ALAG 2019

Symposium:
Genome Instability and Cancer

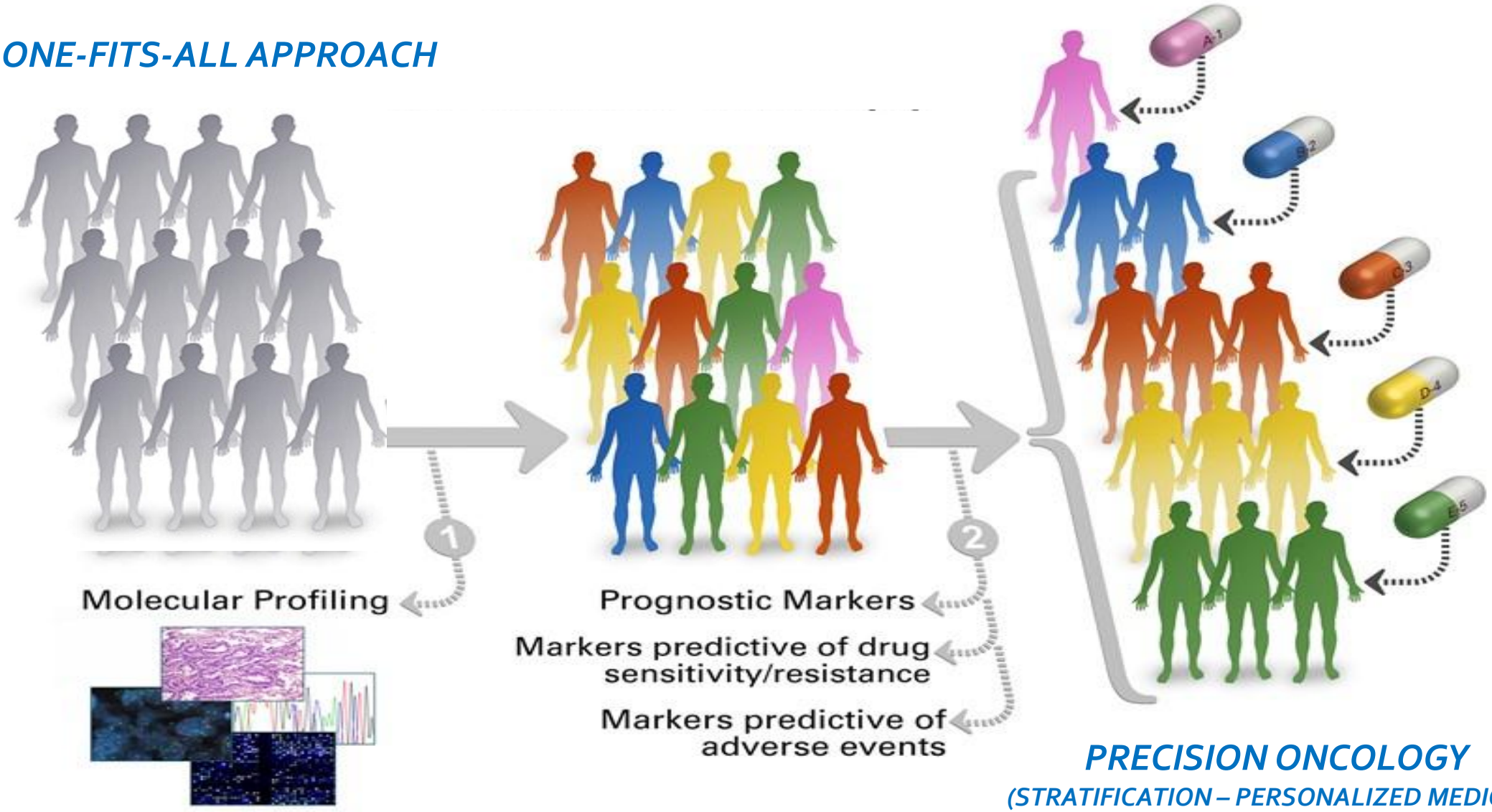
Gastón Soria
Laboratorio
de Letalidad Sintética en Cáncer

CIBICI-CONICET
Universidad Nacional
de Córdoba

Target-ID by Synthetic Lethality Induction for Precision Oncology *of BRCA-deficient Cancers*

The **Evolution** of Cancer Therapeutics

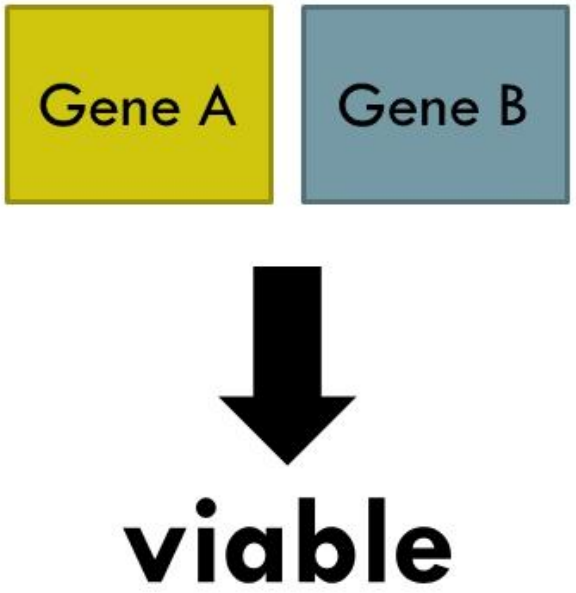
ONE-FITS-ALL APPROACH



PRECISION ONCOLOGY

(STRATIFICATION – PERSONALIZED MEDICNE)

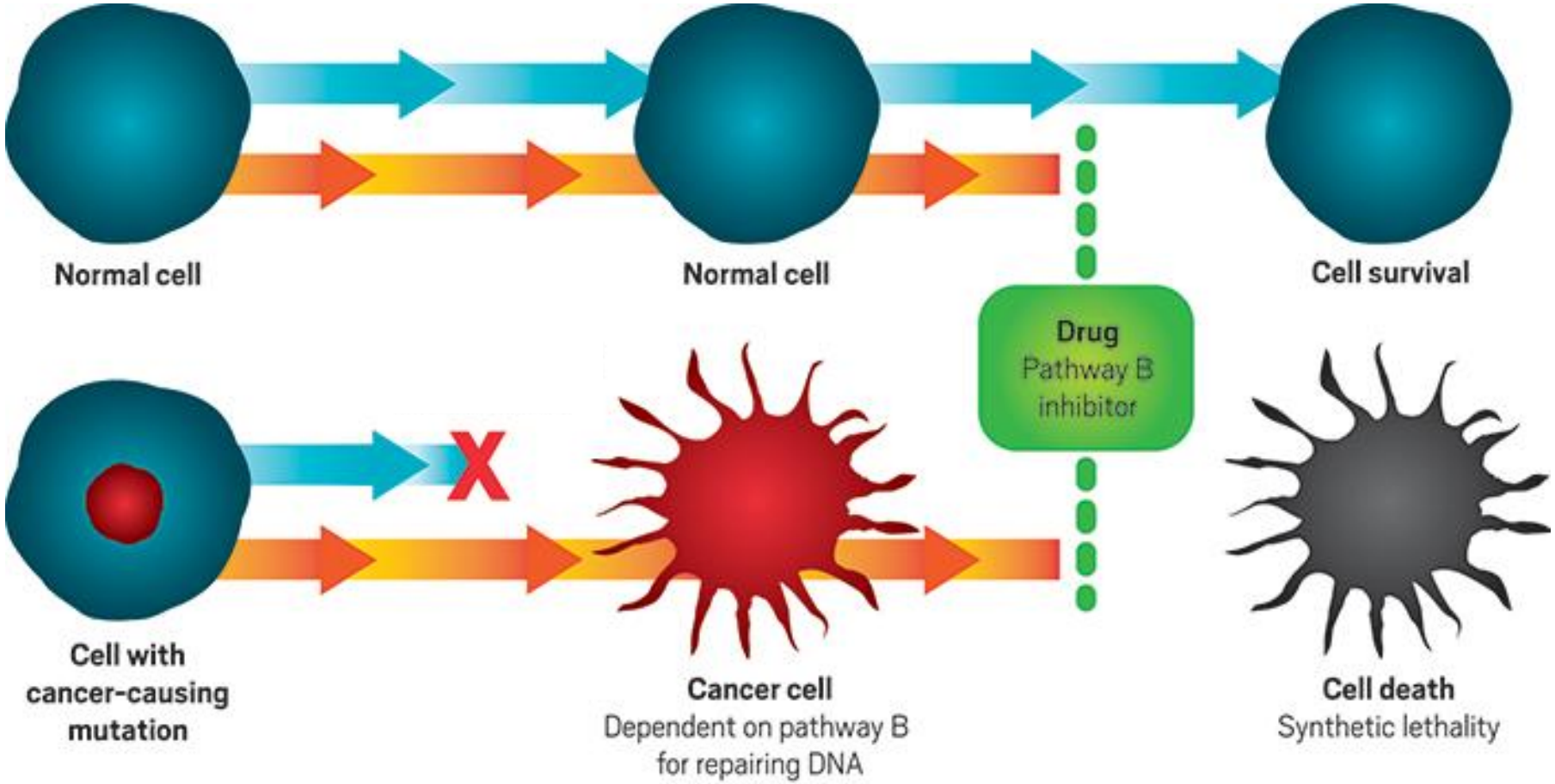
Functional assays based on the concept of ***Synthetic Lethality (SL)***



Yeast and Drosophila historical studies

Source: *Wikipedia*

Exploiting *the principle of pharmacological synthetic lethality (SL) for* ***cancer therapeutics***



A successful SL therapeutic approach should provide:

- **Selective cytotoxicity**
- **Attenuated adverse effects**

Modified from: C&EN American Chemical Society

Proof of concept of ***SL*** as a ***therapeutic strategy***

Targeting the DNA repair defect in *BRCA* mutant cells as a therapeutic strategy

Hannah Farmer[1,2*], Nuala McCabe[1,2*], Christopher J. Lord[2*], Andrew N. J. Tutt[2,3], Damian A. Johnson[2], Tobias B. Richardson[2], Manuela Santarosa[2†], Krystyna J. Dillon[4], Ian Hickson[4], Charlotte Knights[4], Niall M. B. Martin[4], Stephen P. Jackson[4,5], Graeme C. M. Smith[4] & Alan Ashworth[1,2]

Original papers

- Bryant et al. Nature 2005
- Farmer et al. Nature 2005

Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase

Helen E. Bryant[1], Niklas Schultz[2], Huw D. Thomas[3], Kayan M. Parker[1], Dan Flower[1], Elena Lopez[1], Suzanne Kyle[3], Mark Meuth[1], Nicola J. Curtin[3] & Thomas Helleday[1,2]

230 clinical trials – 4 PARPi approved
14 years after its preclinical discovery

Lynparza™ olaparib capsules 50 mg

Rubraca® (rucaparib) tablets

Zejula™ niraparib capsules 100 mg

TALZENNA™ talazoparib 1 mg capsules

WHICH ARE THE MAIN GOALS OF OUR GROUP?

TO DEVELOP HIGH-THOUGHPUT SCREENING ASSAYS TO TEST FUNCTIONAL HYPOTHESES WITH THERAPEUTIC POTENTIAL FOR CANCER TREATMENT

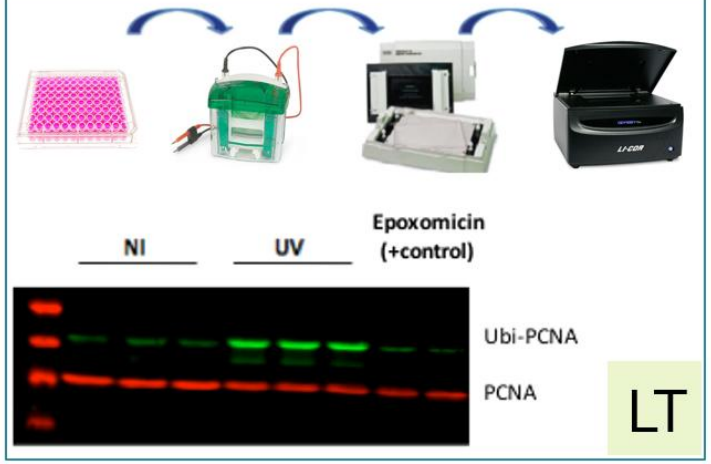
TO IDENTIFY NEW MOLECULAR TARGETS/DRUGS FOR CANCER TREATMENT EXPLOTING THE PRINCIPLE OF SYNTHETIC LETHALITY

Synthetic Lethality in Cancer Lab

http://cibici.fcq.unc.edu.ar/es/letalidad-sintetica-cancer

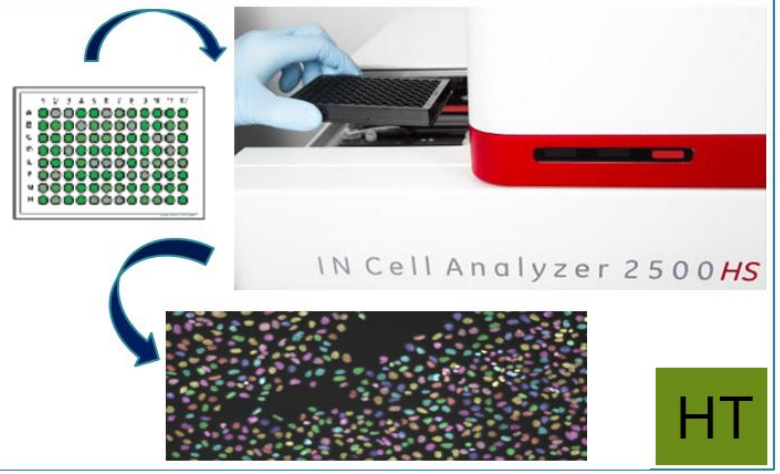
Screening *assays and* ***platforms*** *developed in our Lab*

