ISTITUTO DI CANDIOLO

IRCCS - Istituto di Ricovero e Cura a Carattere Scientifico

UNIVERSITÀ DEGLI STUDI DI TORINO

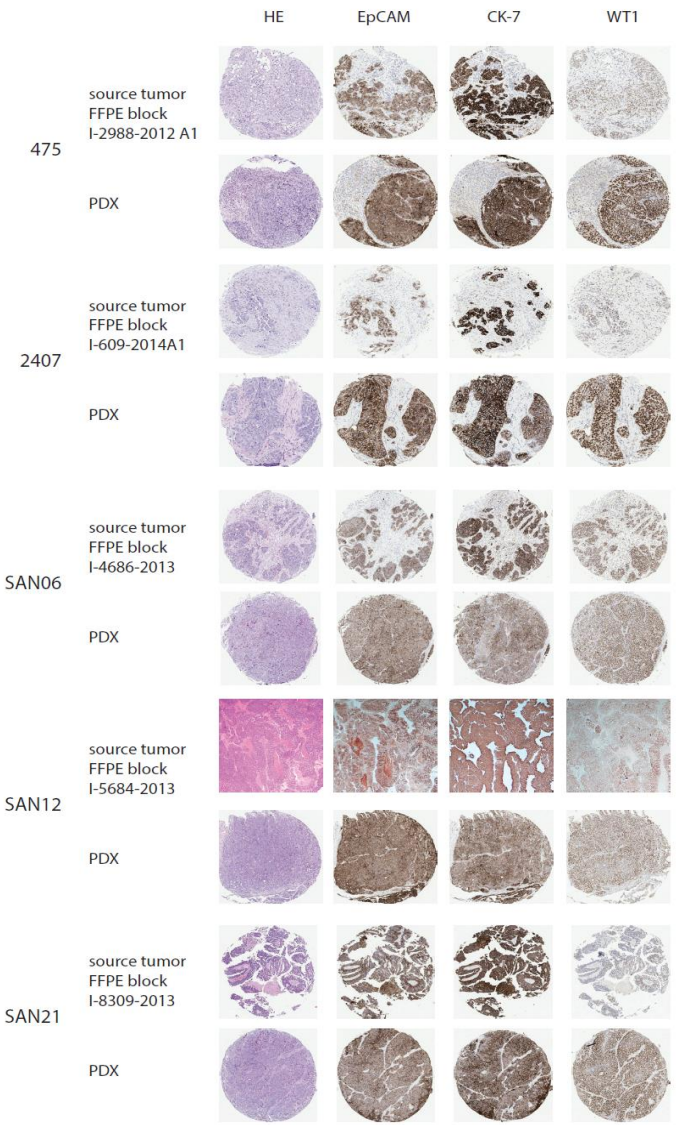
ALMA UNIVERSITAS TAURINENSIS

IDENTIFICATION OF ACTIONABLE CANCER GENES AND TREATMENT OPTIONS FOR METASTATIC OVARIAN CARCINOMAS USING PATIENT DERIVED XENOGRAFTS (PDXs) AND PDX DERIVED TUMOR CELLS (PDTCs)

D'Ambrosio C. Olivero M. Erriquez J. Arigoni M. Capellero S. Mittica G. Borella F. Katsaros D. Privitera S. Berrino E. Vanesio T. Bolla S. Valabrega G. Calogero R. and Di Renzo M.F.

CHARACTERIZATION OF 43 PDX LINES OF OVARIAN CARCINOMA

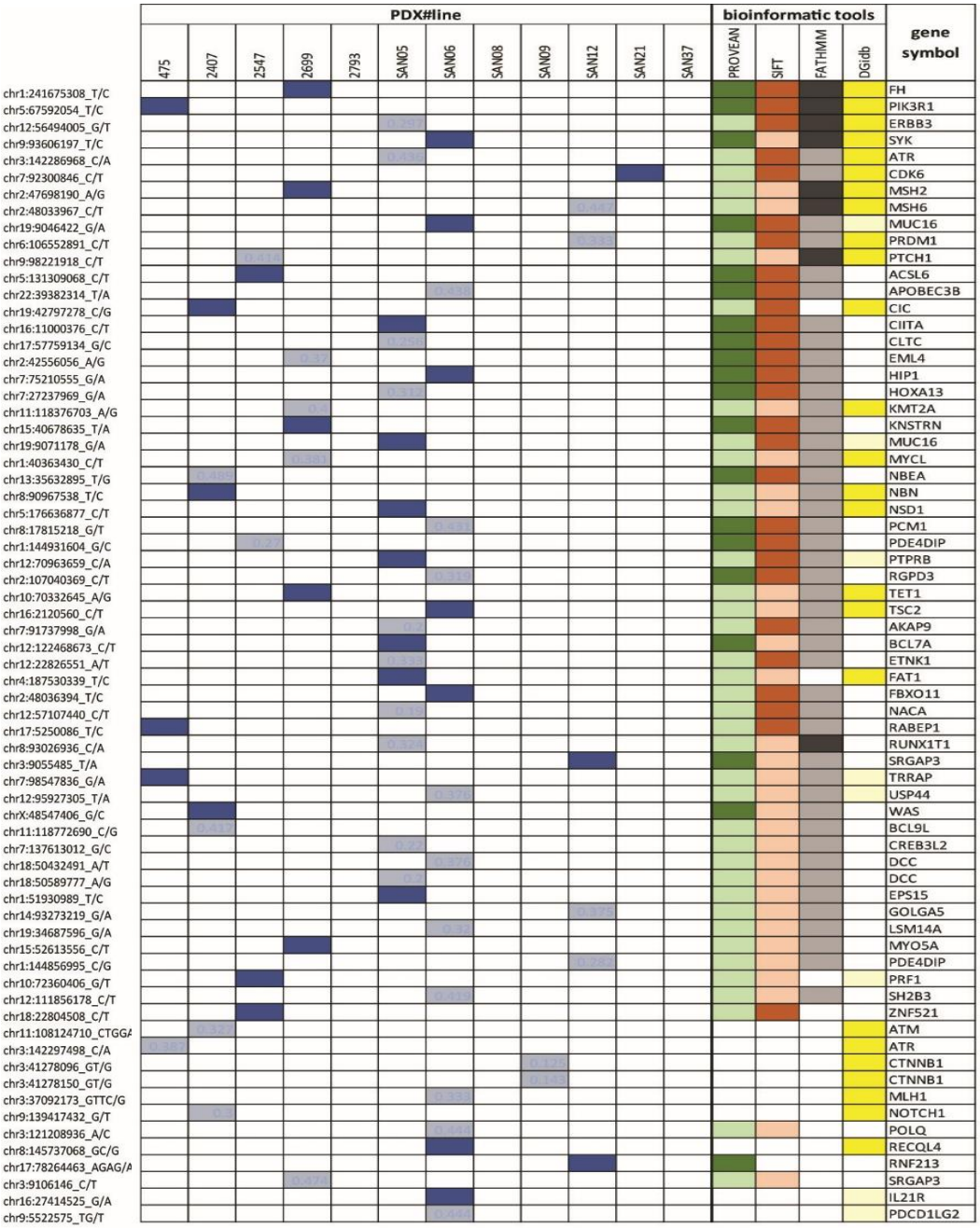
- **Tissue Macro Array (TMA) and Targeted-Next Generation Sequencing (T-NGS) analyses**



