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This is the author's manuscript

Original Citation:

Availability:

This version is available http://hdl.handle.net/2318/1724364 since 2020-01-21T18:39:17Z

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19 April 2024

Identification of Actionable Cancer Genes and Treatment Options for Metastatic Ovarian Carcinomas using Patient Derived Xenografts (PDXs) and PDX Derived Tumor Cells (PDTCs)

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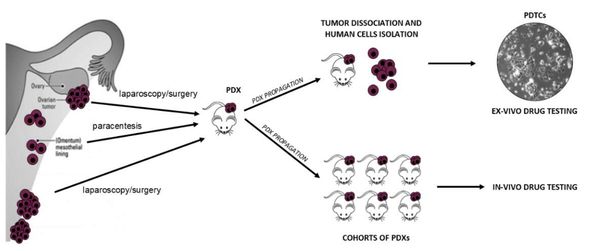
ABSTRACT

Patients with advanced ovarian cancers have experienced little improvement in overall survival and standard treatment has not much progressed beyond cytoreductive surgery and platinum-based combination chemotherapy. Besides targeted anti-angiogenic and anti-PARP1 therapies, matching individual most critical genomic alterations with the best available drugs has not advanced as in other cancers, likely because a handful of cancer-related genes are mutated at high frequency, while many more are found mutated at much lower frequencies. This so called "mutation tail" is not only long but also mostly unexplored. We used Patient Derived Xenografts (PDXs) and PDX Derived Tumor Cells (PDTCs) to identify actionable cancer genes and to accelerate the discovery of treatment options. We envisioned that the alleged weakness of PDX models, i.e. lack of human stromal and immune cells, might be instrumental to identify mutations in cancer genes and to test approved or experimental targeted drugs as monotherapy or in different combinations to link genetic biomarkers to treatments. Fourty-three PDX lines from metastatic epithelial ovarian carcinomas have been propagated and fully characterized as far as histology, immunohistochemistry of epithelial and high-grade serous-specific markers and NGS of TP53 and BRCA1/2. Whole Exome Sequencing (WES) and copy number variations (CNV) analysis of first and late passages were carried out of 12 PDX lines derived from naïve metastatic high-grade serous epithelial ovarian carcinomas. We studied non-synonymous mutations with suitable allele frequencies in cancer genes reported in databases. SNPdb allowed ruling out polymorphisms. SIFT, PROVEAN and FATHMM softwares predicted deleterious or damaging effects onto the protein sequences. DGIdb helped selecting actionable genes. In 8/12 PDX lines 1-4 actionable genes were identified. In one line a possibly driver mutation was found in the PIK3R1 gene, encoding the p85alpha regulatory subunit of PI3K. This likely loss of function missense change had an allele frequency=0.9 in early and late passaged PDXs. Moreover, the mutation was also detected in DNA extracted from two micro-dissected FFPE samples of the source tumor, with an allele frequency nearly identical to that of mutated TP53. Hence, this is likely a trunk mutation in the PDX line and possibly in the source tumor. Notably, CNVs of early and late passages of this PDX line were almost identical. Treatment options were assayed ex-vivo, on short-term cultures of PDTCs of this PDX line. Buparlisib showed the ability to block proliferation of PDTCs and the growth *in vivo* of PDXs in regression preclinical trial. These data proofed the concept that a PDX-based pipeline is able to unveil actionable pathways for the treatment of advanced/metastatic ovarian cancer.

METHODS



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Characterization of PDX lines:

Patient Derived Xenografts (PDXs) have been established from epithelial ovarian carcinomas (EOC) by sampling tumors at diagnostic laparoscopy and/or at cytoreductive surgery. Forty-three PDX lines have been fully characterized within the first three passages, which resulted in loss of human stroma. In this study 12 naïve high-grade serous (HGS) EOC have been included. Whole exome sequencing (WES) was used to detect single nucleotide variants (SNVs) and SNP arrays to detect copy number alterations (CNA). Single Nucleotide Polymorphisms were excluded.