Miniaturized Western Blot



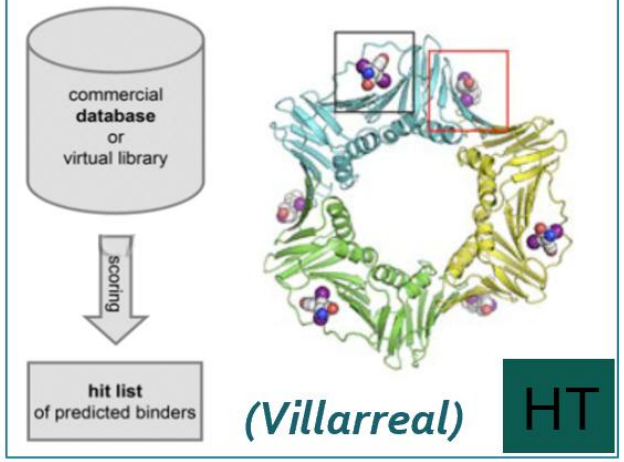
LT

High Content Imaging



HT

Virtual Screening

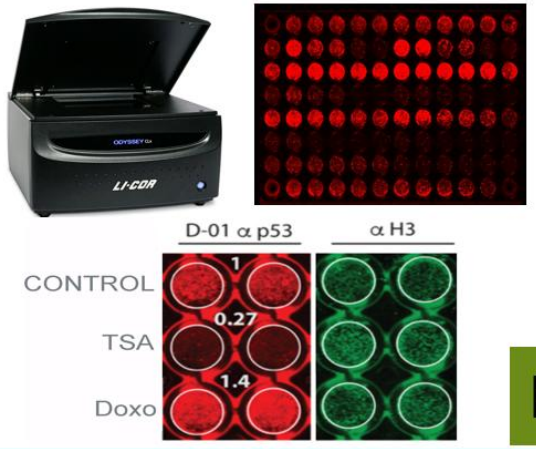


(Villarreal)

HT

Translesion DNA Synthesis (TLS) Inhibitors

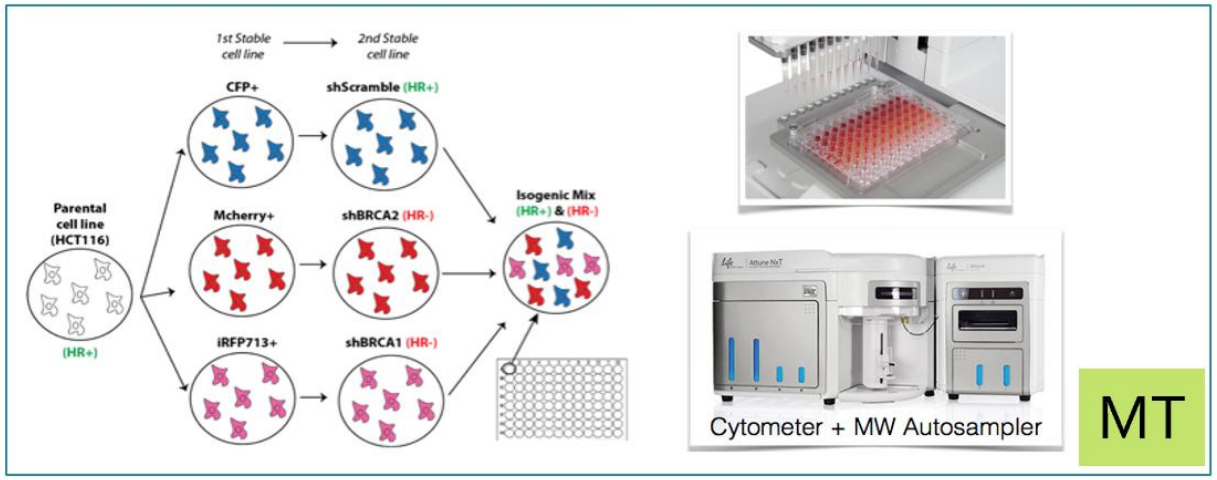
In Cell Western



HT

Mutant p53 downregulation (Girardini)

Automated Flow cytometry



MT

Synthetic lethality in isogenic backgrounds (BRCA1 & BRCA2 status).

Throughput capacity (compounds / week)

200 1000 >5000

Consortium project in collaboration with GlaxoSmithKline

(Synthetic lethality screenings with Natural Products and Compound Libraries)

Screening Node

Members: Soria © & Bocco

Main aim: Setup of the platforms, screening phase and early validation models

Validation Node

Members: Gottifredi ©, Caputto & Gil

Main aim: Molecular validation and characterization of the hits. Complex models

gsk

- Compound libraries
- Chemoproteomics
- Analog compounds
- Compound synthesis

Natural Products Node

Members: Carpinella ©, Joray, García, Nicotra, Ruiz & Barboza

Main aim: preparation of plant extracts. Fractionation and isolation of NP

Bioinformatics Node

Member: Fernandez©

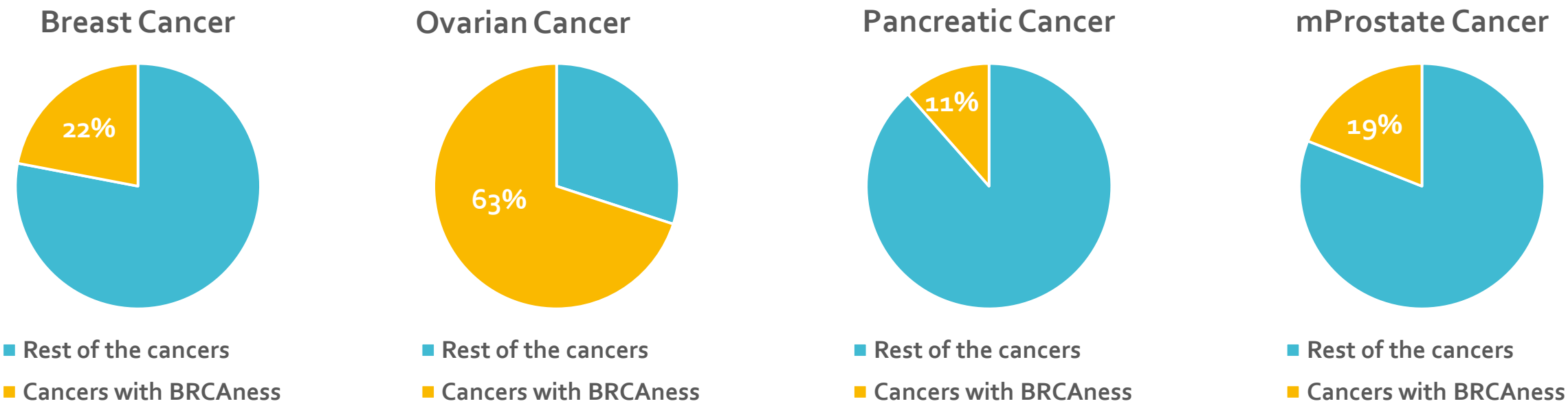
Main aim: Validation using patient databases analysis

BRCA1/BRCA2 deficiencies in Human cancers

nature medicine

DEVELOPMENT OF HR-DETECT Davies et al 2017

BRCAness is much more widespread in human cancers than anticipated



Also detected in: Gastric,Lung, Bladder and other types of cancer.

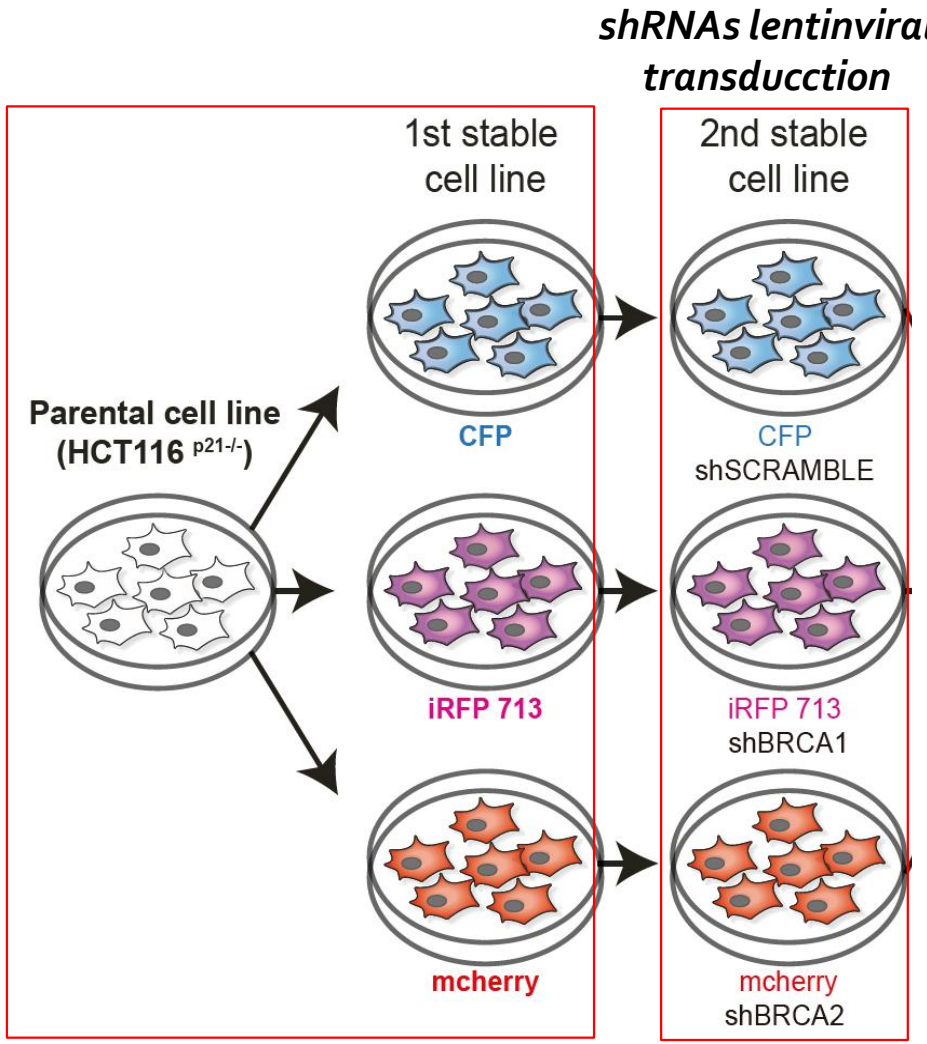
Urgent need to develop targeted therapies against BRCA-deficient cancers

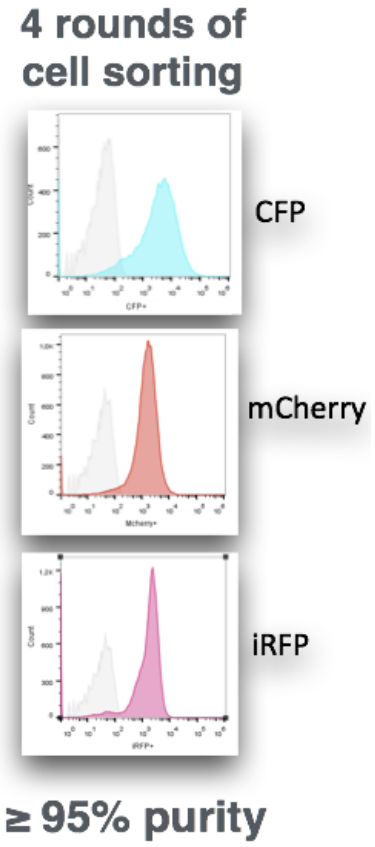
*How can we **exploit BRCA-deficiencies** to develop therapeutic approaches based on synthetic lethality ???*

OUR APPROACH: **THE MERCENARY APPROACH**

KILL(SELECTIVELY) FIRST… ASK QUESTIONS LATER

Development of a **phenotypic screening** platform using high-throughput **flow cytometry**



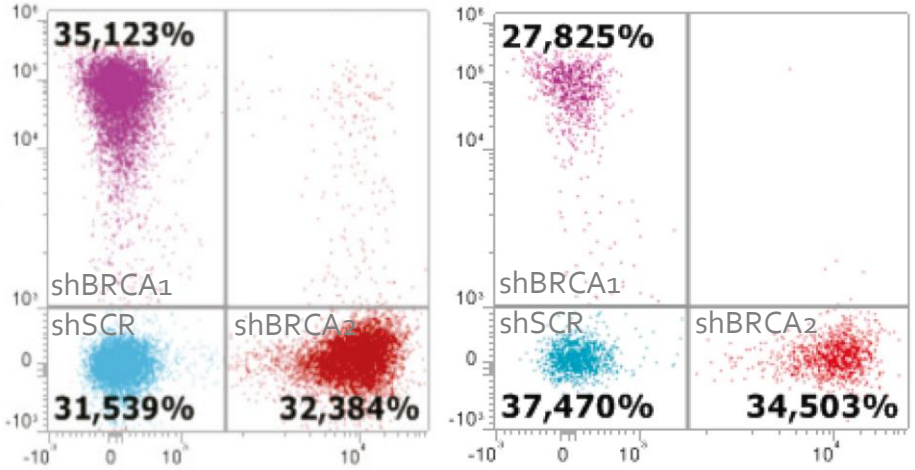


Robotic Arm for cell seeding and high-throughput processing

Automated Flow Cytometer

Development of a **Phenotypic Screening** Platform using High-Throughput **Flow Cytometry**