			TMA analyses					T- NGS		
Patient ID	TISSUE HISTOLOGY	PRIMARY, METASTASIS, RECURRENCE	CD20 IHC	CK7 IHC	WT1 IHC	EPCAM IHC	p53 IHC	TP53 AF	BRCA1 AF	BRCA2 AF
#0150	HGS	metastasis/recurrence	0	1	1	1	1	0.997		
#0172	HGS	metastasis/recurrence	0	1	1	1	1	0.999		
#0209	clear cell	metastasis/recurrence	0	1	ND	1	ND	ND	ND	ND
#0474	HGS	metastasis/recurrence	0	1	1	1	1	0.999		
#0475	HGS	metastasis/primary	0	1	1	1	1	0.988		
#1622	undifferentiated	metastasis/recurrence	0	1	1	1	1	0.999		1
#1658	HGS	metastasis/recurrence	0	1	1	1	1	0.998		
#1864	HGS	metastasis/recurrence	0	1	1	1	1	0.988		
#1897	HGS	metastasis/recurrence	0	1	1	1	1	1		
#1961	HG endometrioid	primary	0	1	1	1	0	0.986		
#1999	mucinous	primary	0	1	0	1	0	ND	ND	ND
#2085	HGS	ascites/recurrence	0	1	1	1	1	0.996		0.976
#2407	HGS	ascites/primary	0	1	1	1	1	0.998		
#2540	clear cell	primary	0	1	0	1	1	ND	ND	ND
#2547	HGS	primary	0	1	1	1	1	0.998		0.999
#2699	HGS	primary	0	0	1	0	0	0.999		
#2793	HGS	primary	0	1	1	1	1	0.998		
#2830	HGS	metastasis/primary	0	1	1	ND	0	0.998		
#2834	HGS	metastasis/primary	0	1	1	1	1	0.984	0,994	
#2976	HGS	metastasis/recurrence	0	1	1	1	1	ND	ND	ND
#2991	HGS	primary	0	1	0	1	0	ND	ND	ND
#2995	LGS	primary	0	1	1	ND	1	ND	ND	ND
#3213	undifferentiated	metastasis/recurrence	0	1	ND	1	ND	1	1	
#3679	HGS	metastasis/recurrence	0	1	1	1	0	ND	ND	ND
#3727	borderline serous	metastasis/recurrence	0	0	ND	1	ND			
#3915	unknown	primary	0	1	0	1	0			
#3982	HGS	metastasis/recurrence	0	1	1	1	0	ND	ND	ND
#SAN05	HG pleiomorphic	primary	0	1	1	1	1	1		0.999
#SAN06	HGS	primary	0	1	1	1	1	0.993		0.997
#SAN08	HGS	primary	0	1	1	1	1	1		
#SAN09	HG endometrioid	primary	0	1	0	ND	1	1		
#SAN12	HGS	primary	0	1	1	1	0	0.994		
#SAN20	HGS	primary	0	1	0	ND	0	0.923		
#SAN21	HGS	primary	0	1	1	1	1	0.982		
#SAN24	unknown	pleural effusion/recurrence	0	1	1	ND	1	0.996		
#SAN25	HGS	metastasis/primary	0	1	1	1	1	0.988		
#SAN31	unknown	metastasis/recurrence	0	1	1	ND	1			
#SAN37	HGS	primary	0	1	1	1	0	1		
#SAN40	HGS	metastasis/primary	0	1	1	1	1	0.962		
#SAN44	undifferentiated	metastasis/primary	0	1	1	1	0	0.993		
#SAN46	mucinous	primary	0	1	0	1	1	ND	ND	ND
#SAN47	clear cell	primary	0	1	0	1	1	ND	ND	ND
#SAN60	clear cell	primary	0	1	ND	1	ND	ND	ND	ND

Bolla S. *Department of Pathology IRCCS Candiolo*
Arigoni M. *Bioinformatics and Genomics laboratory (B & Gu) MBC*

IDENTIFICATION OF PUTATIVE DRIVER AND ACTIONABLE CANCER GENES



WES AND CNA ANALYSES OF 12 PDX lines
(naive,TP53 mutated, HGS-EOCs, collected from patients treated with cytoreductive surgery and platinum-based chemotherapy)