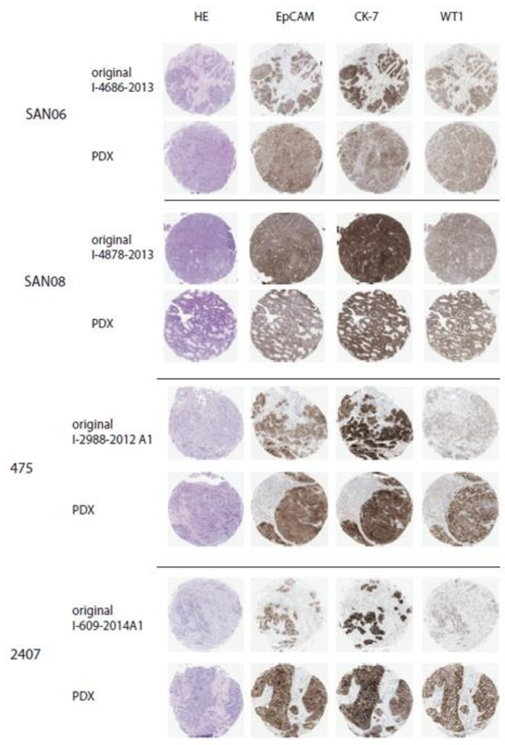
Ex vivo and in vivo assays of susceptibility to predictably active drugs:

The analysis of biological effects of possible druggable mutations was carried out *ex vivo* and *in vivo*. PDX Derived Tumor Cells (PDTCs) were propagated as short term cultures for *ex vivo* assays. These cultures and control cell lines (A2780, OVCAR8 and LNCaP) were exposed to different targeted drugs for 72 hours and analyzed with CellTiter-Glo® Assay. Drug response was studied using GR metrics calculator. *In vivo* studies were carried out treating PDX lines with different doses of selected targeted drugs.

RESULTS

***Results 1:* Characterization of PDX lines**

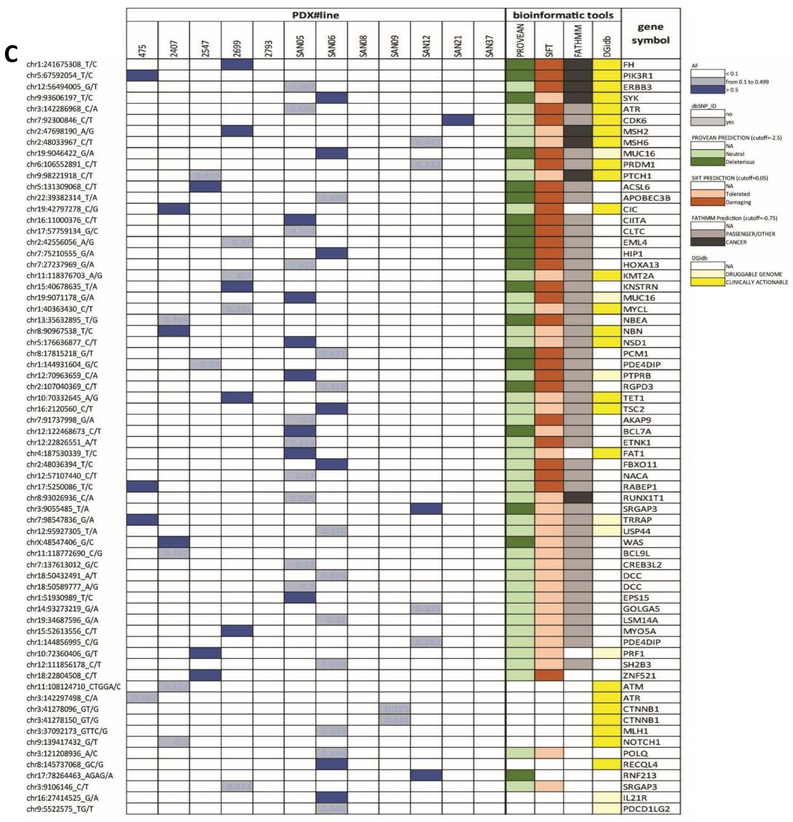
A



B

PDX LINE	NON SYNONYMOUS SNV/ PDX LINE	TP53	BRCA2
475	63	p.V173L	
2407	138	p.R273H	
2547	50	p.C176W	p.Y1894*
2699	119	p.E198*	
2793	26	p.G262D	
SAN05	181	p.C275Y	pT219fs
SAN06	156	p.Y234C	pG1529R
SAN08	23	pS127F	
SAN09	15	pS127F	
SAN12	92	c.97-2A>T	
SAN21	12	p.Q167*	
SAN44	146	p.C242fs	p.T2662R

