factor, alcohol and physical inactivity. Besides lifestyle risk factor, there are some genetic disorders that increasing PDAC risk: Lynch syndrome, hereditary breast and ovarian cancer syndrome, Li-Fraumeni syndrome and familial adenomatosus polyposis (FAP).

DDR Panel design

We designed a custom panel (Fig.1) using QIAGEN QIAseq technology including 65 genes involved in the DNA Damage Repair (DDR) (Tab. 1) (BRCA1; BRCA2; ATM among others) and sequenced the isolated PBMC of 48 PDAC patients.
Raw data was analyzed using the QIAGEN Analysis Workbench Cloud software, and mutation VCFs were annotated via ANNOVAR. Custom Python scripting was used to merge our dataset with EXCHANGE data (from ENIGMA/LOVD BRCA consortia) and other publicly available mutational datasets.

GENE PANEL								
AKT1	BRCA1	CTNNA1	HOXB13	MUTYH	PMS2	RAD51B	SDHB	XRCC2
APC	BRCA2	FAM175A	MEN1	NBN	POLD1	RAD51C	SDHC	
ATM	BRIP1	FANCM	MET	NF1	POLE	RAD51D	SDHD	
ATR	CDH1	FH	MITF	NTHL1	POT1	RB1	SLX4	
AXIN2	CDK4	FLCN	MLH1	PALB2	PRKAR1A	RECQL	SMAD4	
BAP1	CDKN2A	GALNT12	MRE11A	PALLD	PRSS1	RET	SMARCA4	
BARD1	CHEK1	GEN1	MSH2	PDGFRA	PTCH1	RINT1	TP53	
BMPR1A	CHEK2	GREM1	MSH6	PIK3CA	PTEN	RPS20	VHL	

Tab.1: 65 genes involved in the DNA Damage Repair (DDR)

Panel performance

- panel size: ca. 200 kb (AmpliSeq Solid Tumor 15kb)
- each patient sequenced with ca. 10 Mio reads
- 8.7 Mio reads on target and high quality
- mean primer read fragment depth: 3302 x
- Molecular Tags for each sample ca. 2.6 Mio = 3 per DNA fragment = LOD 5%
- 99% of primers >= 30% of mean read fragment depth => 990 x
- 2663 amplicons: 12 amplicons below threshold (OM109) = 0.5%

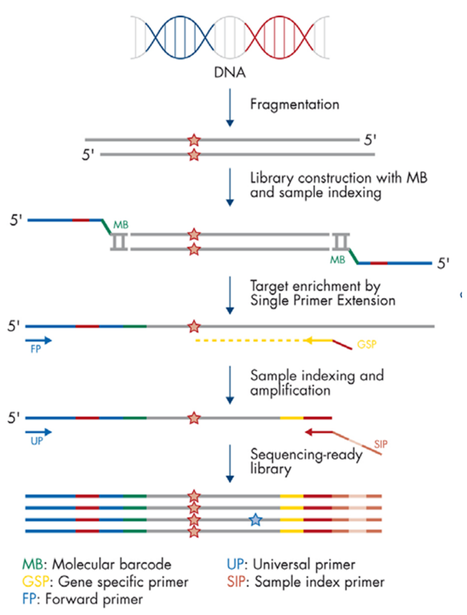

Fig 1: panel

PDAC germline guidelines

There is no accepted guideline for germline hereditary screening of Pancreatic Adenocarcinoma (PDAC), even if in the last years several evidences have been reported that link germline defects in the DNA Repair machinery to PDAC pathogenesis (Shindo et. Al JCO 2017; Pihlak et. Al Oncotarget 2017) (Fig. 2). A subgroup of these patients could be effectively characterized as it happens with subsets of Breast and Colorectal Cancers.
For this reason, we developed a custom panel in order to investigate the germline features of DDR genes in PDAC patients.

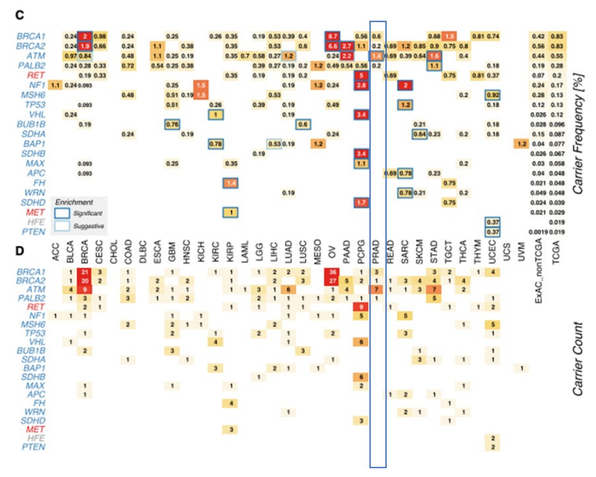

Fig 2: Pathogenic germline variants in 10.389 adult cancers

Preliminary results

In order to keep only somatic variations, we selected only protein damaging events, with a MAF filter less than 1% in general population (Fig. 3) Then, we annotated our variants with germline pathogenicity databases.