↓

SOMATIC SNVs IDENTIFICATION

↓

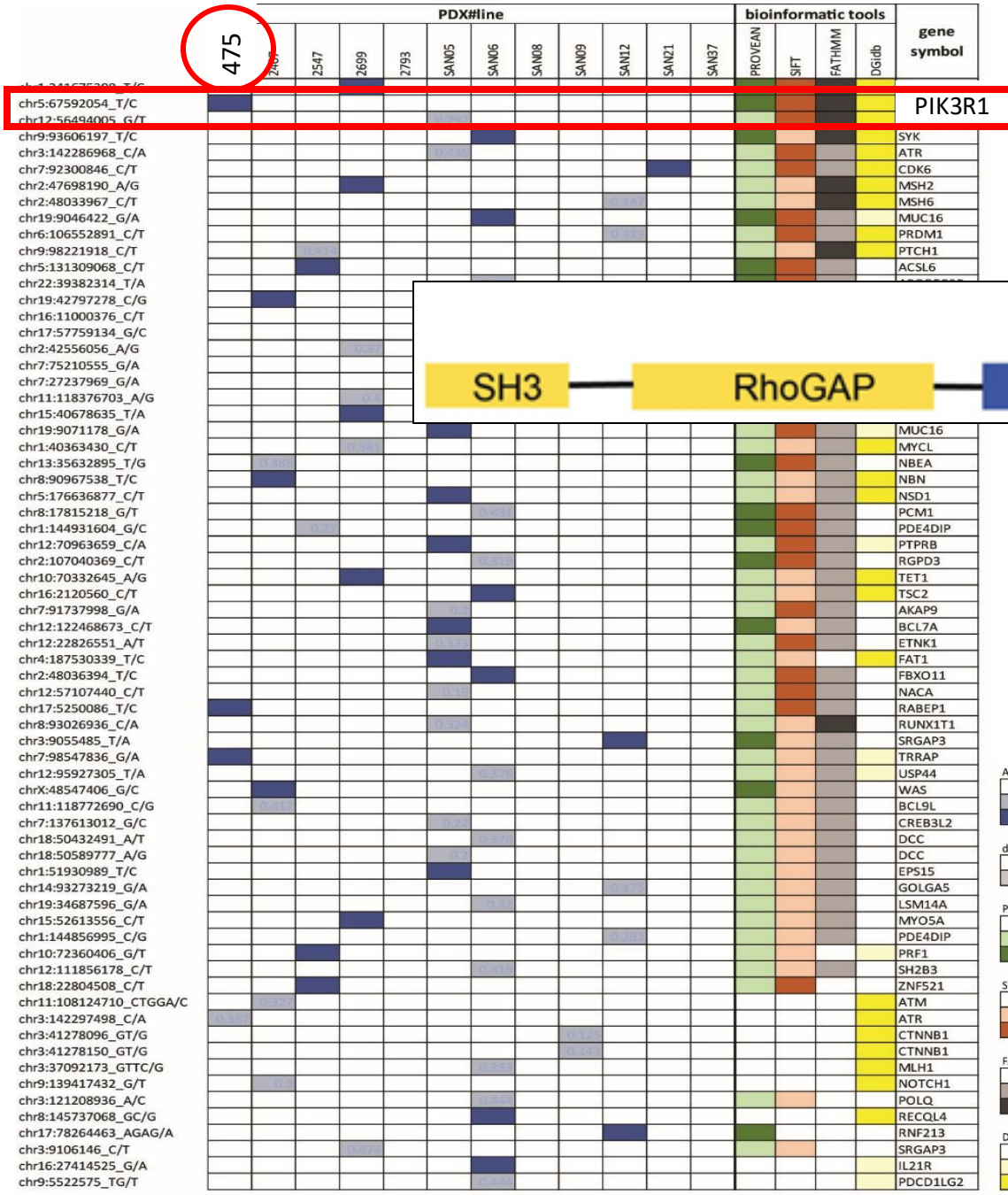
SELECTION OF THE IDENTIFIED SNVs WITH AF ≥ 0.1 IN CANCER-RELATED GENES LISTED IN COSMIC

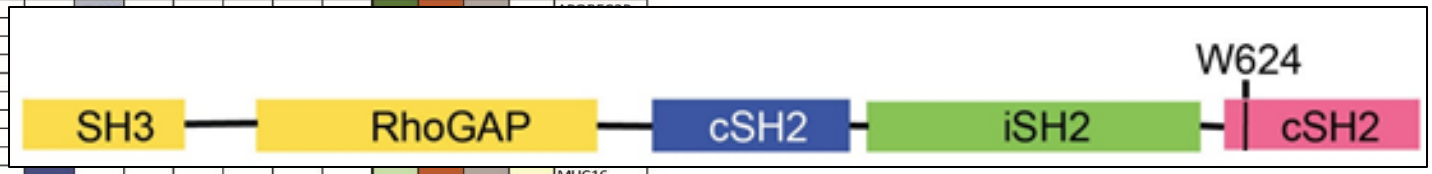
↓

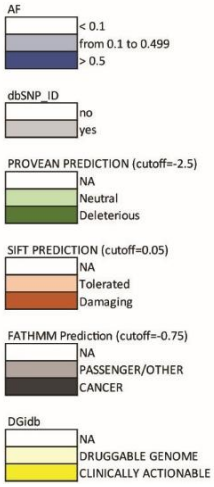
IDENTIFICATION OF PUTATIVE ACTIONABLE DRIVERS

Olivero M. & Arigoni M.

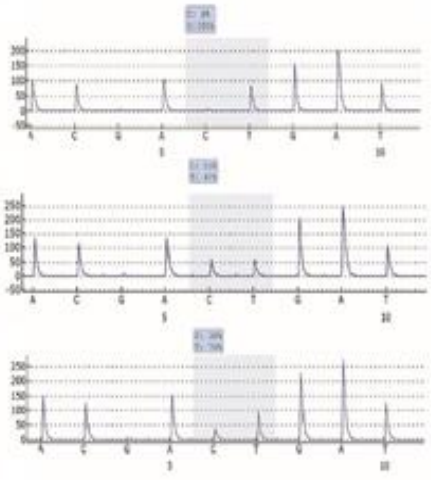
PUTATIVE DRIVER AND ACTIONABLE SNVs IN CANCER GENES