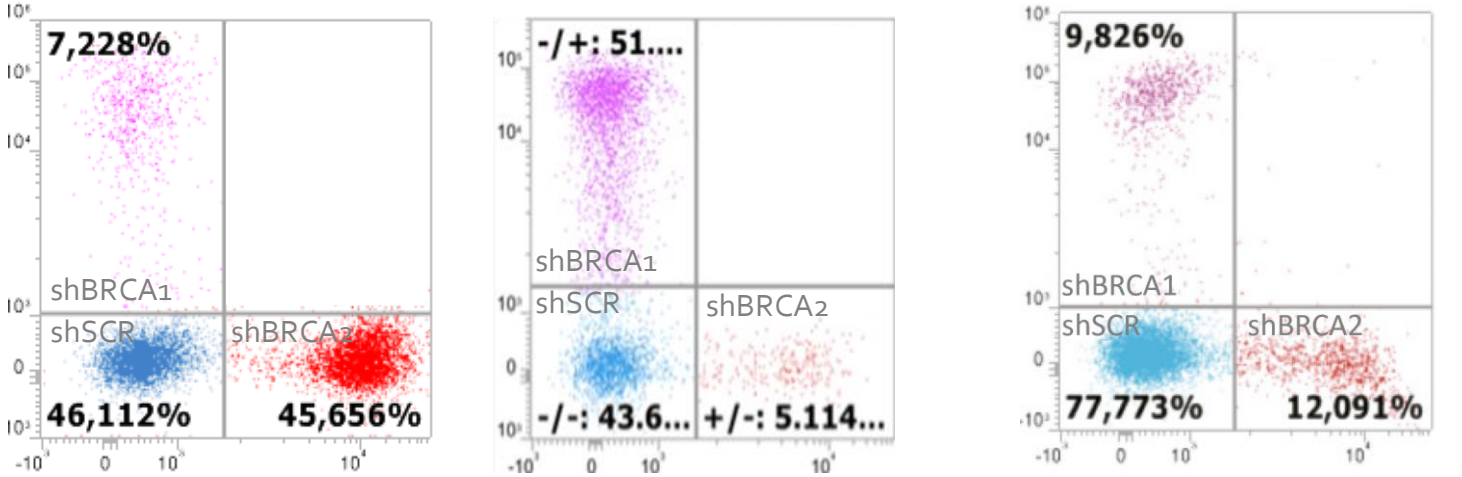
DISCARDED



Non-toxic

Toxic treatment (non selective)

SYNTHETIC LETHAL



BRCA1 single hit

BRCA2 single hit

Double hit

- **Divergent** functions of BRCA1 and BRCA2
- Smaller, yet more specific patients' coverage
- **Different** mechanism than PARPi

- **Convergent** functions of BRCA1 and BRCA2
- Mechanis might be **similar** to PARPi

*WHICH ARE THE **SOURCES** OF COMPOUNDS/DRUGS FOR OUR **SCREENINGS**?*

1) Pure natural products and plant extracts.

2) A 13K collection of natural products from GSK.

Unknown targets

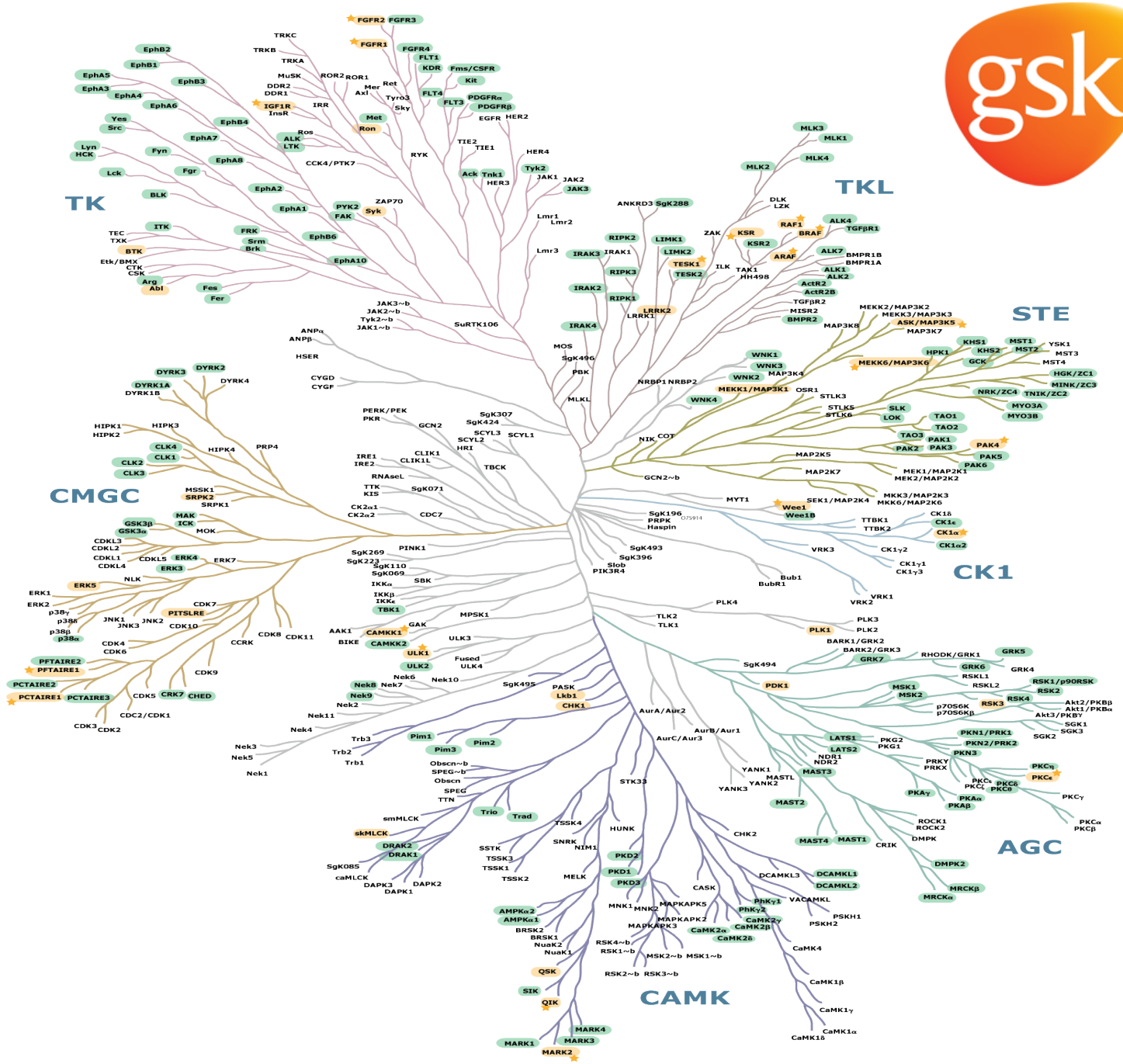
3) A 1.7K collection of FDA and EMA approved drugs.

4) An open source collection of 688 Kinase inhibitors.

known targets

PKIS2 Library

Targeting the Human Kinome (688 kinase inhibitors)