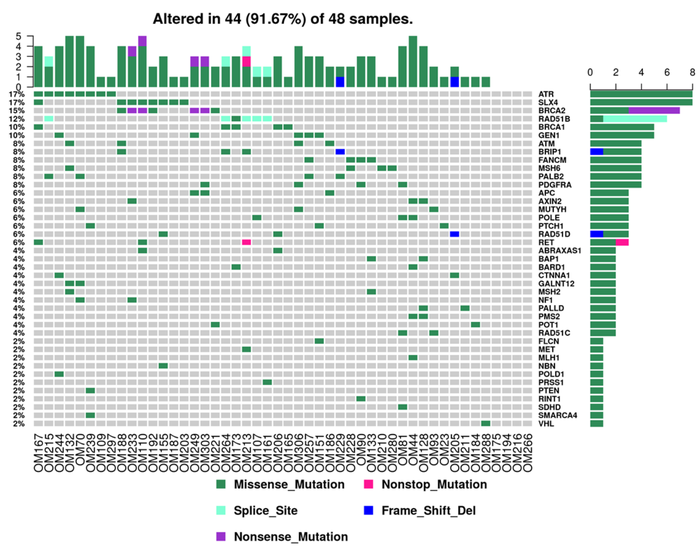

Fig 3: oncoprint of the whole gene panel

Out of 48, 2 patients (4%) harbored BRCA1 mutations considered to be pathogenic for Breast Cancer Susceptibility These variants were known and annotated in the EXCHANGE dataset and ClinVar (Fig. 4). Furthermore, we managed to compare our variants with the ones recently reported in the last CELL/TCGA issue as being genetically predisposing for cancer according to advanced bioinformatic integration of 10389 cancers (Huang. Et. Al 2018, Cell). Only the two BRCA1 variants were a match according to this dataset.

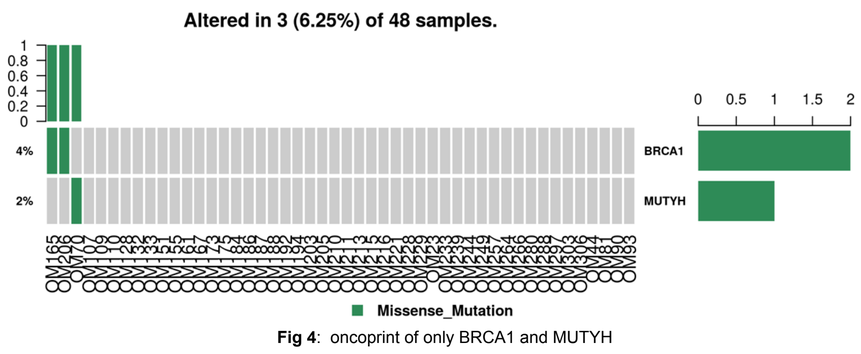

Fig 4: oncoprint of only BRCA1 and MUTYH

Clinical evidence and future perspectives

One out of the three DDR-positive patients had a family history (Mother: Endometrial Cancer; Grandmother on mathernal line: Breast Cancer). The other two had no such information available on our internal records. Furthermore, other 8 patients were positive with mutations flagged as Likely Pathogenic in **ClinVar**; of those we don't know yet how many could have a clinical evidence of increased incidence. This preliminary data shows that a subset of PDAC patients could carry pathogenic germline variants, that could benefit from familiar genetic counseling. Sequencing of approximately 250 additional patients in our pancreatic cancer biobank is ongoing and results will be correlated with familiar history, somatic genomic profiling of tumors and clinical data.

References

Ilic, Milena, and Irena Ilic. "Epidemiology of pancreatic cancer." *World journal of gastroenterology* 22.44 (2016): 9694.
Huang, K. L., Mashl, R. J., Wu, Y., Ritter, D. I., Wang, J., Oh, C., ... & Scott, A. D. (2018). Pathogenic germline variants in 10,389 adult cancers. *Cell*, *173*(2), 355-370.
Carrato, A., et al. "A systematic review of the burden of pancreatic cancer in Europe: real-world impact on survival, quality of life and costs." Journal of gastrointestinal cancer 46.3 (2015): 201-211.
https://rarediseases.info.nih.gov/diseases/pages/31/faqs-about-rare-diseases