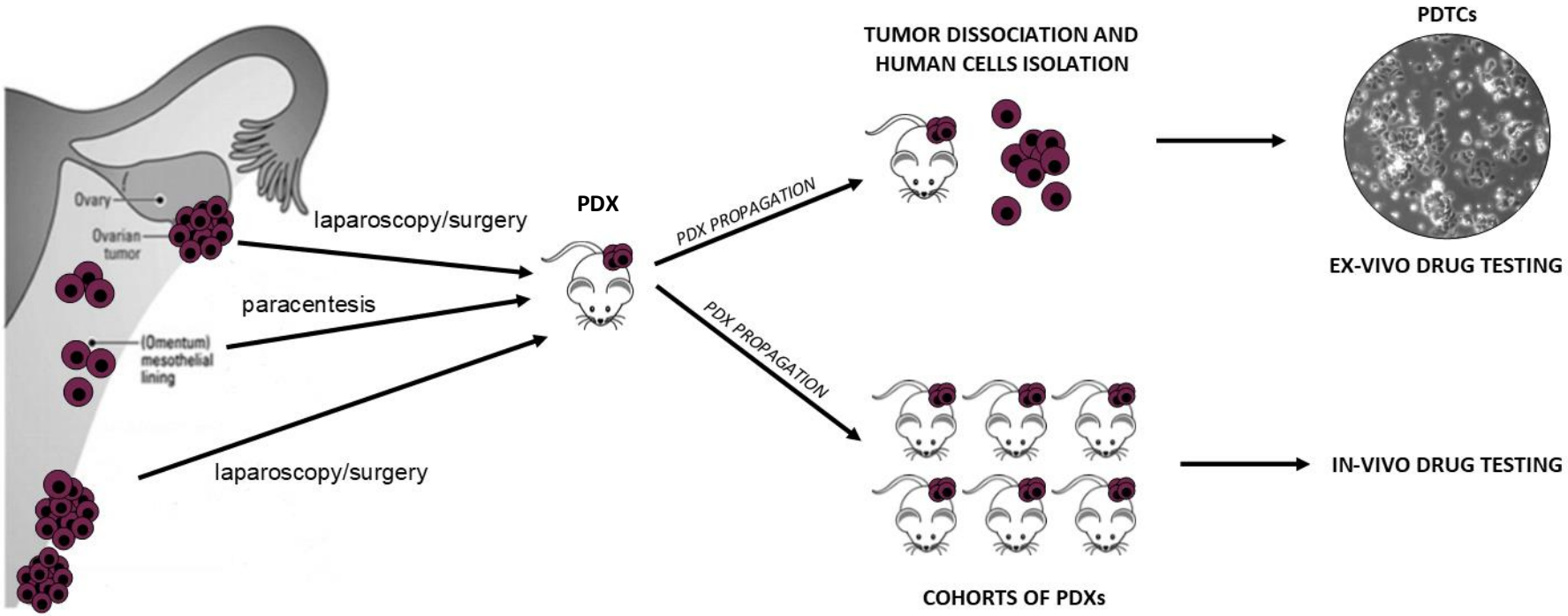


PYROSEQUENCING OF SOURCE TUMOR SAMPLES



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

EX VIVO AND *IN VIVO* ASSAYS OF PI3K INHIBITORS ON #475 PDX LINE

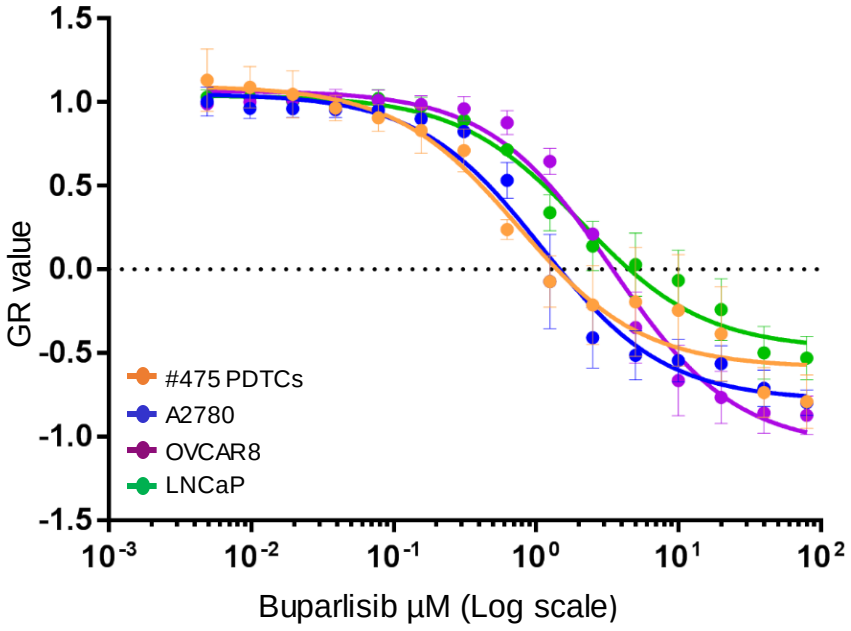


Modified from: "**Shield K.** Gynecol Oncol 2009 "

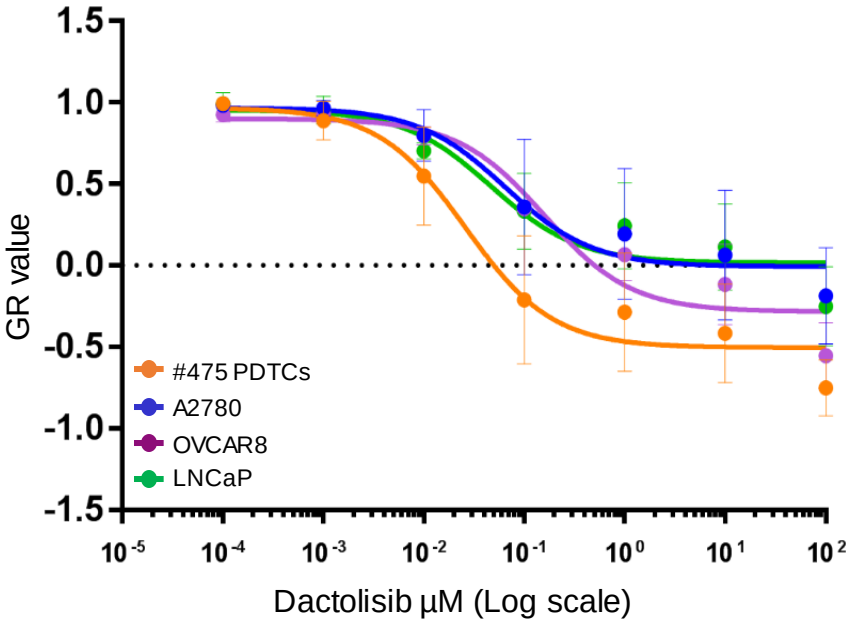
EX-VIVO ASSAYS OF PI3K INHIBITORS ON #475 PDTCs

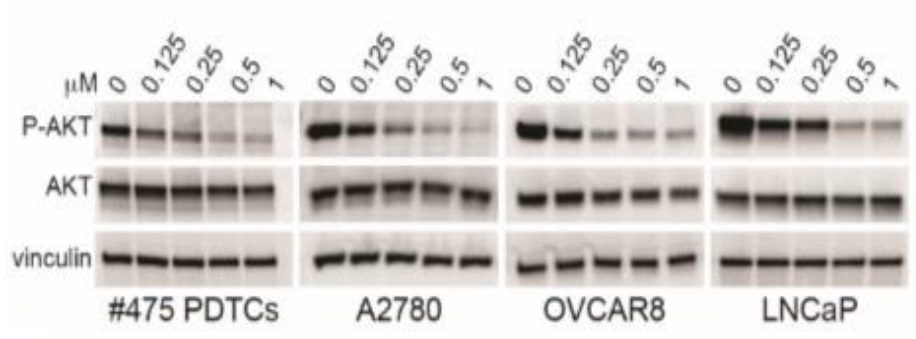
- **Buparlisib and Dactolisib (72h Viability assay and Western Blot analysis)**