AGC: PKA/PKG/PKC-family kinases; CAMK, calcium/calmodulin-dependent kinases

CK1: casein kinases; CMGC, CDK/MAPK/GSK3/CLK-family kinases

RCG: receptor guanylate cyclases

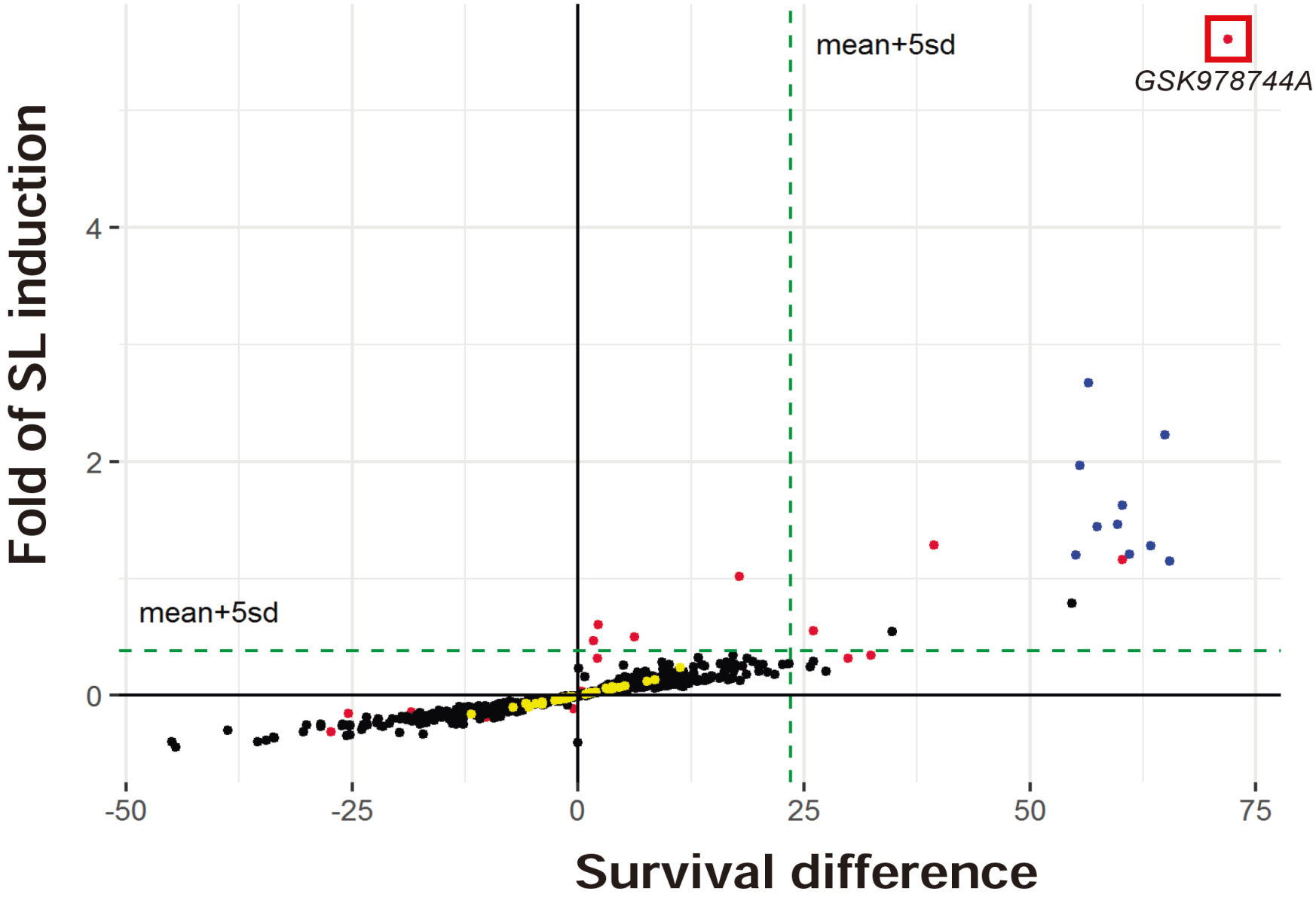
STE: sterile homologue kinases

TK: tyrosine kinases

TKL: tyrosine kinase-like kinases; atypical protein kinases

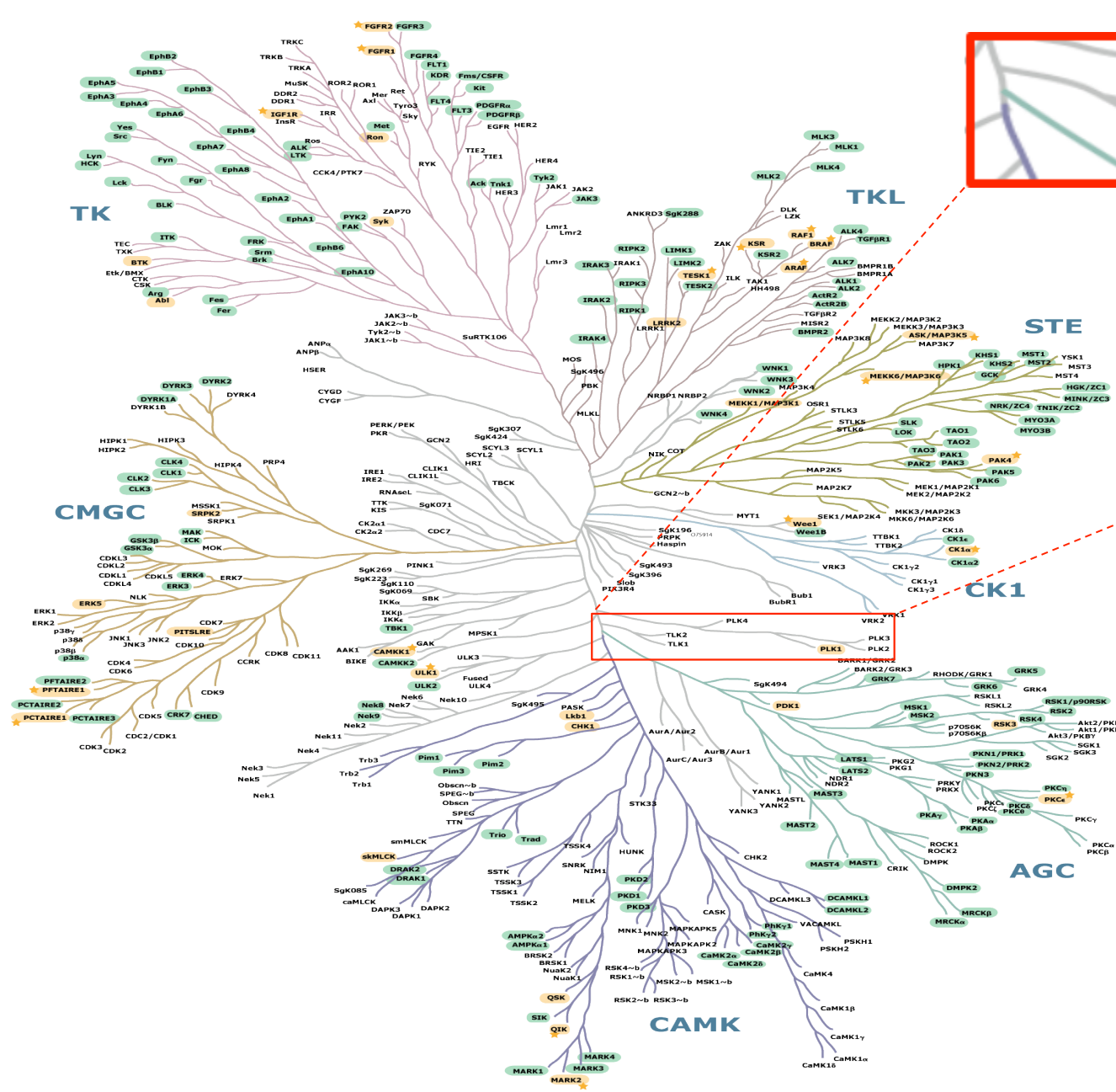
Adapted from Manning et al, 2002

PKIS2 Screening at 0.1μM in BRCA1 deficient cells

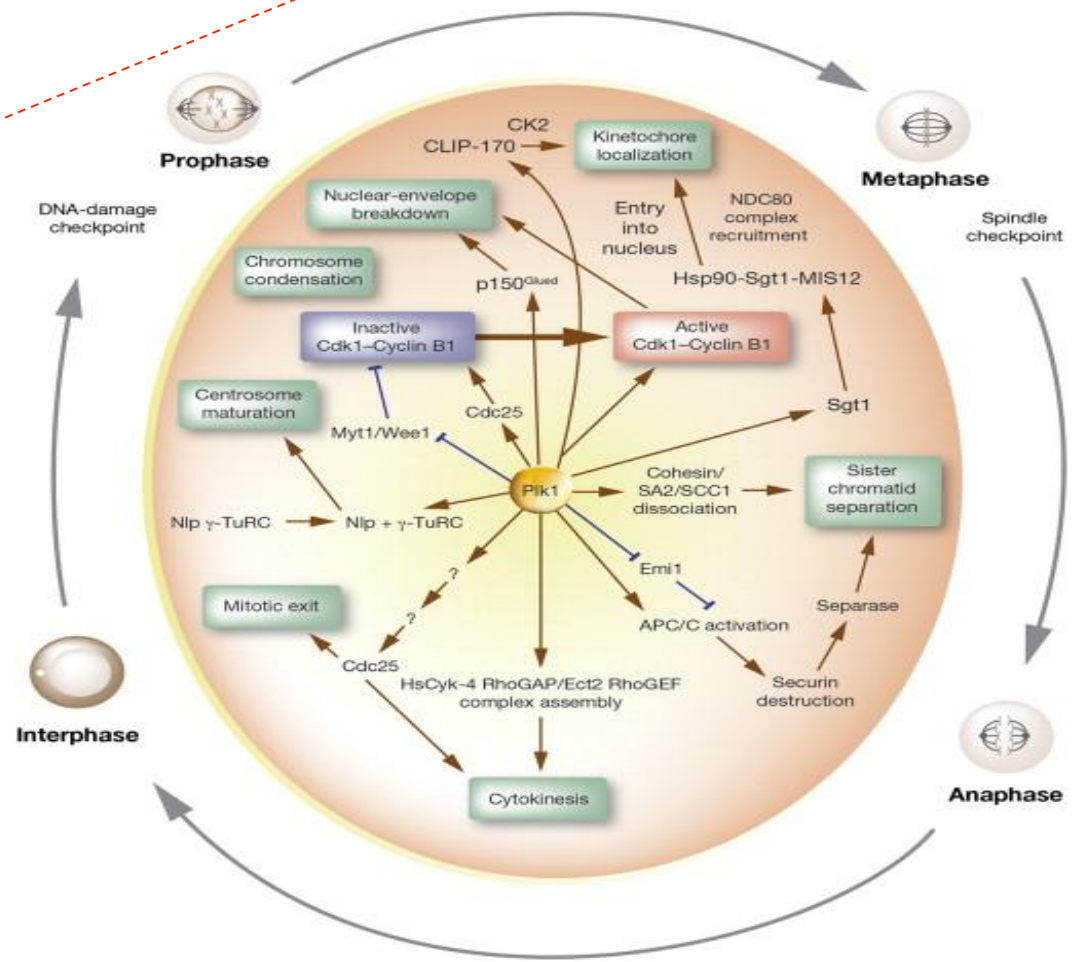


- (-) control (Non Treated)
- (+) control (Olaparib)
- Others inhibitors
- PLK1 inhibitors

Compound code	Putatitive target
GSK978744A	PLK1
GSK326090A	PLK1
GSK483724A	PLK1
GSK479719A	PLK1
GSK2008607A	rTbIPMK
GSK641502A	PLK1
GW852849X	PLK1
GSK1322949A	AURKC / Pleiotropic
GSK1576028A	PLK1 / Pleiotropic
GSK571989A	PLK1
GSK2110236A	PLK1
GSK312948A	PLK1
GSK619487A	AKT
SB-590885-AAE	B-RafV600E
GSK1751853A	IGF1R

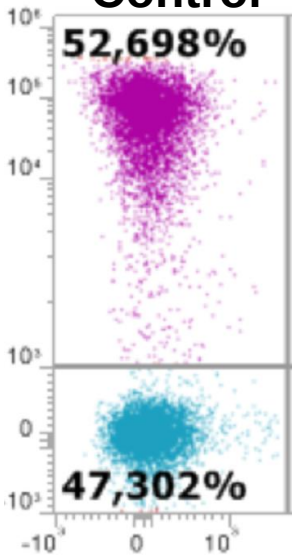

TK
TKL
STE
CMGC
CK1
AGC
CAMK
PLK4
TLK2
TLK1
PLK3
PLK1
PLK2
VRK2


PLK4
VRK2
TLK2
TLK1
PLK3
PLK1
PLK2

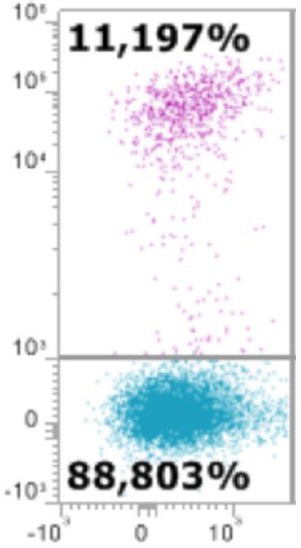

Prophase
Metaphase
Anaphase
Interphase
DNA-damage checkpoint
Spindle checkpoint
CK2
CLIP-170
Kinetochore localization
Nuclear-envelope breakdown
Entry into nucleus
NDC80 complex recruitment
Chromosome condensation
p150Glued
Hsp90-Sgt1-MIS12
Inactive Cdk1–Cyclin B1
Active Cdk1–Cyclin B1
Centrosome maturation
Myt1/Wee1
Cdc25
Sgt1
Plk1
Cohesin/ SA2/SCC1 dissociation
Sister chromatid separation
Nlp γ-TuRC
Nlp + γ-TuRC
Emi1
Mitotic exit
APC/C activation
Separase
Cdc25
HsCyk-4 RhoGAP/Ect2 RhoGEF complex assembly
Securin destruction
Cytokinesis

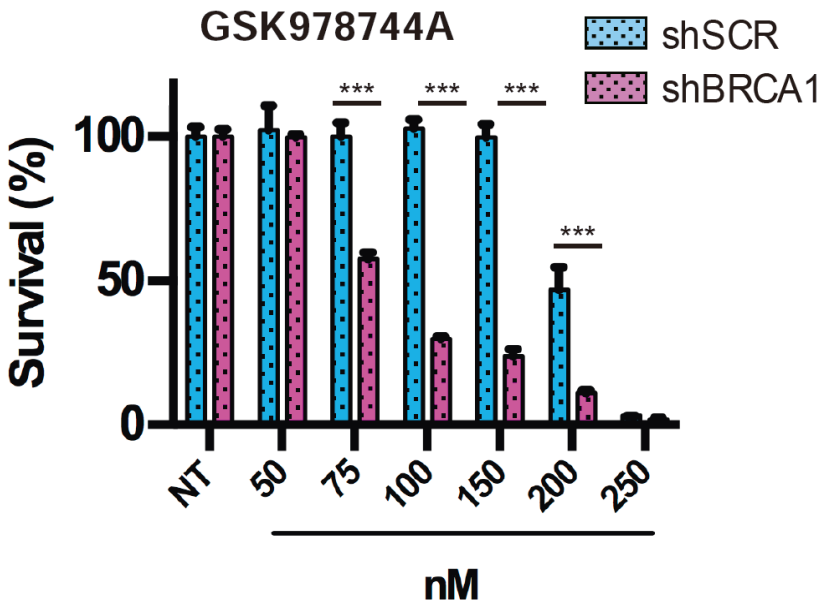
PLK1 inhibition triggers **strong SL** in BRCA1-deficient cells