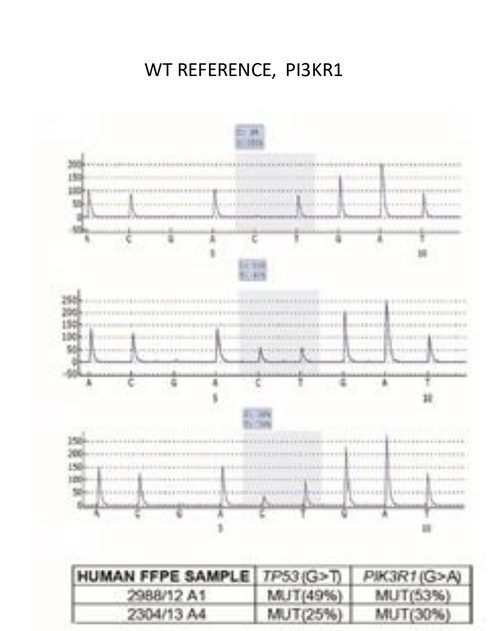
C



(**A**) PDX lines derived from EOC were analyzed using immunohistochemistry with antibodies against CK-7, EpCAM, WT1 to confirm the preservation of the histotypes of the source tumors. **(B)** Targeted NGS of TP53 showed the presence of pathogenic mutations with AF=1 in all the 12 PDX lines selected. WT1 staining and TP53 mutations confirmed the diagnosis of high-grade serous histology. Targeted NGS of BRCA1/2 showed the presence of BRCA1/2 mutations with the expected frequency (approx. 15%). Table **(C)** shows mutations in cancer genes reported in COSMIC found in 8/12 PDX lines and the prediction of their possible pathogenic role based on SIFT, PROVEAN and FATHMM softwares. In the PDX line #475 the W624R mutation of the PIK3R1 tumor suppressor gene was found with an AF =0.9 and predicted to be deleterious and damaging by softwares.

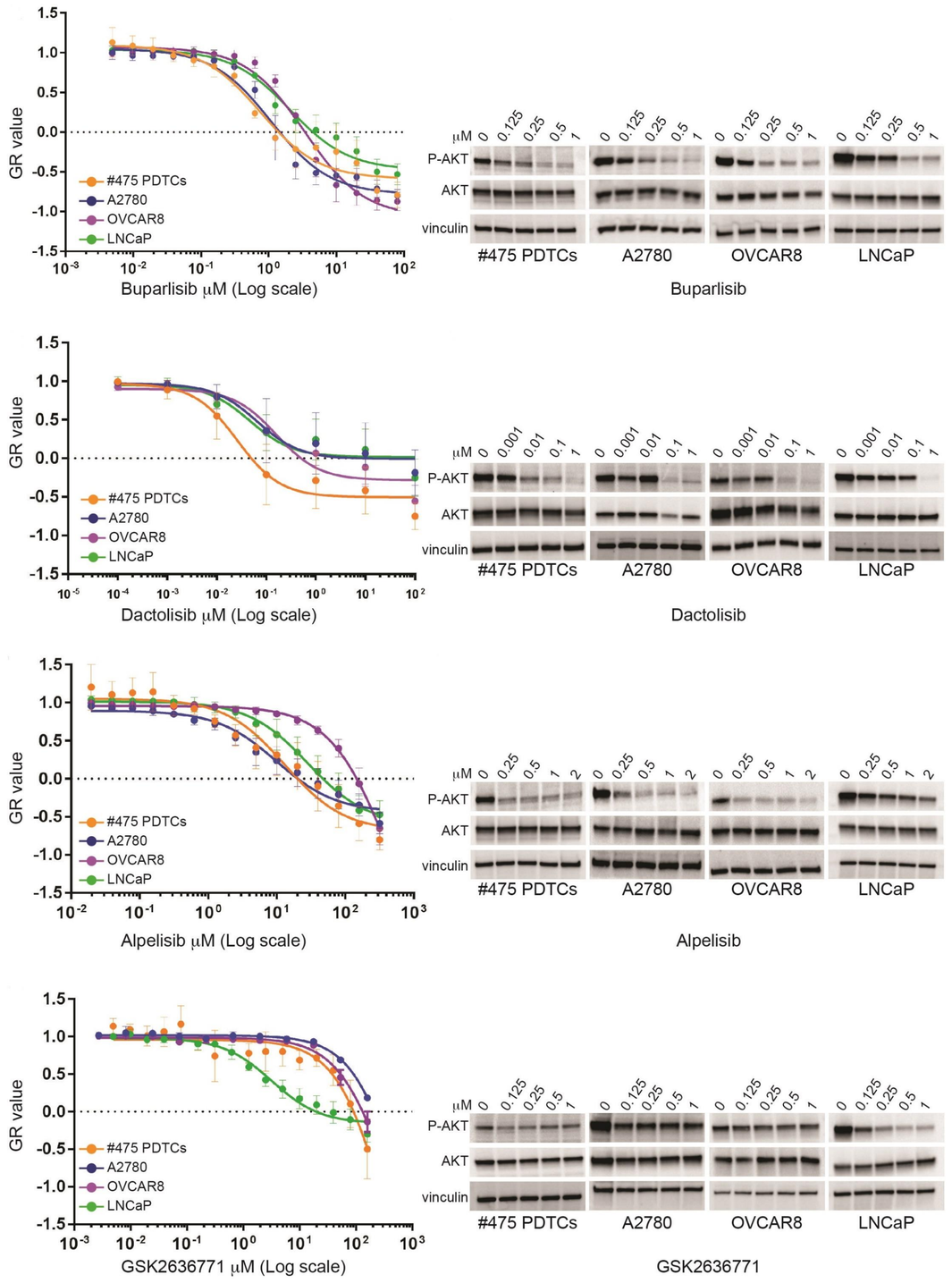
***Results 2:* Ex vivo and in vivo assays of susceptibility to predictably active drugs of the #475 PDX line carrying the W624 mutated PIK3R1**