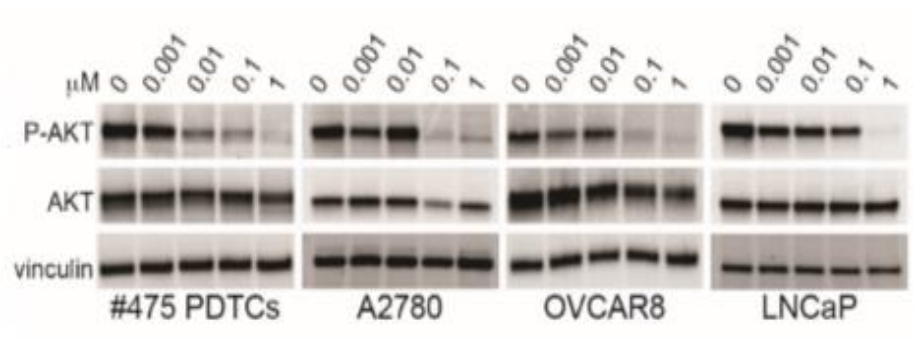
BUPARLISIB (BKM120)
Pan-class I PI3K inhibitor



DACTOLISIB (BEZ235)
Dual mTOR/PI3K inhibitor



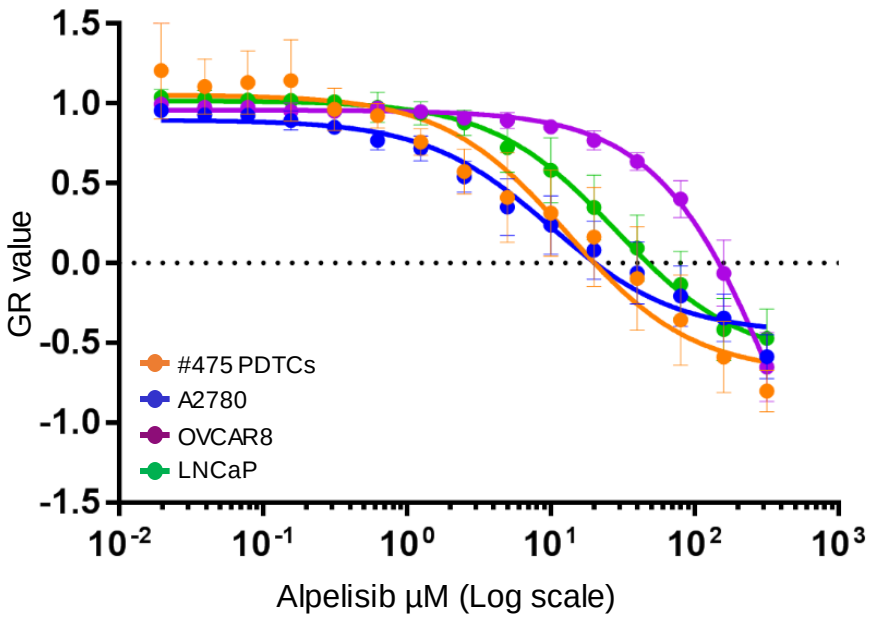




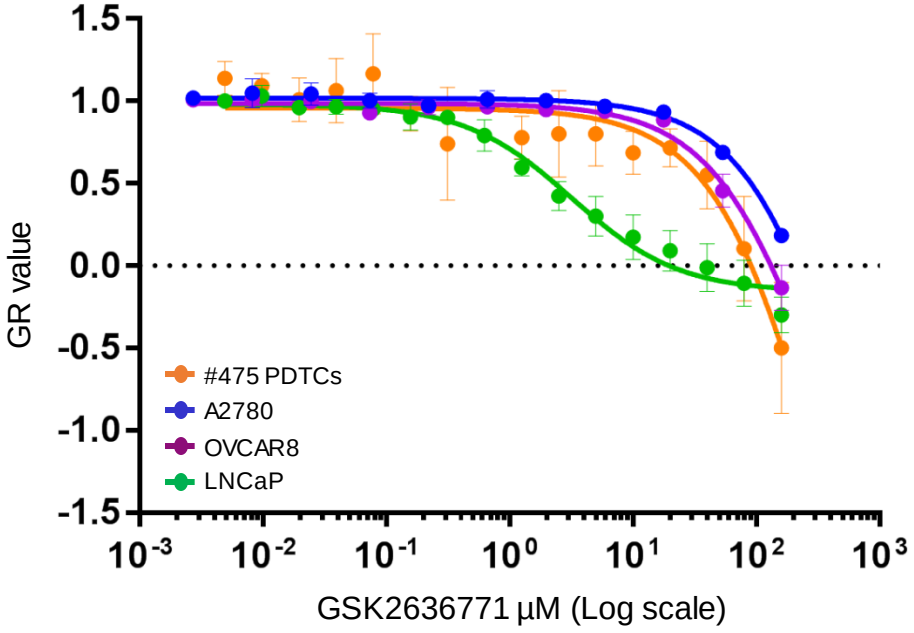
EX-VIVO ASSAYS OF PI3K INHIBITORS ON #475 PDTCs

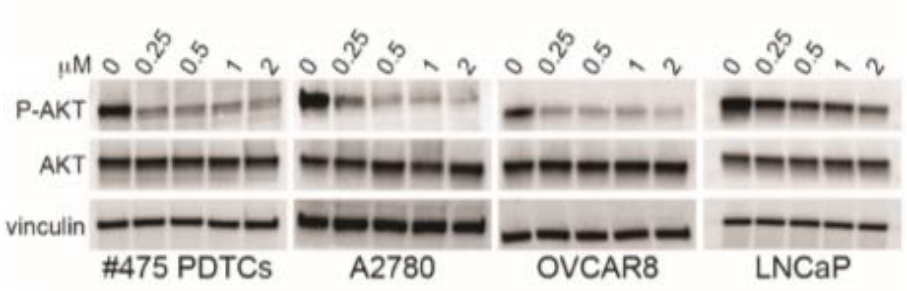
- **Alpelisib and GSK2636771 (72h Viability assay and Western Blot analisys)**

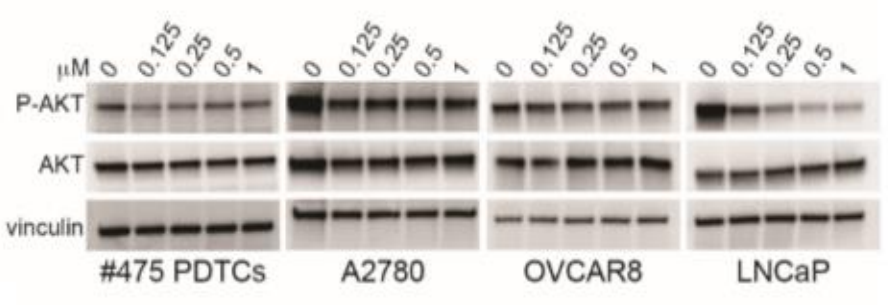
ALPELISIB (BYL719)
P110α selective inhibitor