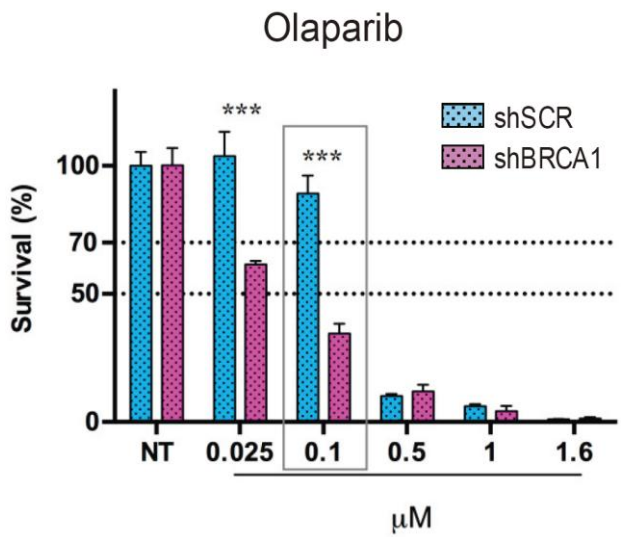
Control

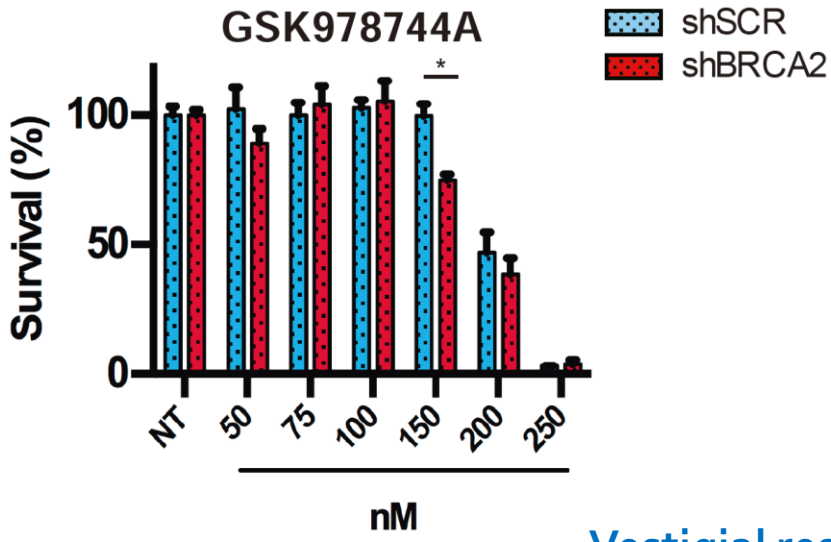


GSK9787744A

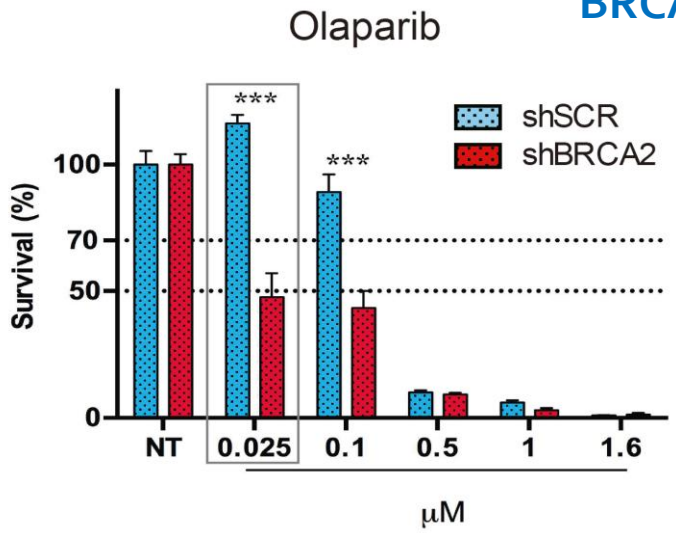






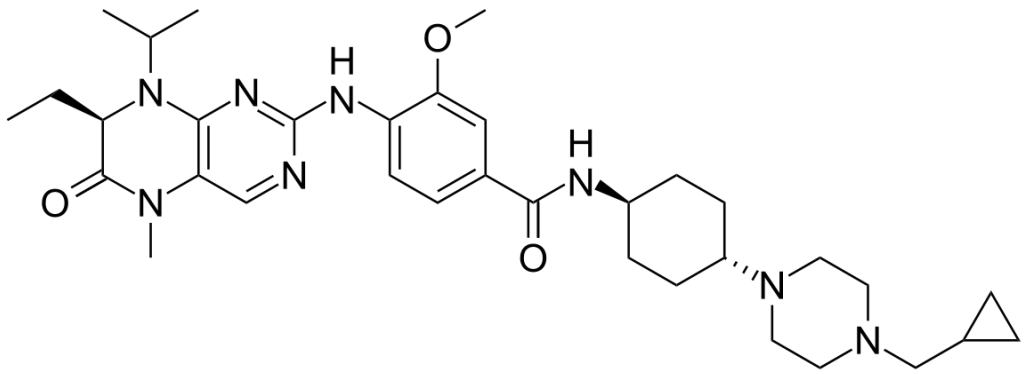


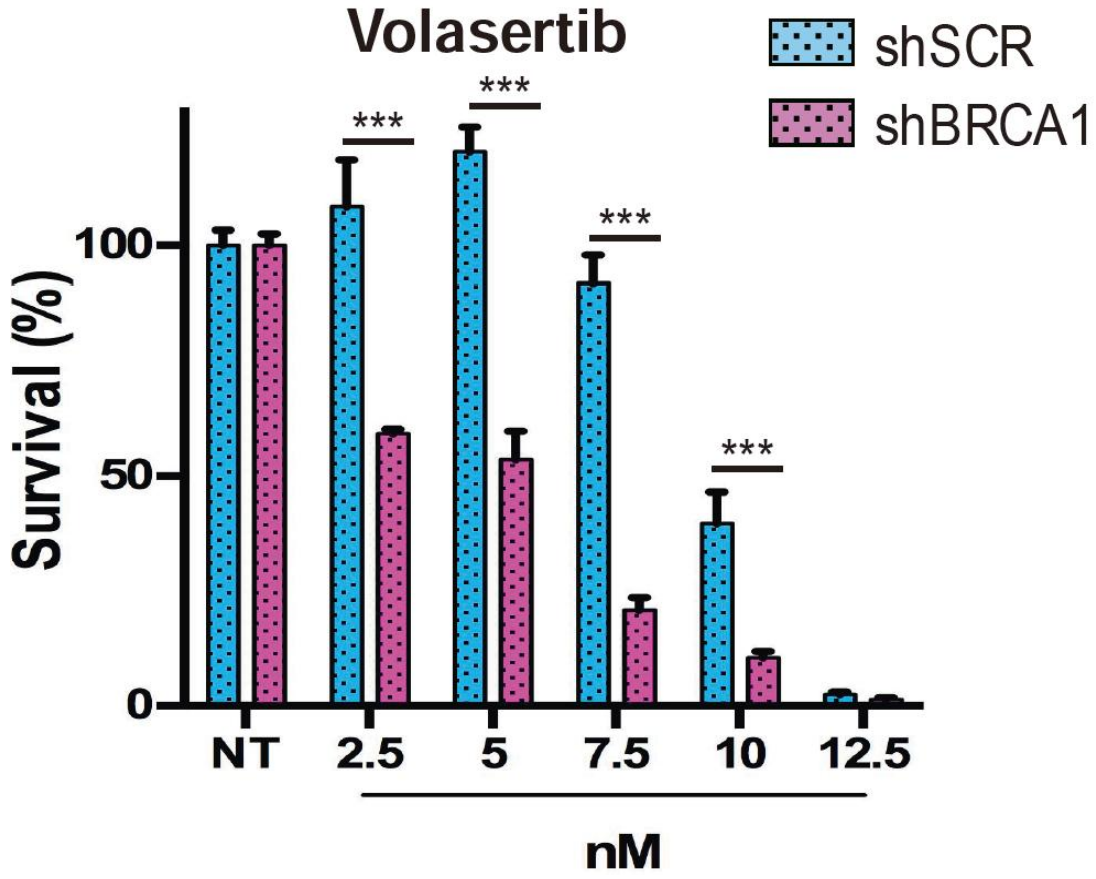
Vestigial response in BRCA2-deficient cells



PLK1 inhibition tiggers **strong SL** in BRCA1-deficient cells

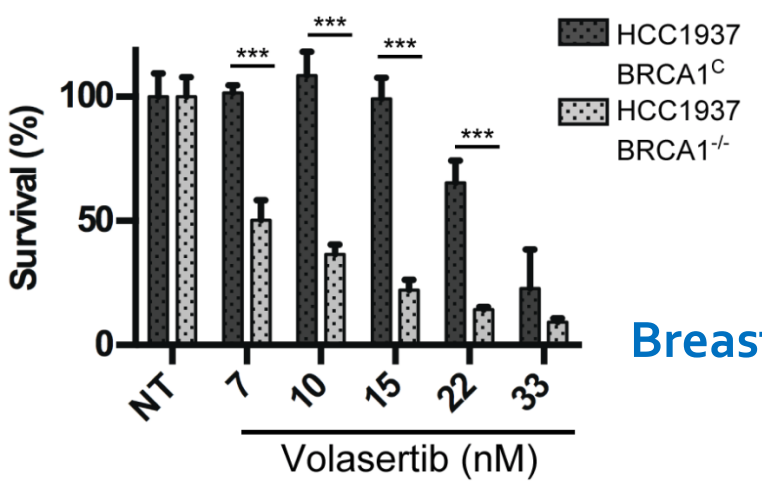
A PLK1 inhibitor in Phase III clinical trials from Boehringer Ingelheim