A

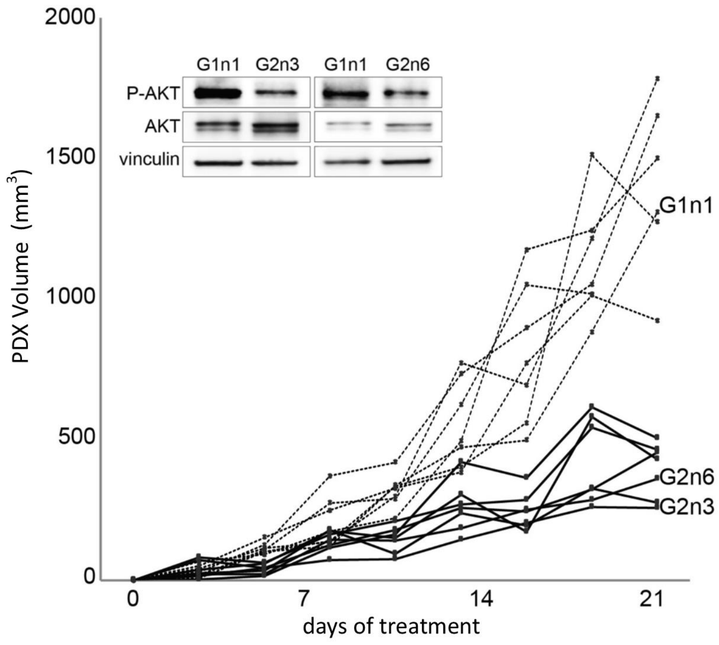


HUMAN FFPE SAMPLE	*TP53* (G>T)	*PIK3R1* (G>A)
2988/12 A1	MUT(49%)	MUT(53%)
2304/13 A4	MUT(25%)	MUT(30%)

B



C



(A) Pyrosequencing analysis confirmed the presence of TP53 and W624R PIK3R1 mutations in the two samples of the source tumor of the #475 PDX line with the same AF. The AF of TP53 mutation was considered as a proxy of the percentage of tumor cells in the human tumor sample. This gene encodes the p85a regulatory subunit of the P110a catalytic subunit of the PI3K complex. **(B)** The effect of different PI3K pathway inhibitors (Buparlisib, Alpelisib, Dactolisib and GSK2636771) was assayed on short term cultures of PDX Derived Tumor Cells (PDTC) of the #475 PDX line and on control cell lines (A2780, OVCAR8 and LNCaP). CellTiter-Glo® Assay revealed that *ex vivo* #475 PDTCs were exquisitely sensitive to Buparlisib, Alpelisib and Dactolisib. Conversely the p110β selective inhibitor GSK2636771 did not affect the #475 PDTCs. In line with viability assays, Buparlisib, Alpelisib and Dactolisib but not GSK2636771 affected AKT phosphorylation in #475 PDTCs. **(C)** *In vivo* growth inhibition of #475 PDX line by Buparlisib. Cohorts of mice carrying the #475 PDXs were randomized and divided in two cohorts left untreated (dashed line) or treated (solid line) with Buparlisib at 20 mg/Kg. In the inset inhibition of AKT phosphorylation in the indicated xenografts is shown.

CONCLUSIONS

The PDX based pipeline shown here has been able to unveil mutated actionable cancer genes in epithelial ovarian cancer. *Ex-vivo* assays have been instrumental to accelerate the discovery of new treatment options, validated in the following *in vivo* assays. The W624R mutation of the tumor suppressor PIK3R1 gene was found in one PDX line as putative trunk mutation also in its source tumor. This mutation was previously reported in one colorectal cancer and mutations of the same residue in one stomach and one NSCL. We show here that this mutation makes ovarian cancer cells responsive to PI3K inhibitors, suggesting new potential therapeutic strategies for the treatment of advanced ovarian cancer carrying mutations of the PIK3R1 gene.

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ACKNOWLEDGEMENTS This work has been supported by an IG grant of the Italian Association for Cancer Research (AIRC) to MFD

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