GSK2636771
P110β selective inhibitor

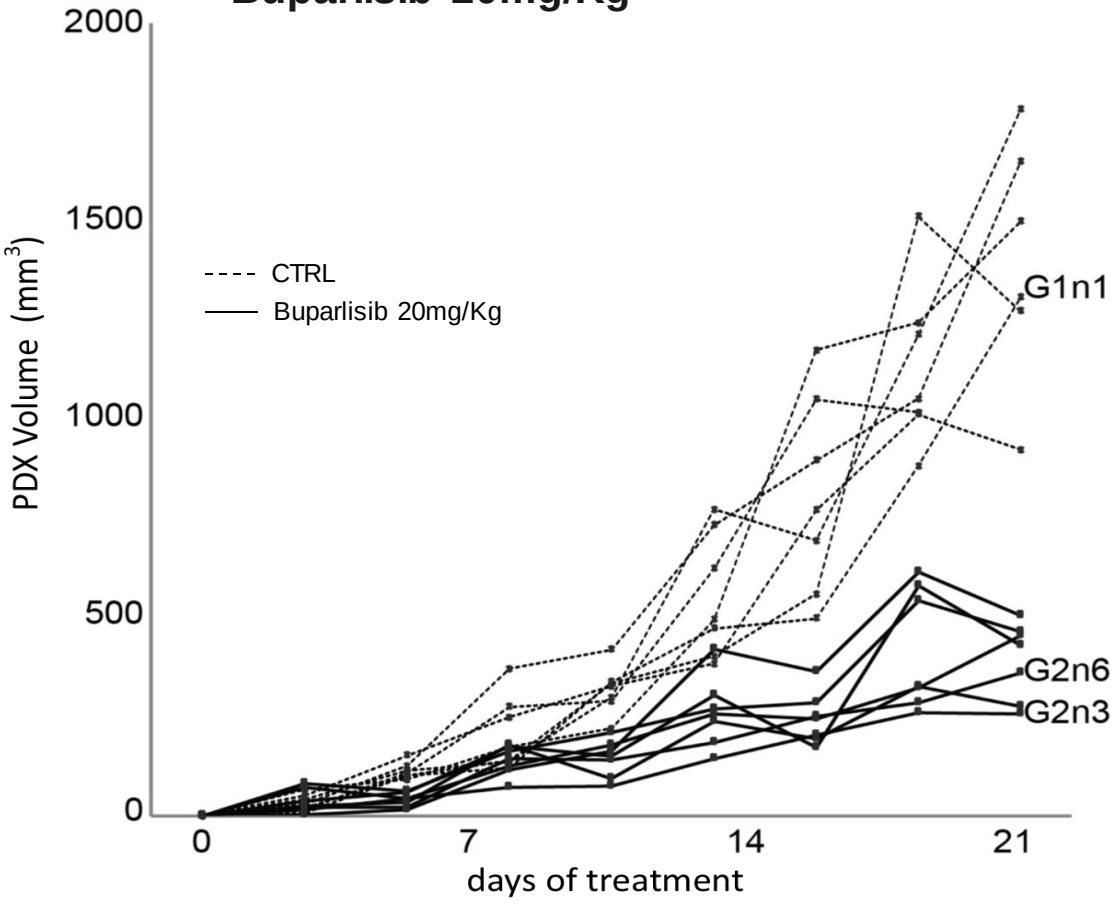




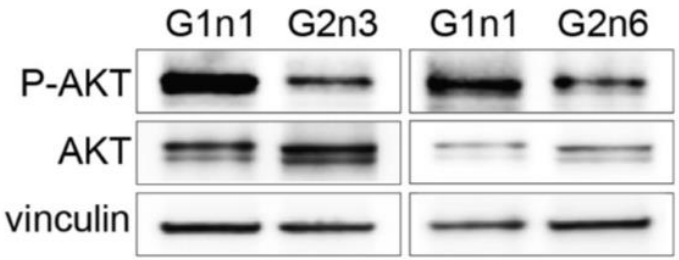


IN VIVO ASSAY OF BUPARLISIB ON #475 PDXs

- **Buparlisib 20mg/Kg**



- **Western Blot analysis**
at the end of *in vivo* treatment



CONCLUSIONS

- PIK3R1^{W624R} mutation is an actionable mutation in HGS-EOC and it makes the tumor susceptible to PI3K inhibitors

- We show the utility of a PDX-based pipeline to study and identify new driver and actionable mutations in HGS-EOC

- PDXs and PDTCs models of ovarian cancer are invaluable tools to assay actionability when sequence- and structure-based* prediction are inadequate

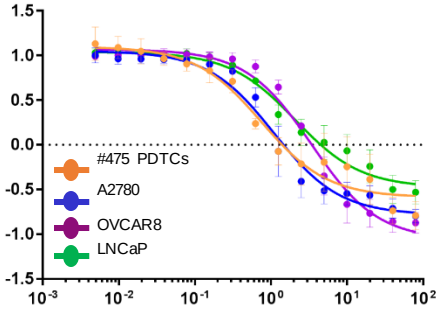
* data not shown

THANK YOU!