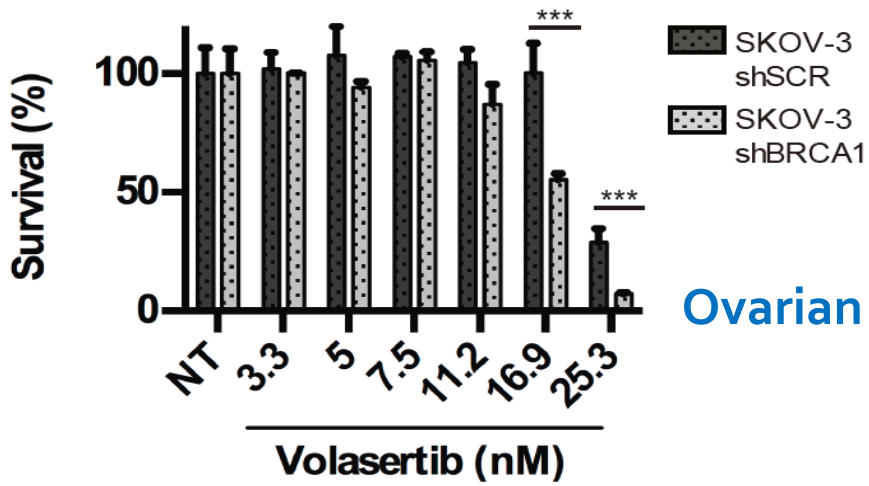


Validation of SL induction in other cellular models

ISOGENIC VALIDATION MODELS

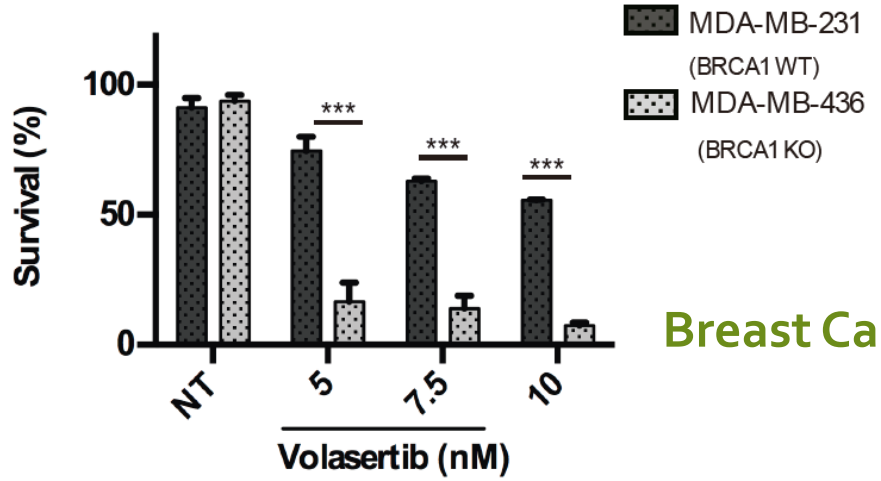


Breast Cancer



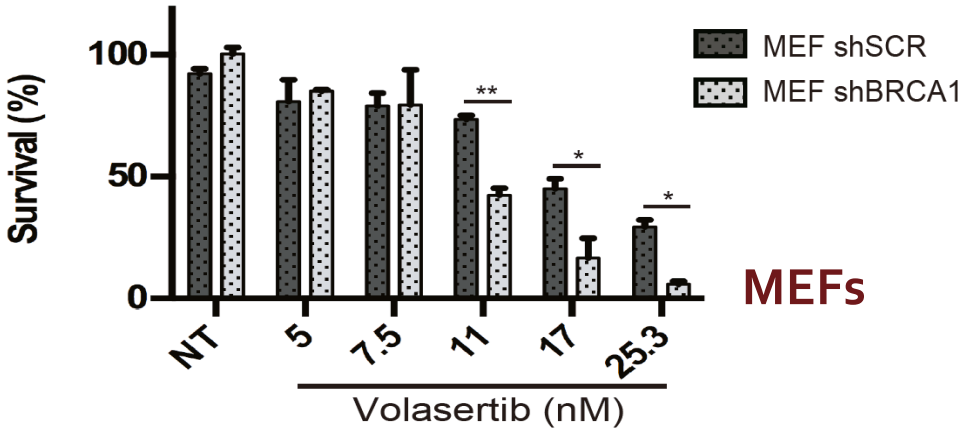
Ovarian Cancer

NON-ISOGENIC VALIDATION MODEL



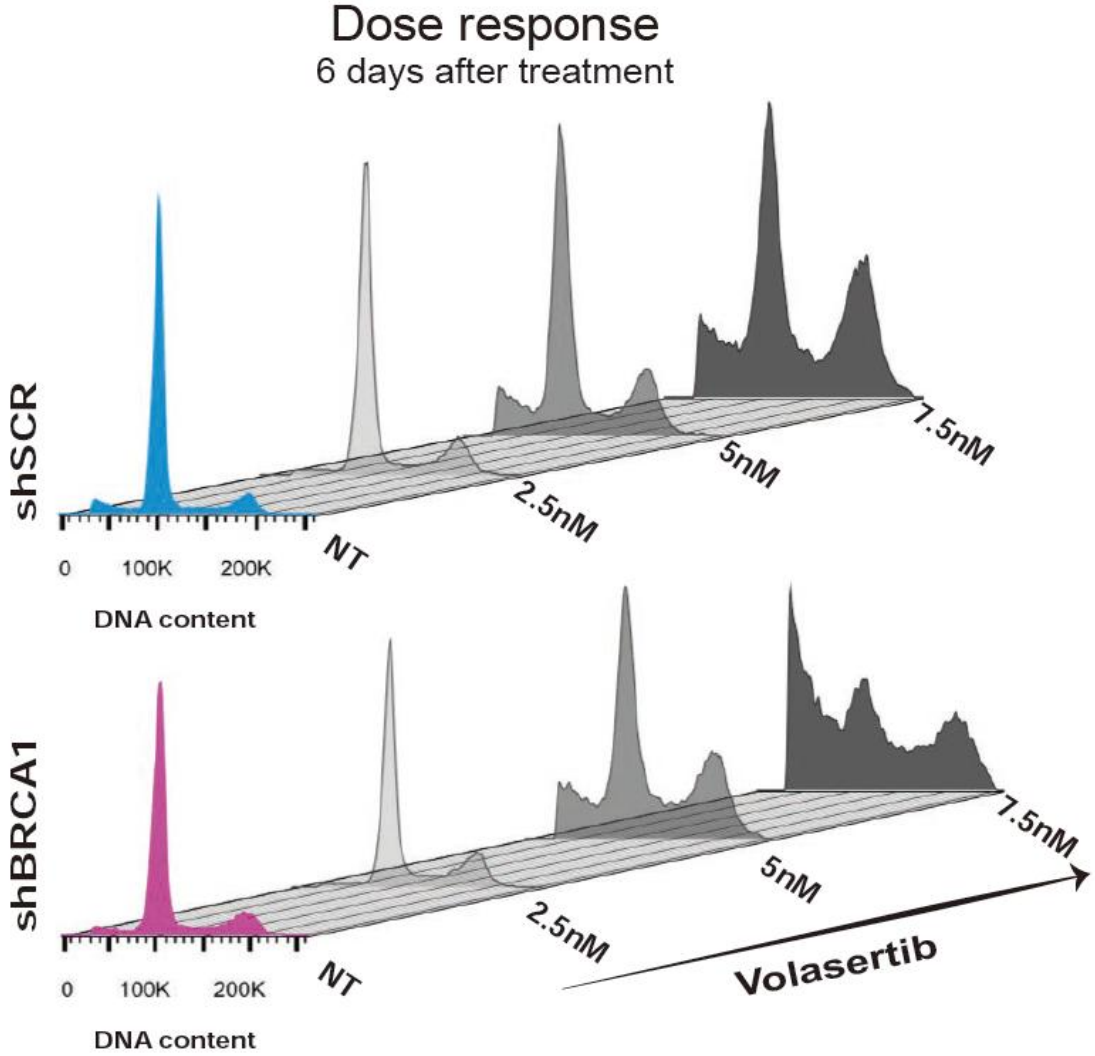
Breast Cancer

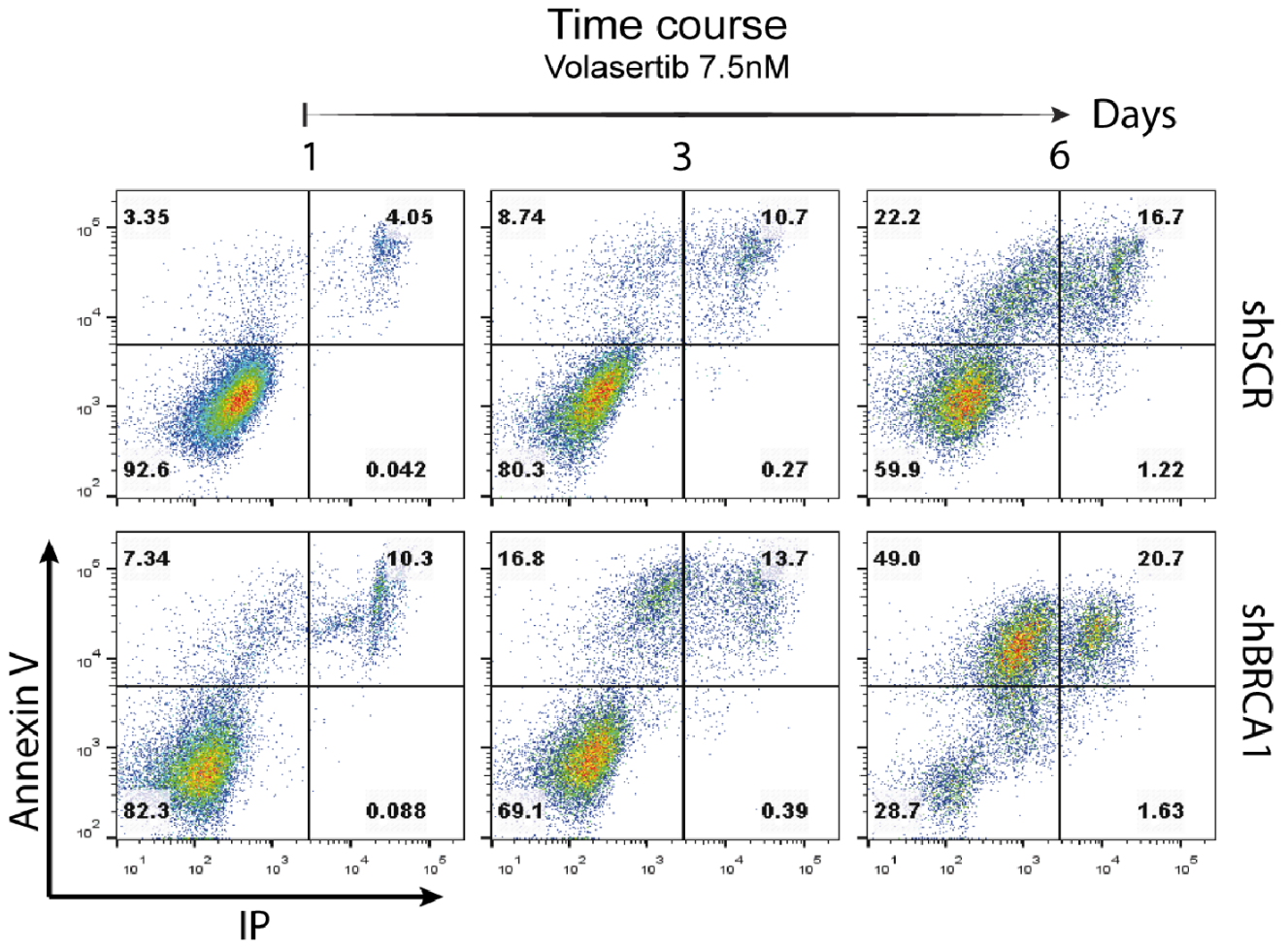
NON-TUMORAL ISOGENIC MODEL



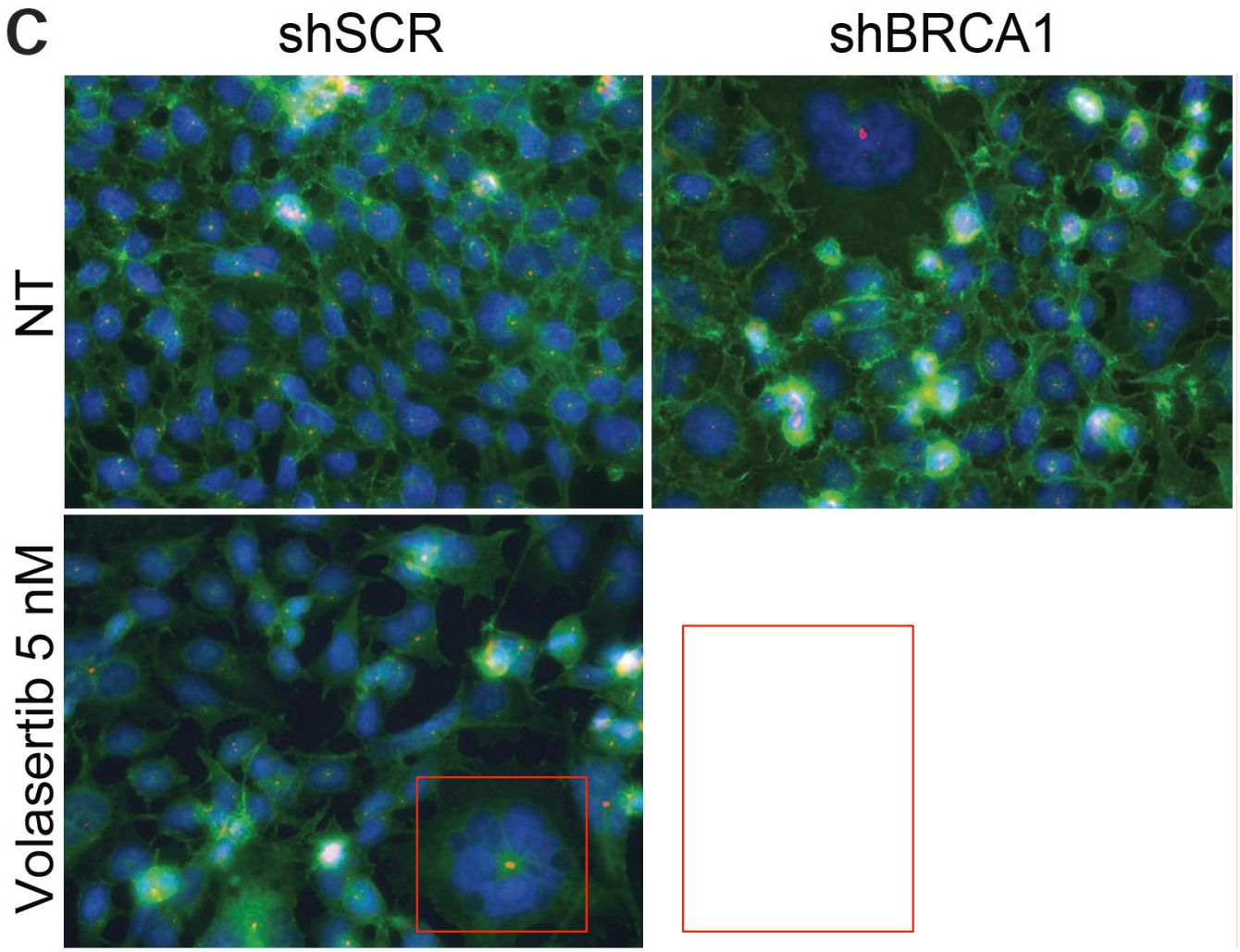
MEFs

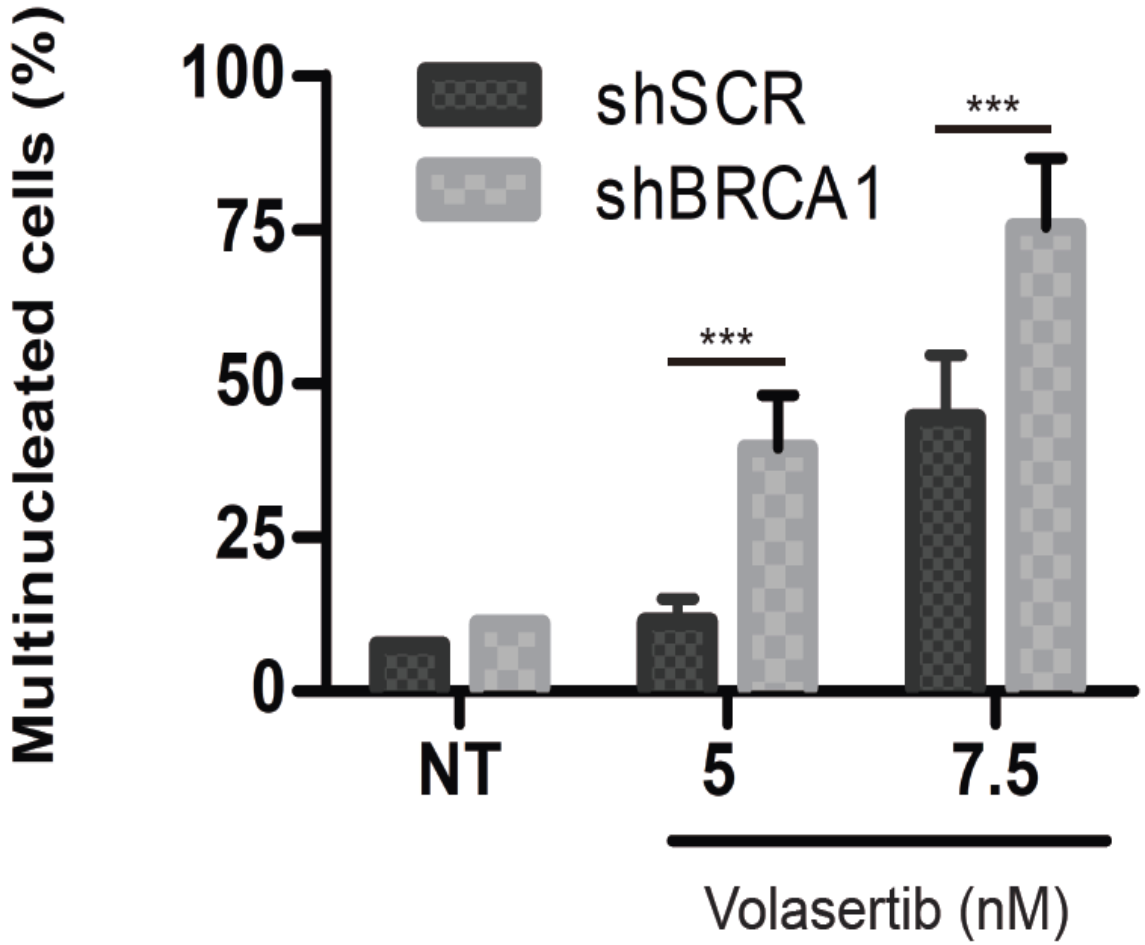
BRCA1-deficient cells **impaired recovery** from M-phase arrest induces apoptosis



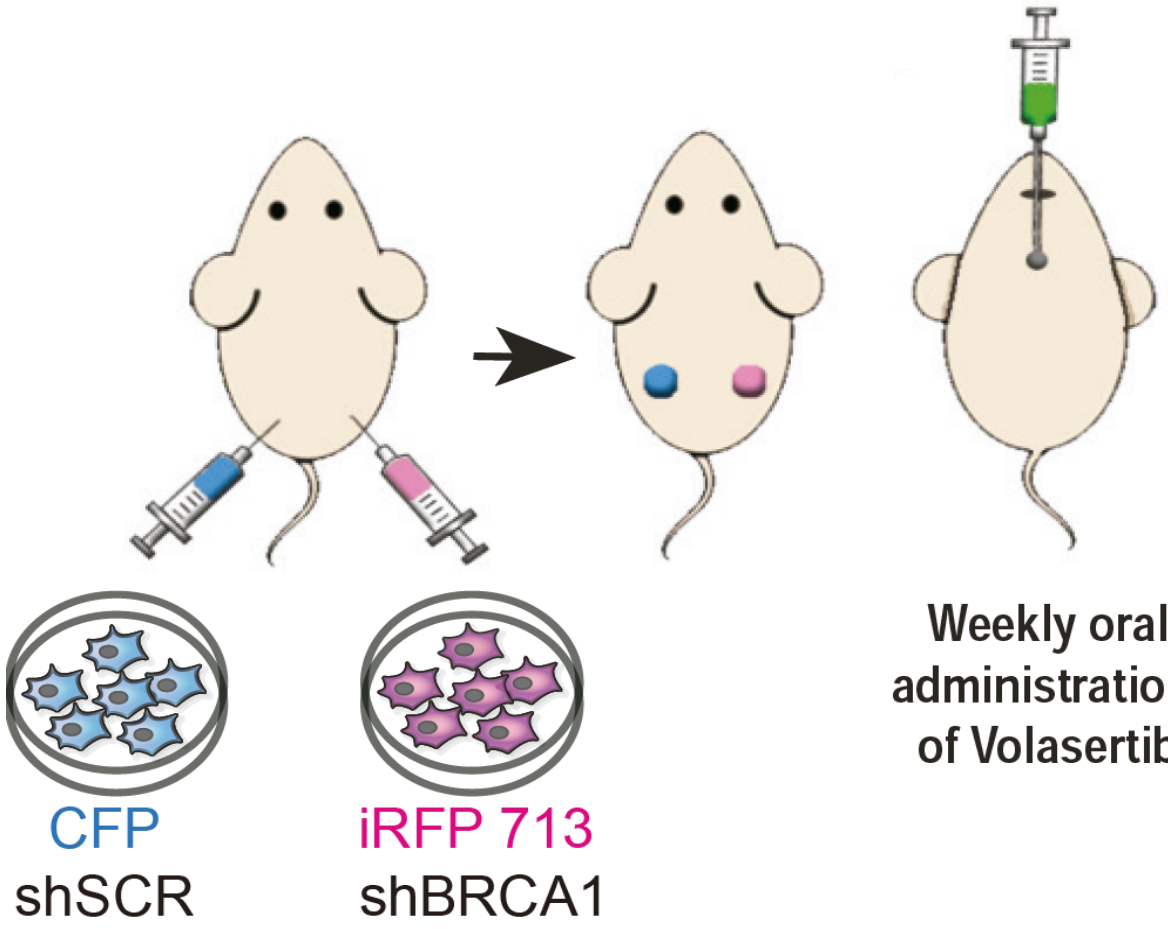


BRCA1 deficiency and PLK1 inhibition trigger alterations of **centrosomal duplication** and **cytokinesis**

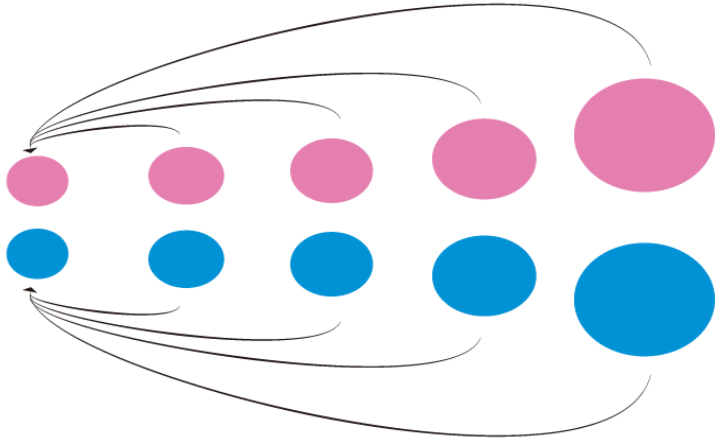




Development of a model to study **SL** induction ***in vivo***



Tumor growth normalized within the same animal

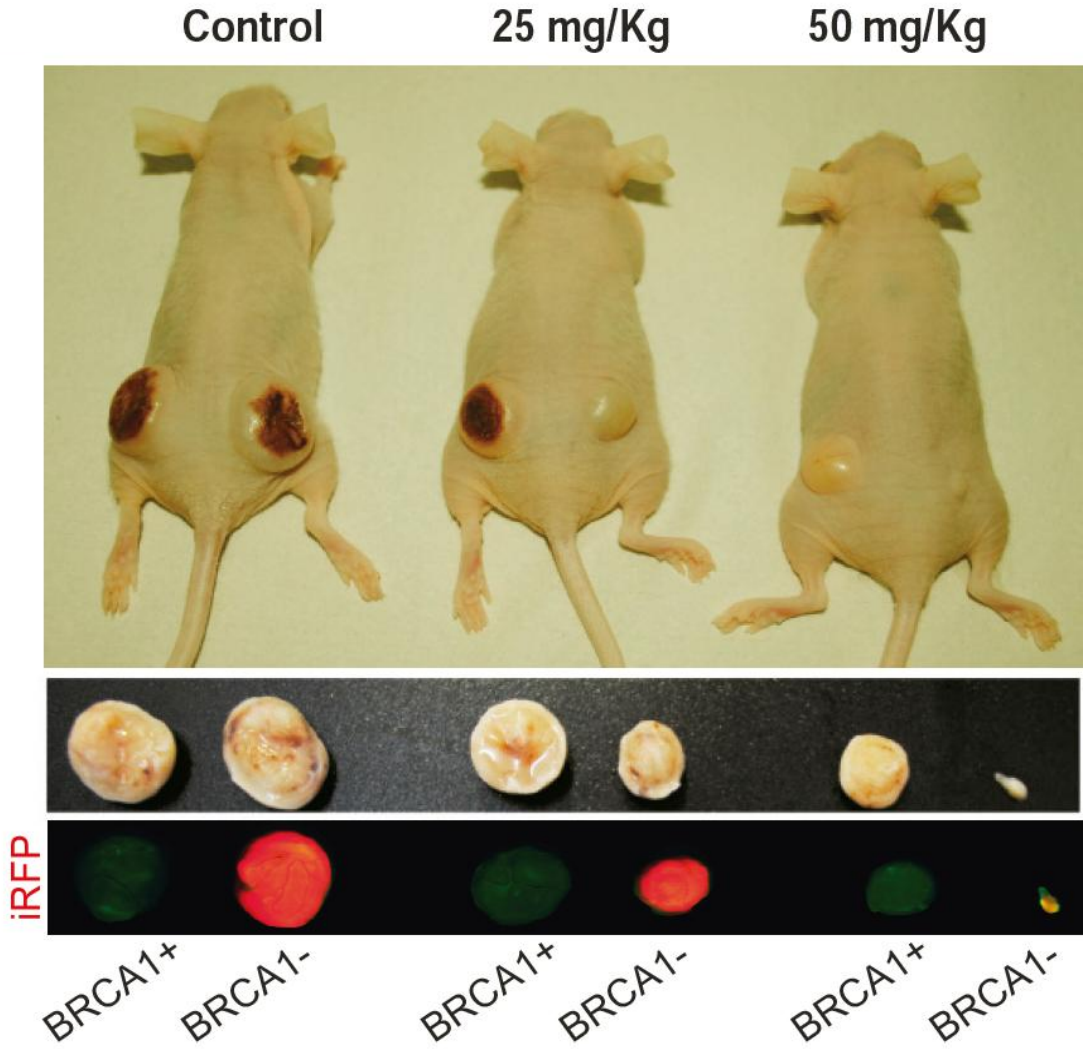


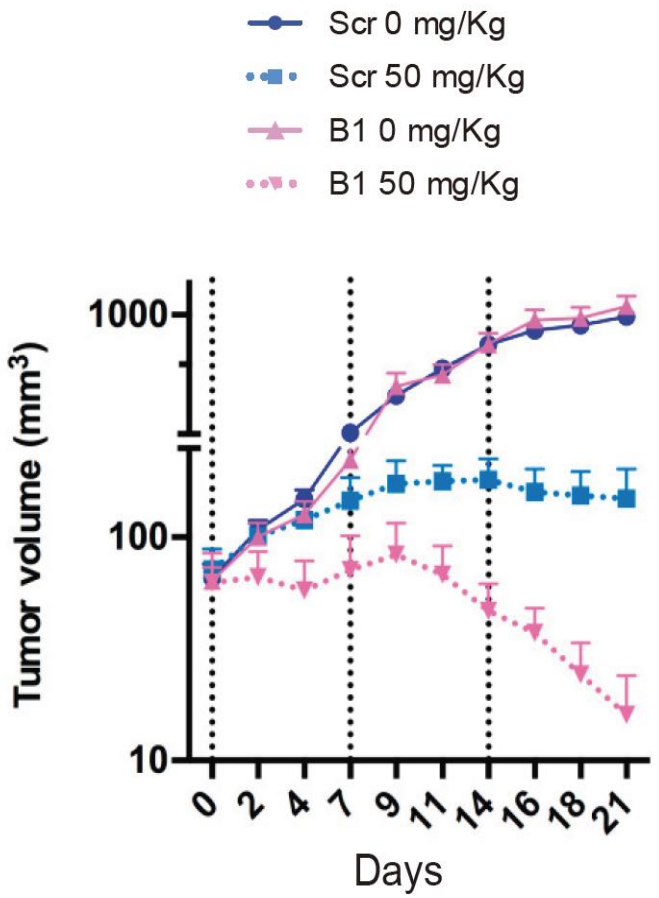
Measurable tumor (≈50 mm³) (Day 0)

End of experiment (Day 21)

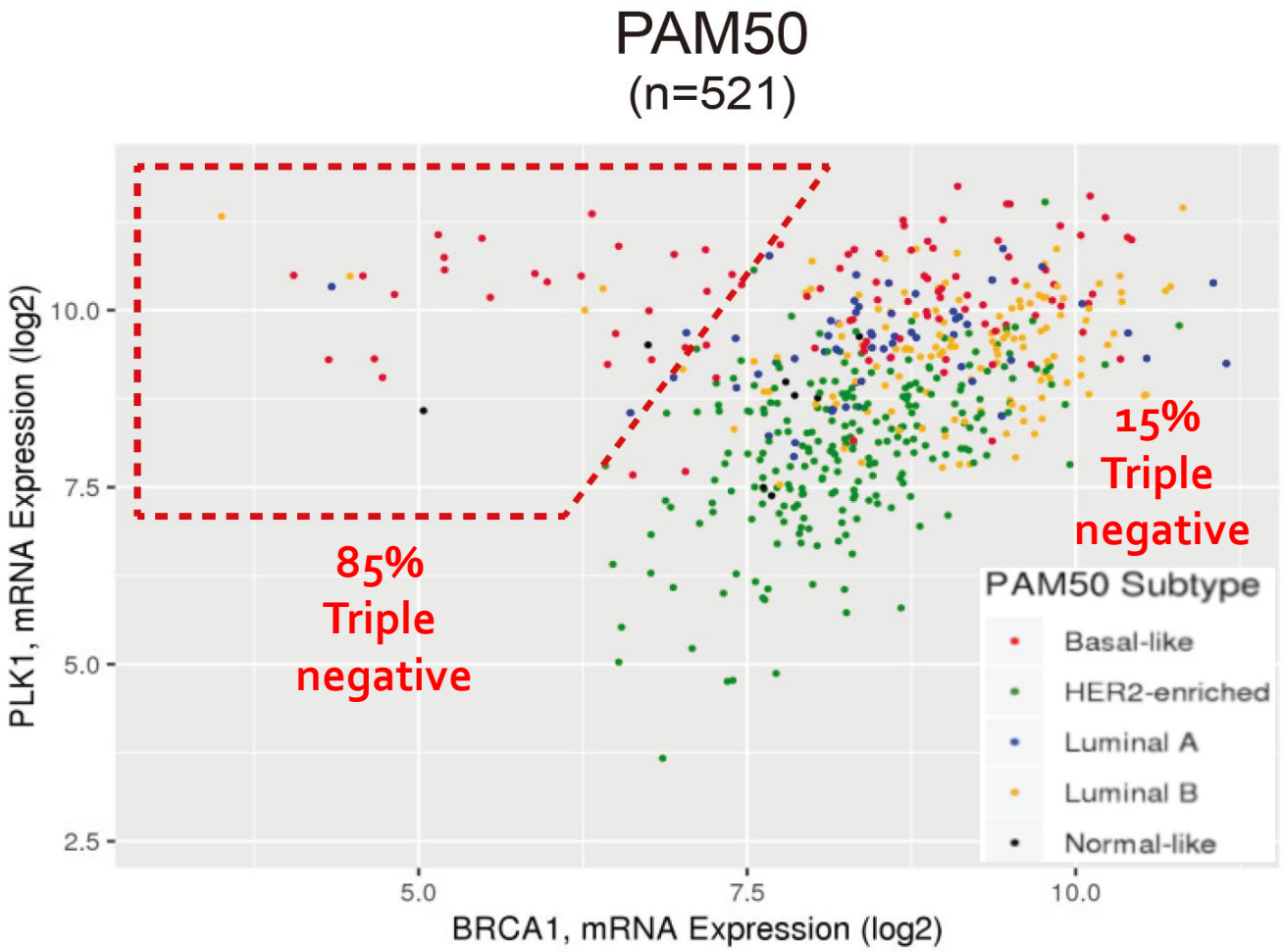
(In collaboration with Gil´s Lab)

Validation of Volasertib SL-inducing activity using ***dual tumor xenogratfs***

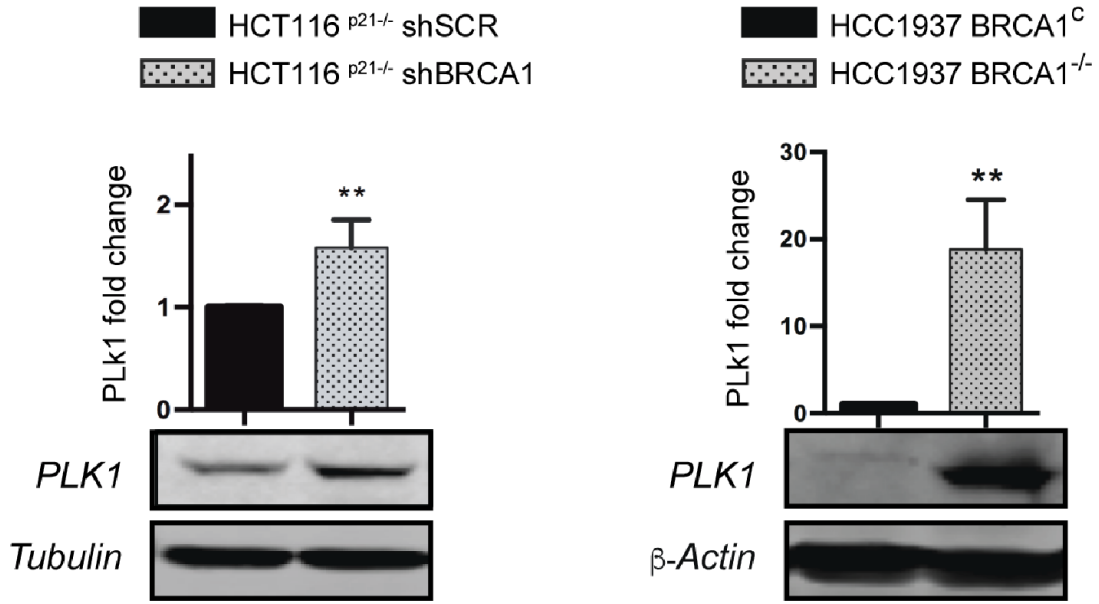




TCGA breast cancer *database validates the* ***therapeutic potential*** *of PLK1 inhibition in patients with low BRCA1 expression*



PLK1 addiction in BRCA1-deficient cells is also evident *in vitro*



Carbajosa, Pansa et al, Clinical Cancer Research, In press 2019

(In collaboration with Fernandez´s Lab)

GRACIAS!