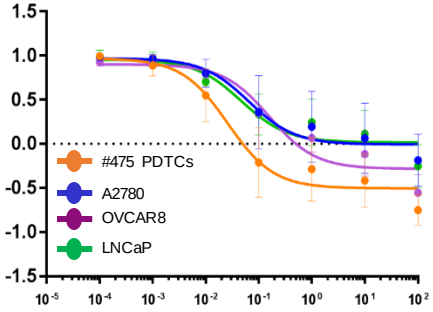
EX-VIVO ASSAYS OF PI3K INHIBITORS ON #475 PDTCs

- **GR50 Values PI3K inhibitors on CTRL Cell lines and #475 PDTCs**

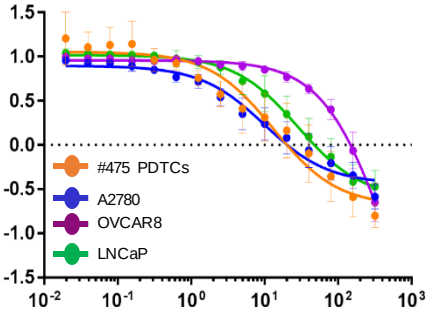
Buparlisib	
Cell line	**GR 50**
A2780	0.568
#475	0.297
OVCAR8	1.595
LNCaP	1,6



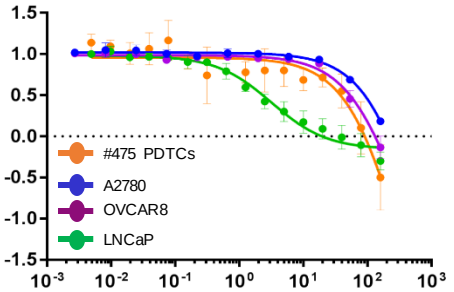
Dactolisib	
Cell line	**GR 50**
A2780	0.023
#475	0.024
OVCAR8	0.087
LNCaP	0,291



Alpelisib	
Cell line	**GR 50**
A2780	3.353
#475	6.741
OVCAR8	48.525
LNCaP	17,3



GSK2636771	
Cell line	**GR 50**
A2780	93.75
#475	24.65
OVCAR8	55.55
LNCaP	2.11

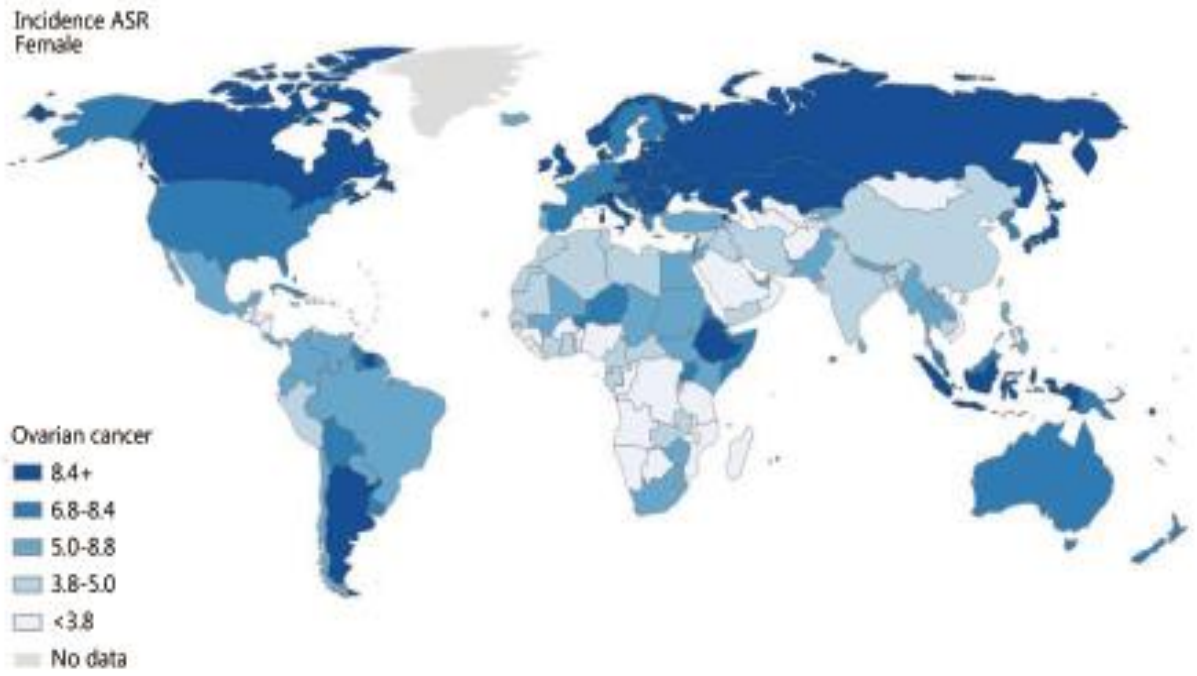


INTRODUCTION

Slide da decidere se levare per questioni di tempo

Ovarian Cancer (OC): Fifth most lethal gynecological cancer among woman in the world derived from:

- Epithelial cells (EOC): more than 90% mostly malignant
- Stromal cells: 5-6%
- Germ cells: 2-3%

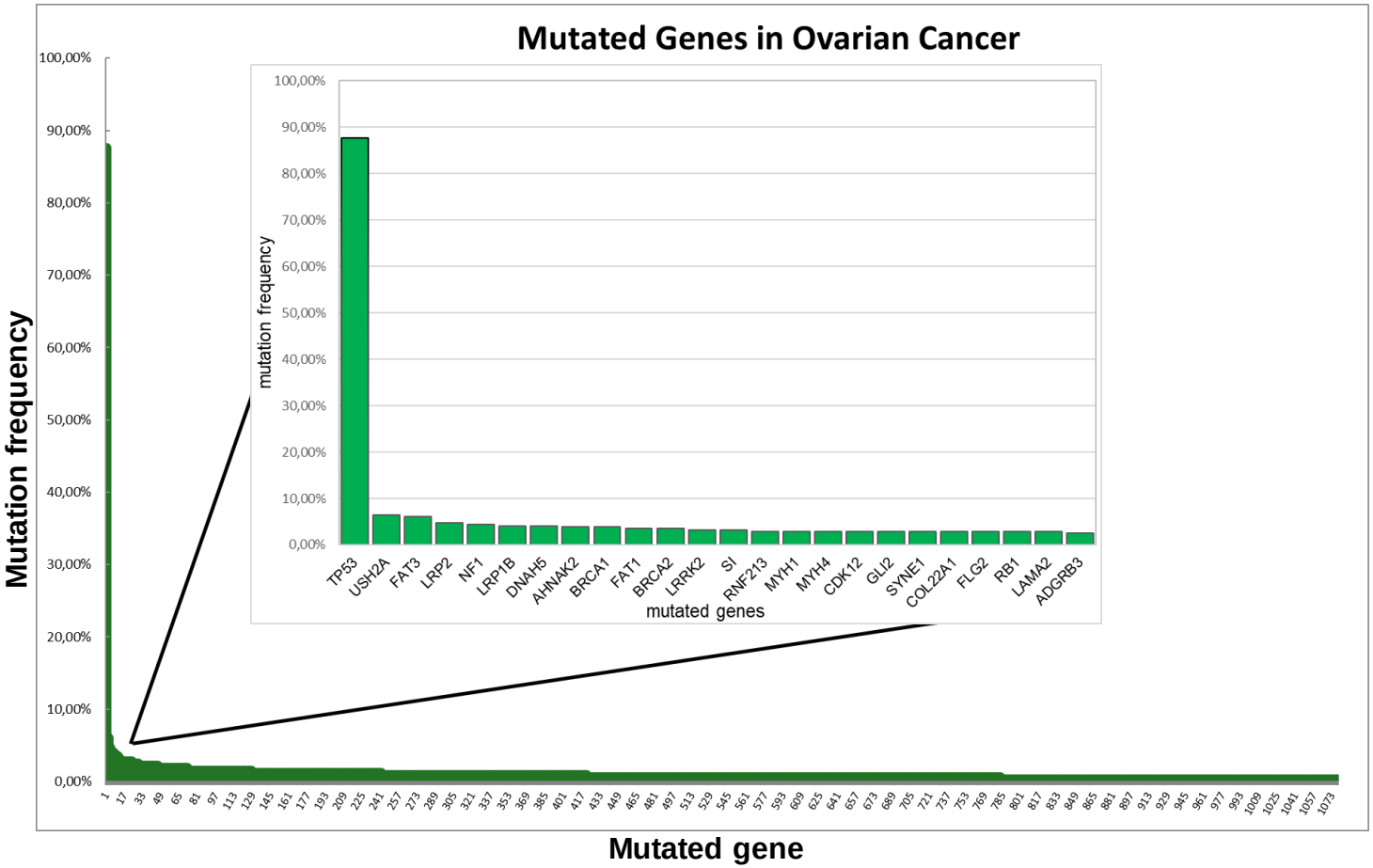


Adapted from: **Brett M. Reid et al.** *Cancer Biol Med 2017*

INTRODUCTION

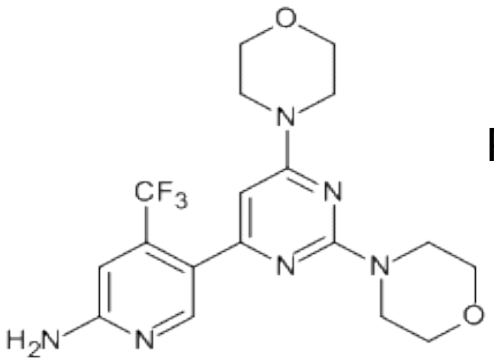
- **Long Tail Distribution of mutated cancer genes in ovarian cancer**

1078 mutated genes in 315 High Grade Serous Ovarian Cancer reported in TCGA analyses with WES

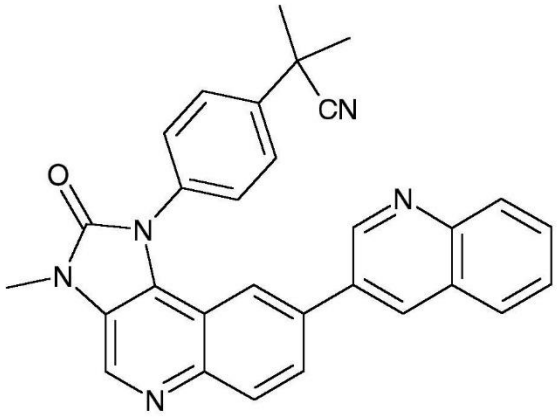


Adapted from: ***TCGA data*** *(https://cancergenome.nih.gov)*

ASSAYED PI3K INHIBITORS



Buparlisib (BKM120)
Pan-class I PI3K inhibitor



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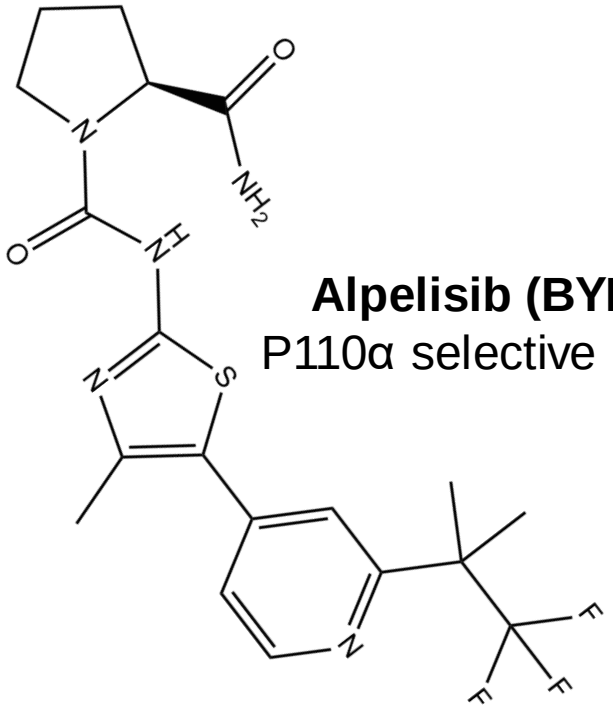

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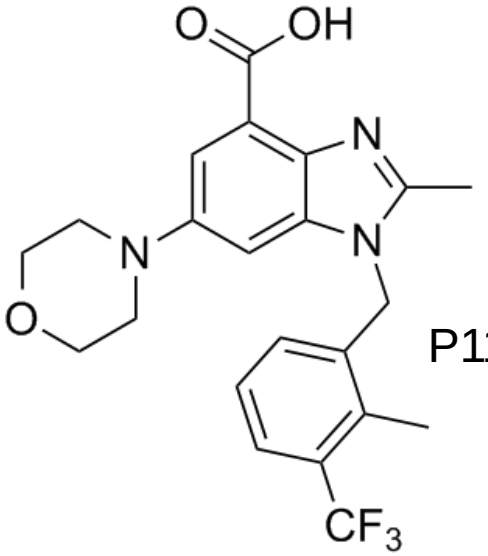
P110α selective inhibitor



GSK2636771
P110β selective inhibitor

Adapted from: http://www.**selleckchem**.com/products.html

AIM OF WORK

IDENTIFICATION OF ACTIONABLE CANCER GENE MUTATIONS
IN PATIENT DERIVED XENOGRAFTS (PDXs) DERIVED FROM HIGH GRADE SEROUS EPITHELIAL OVARIAN CANCER (HGS-EOC)

1. Characterization of PDX lines
2. WES and CNA analyses of selected PDX lines
3. Identification of SNVs in cancer genes
4. Evaluating the functional impact using SIFT, PROVEAN and FATHHMN algorythms
5. *Ex vivo* assays of targeted drugs on PDX-Derived Tumor cells (PDTCs)
6. *In vivo* assays of targeted drugs on PDXs