***S**ynthetic **L**ethal **T**eam*

Virginia Agniolini, Laura Guantay, ***Sofía Cabajosa, Maria Florencia Pansa***

Florencia Villafañez, Alejandra García, Candelaria Llorens

CONICET

AGENCIA

gsk GlaxoSmithKline

INC INSTITUTO NACIONAL del CÁNCER

5 - 8 November 2019

SALTA-ARGENTINA

CONVENTION CENTER

1ST WORKSHOP ON DRUG DISCOVERY

INFORMATION DD

5 de Noviembre de 9 a 16

WORKSHOPS REGISTRATION FEE U$D 20

ASSISTANTS TO SAIB-PABMB MEETING ARE FREE OF CHARGE

BUENOS AIRES
BREAST CANCER
SYMPOSIUM

BA BCS 2020

Buenos Aires Breast Cancer Symposium 2020

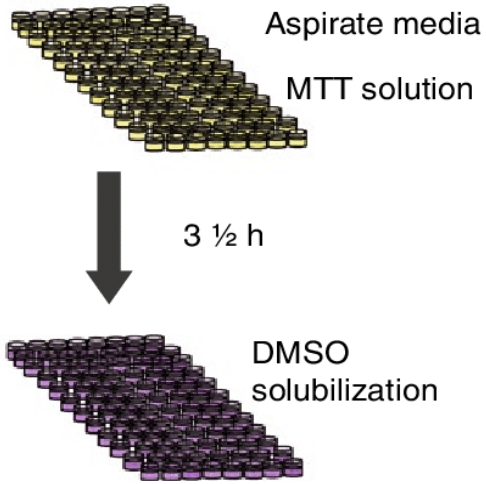
Buenos Aires Breast Cancer Symposium / BA-BCS2020

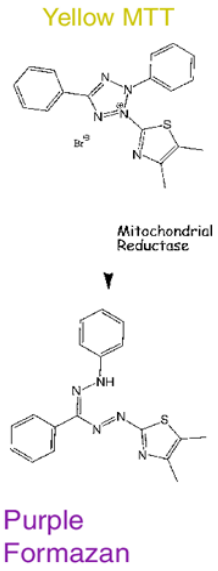
La Usina del Arte, Buenos Aires, Argentina
May 18 – 21, 2020

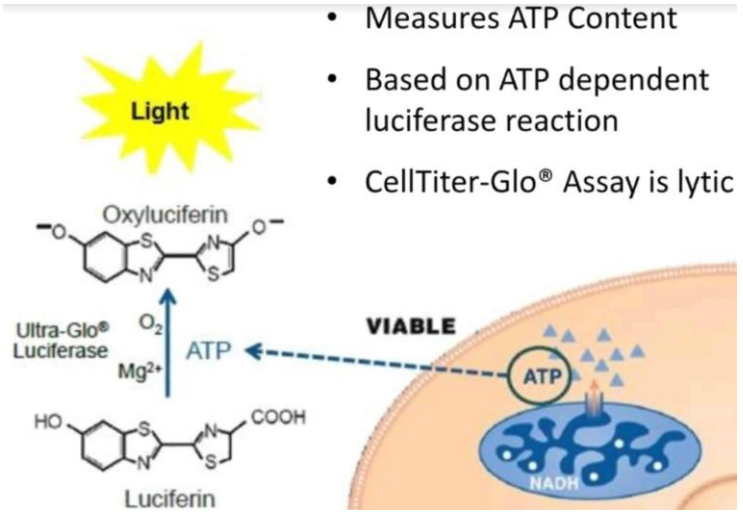
The main goal of this nonprofit meeting is to expose young Latin American basic investigators and oncologists to the state of the art research and management in breast cancer, as presented by paradigmatic leaders in the field.

*Different readouts for **cell survival:** high-throughput and population info*

Metabolic



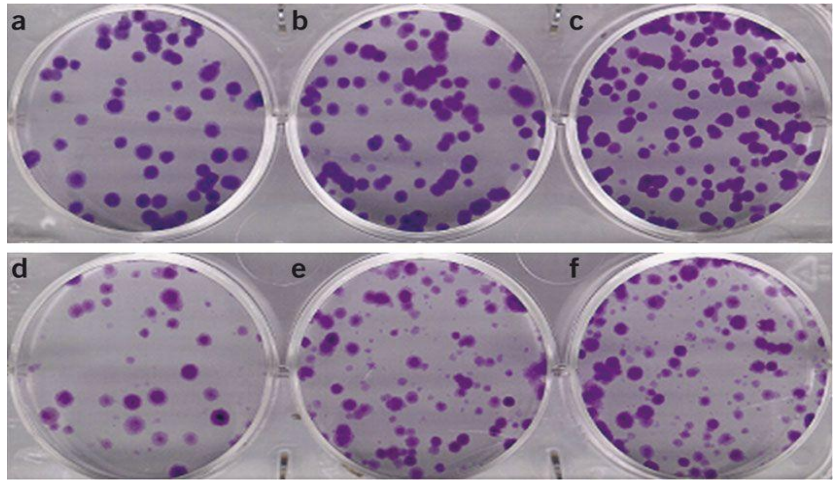




ViewLux Plate reader tower

Protein conc : cell number?

Sulforhodamine B (SRB)



Clonogenic assay – Cristal violet

Critical variables *that a phenotypic survival assay should have to screen for* ***synthetic lethal relationships***

1) Sufficient experiment length.

2) Isogeneity.

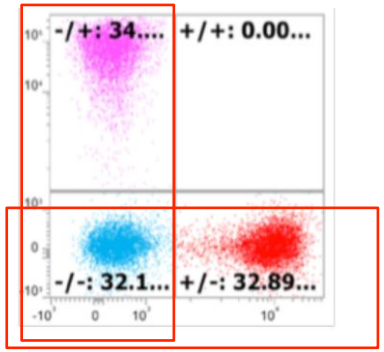
3) High sensitivity and comparability.

4) Heterogeneity.

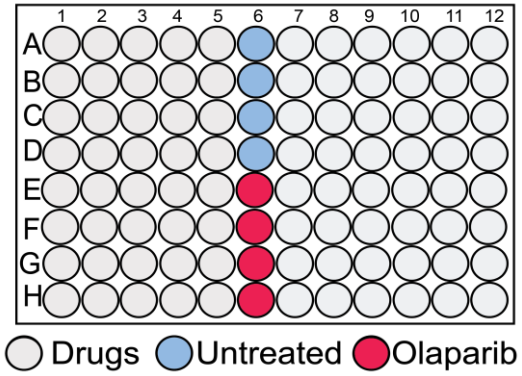
Development of a **phenotypic screening** platform using high-throughput **flow cytometry**

CRITICAL SURVIVAL INFORMATION TO DEFINE A SUCCESFULL INDUCTION OF SYNTHETIC LETHALITY

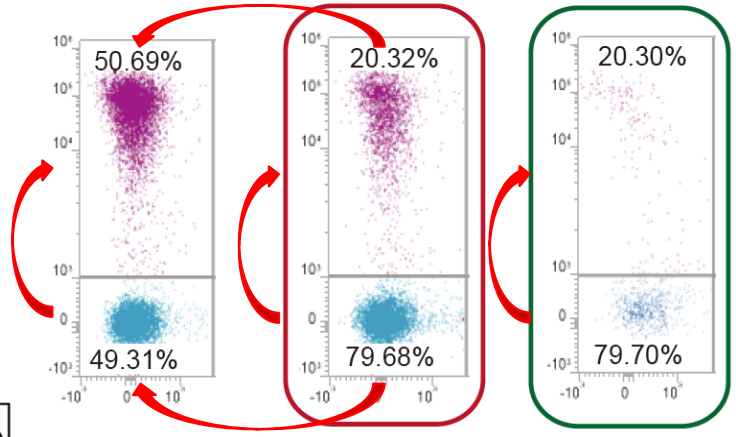
Gate BRCA1



Gate BRCA2



88 drugs screened / plate

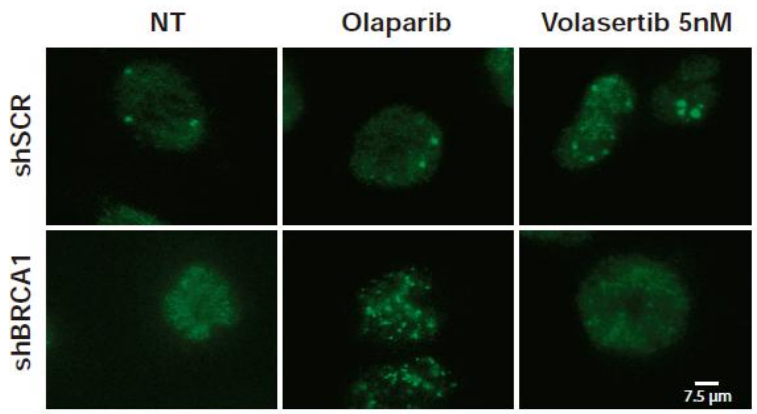


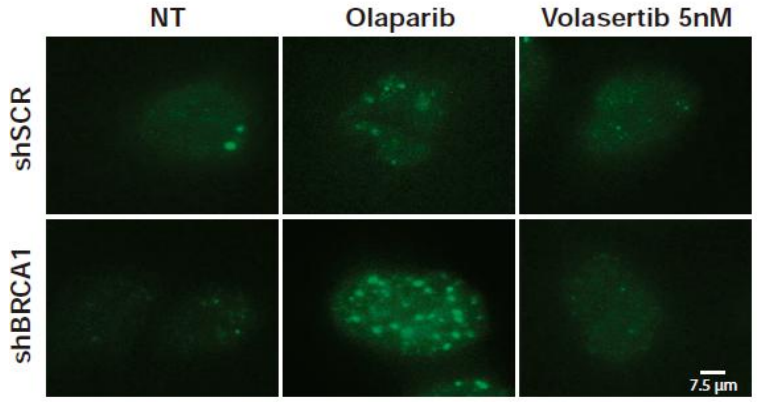
NT	Type 1 Compound	Type 2 Compound	
0	3	3	**Fold of SL** (Intra-well)
100	22	2	Survival shBRCA1 (%)
100	90	6	Survival shSCR (%)
0	68	4	**Survival difference (%)** (intra-plate)

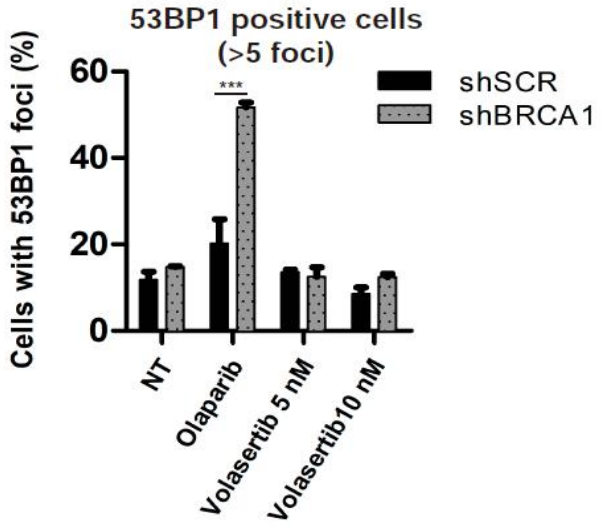
INDUCTION OF SL IN BRCA1-DEFICIENT CELLS BY PLK1 INHIBITION

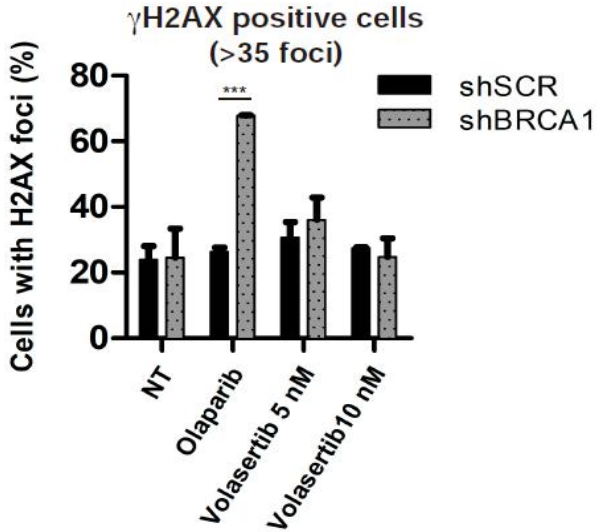
HOW DOES IT WORKS?

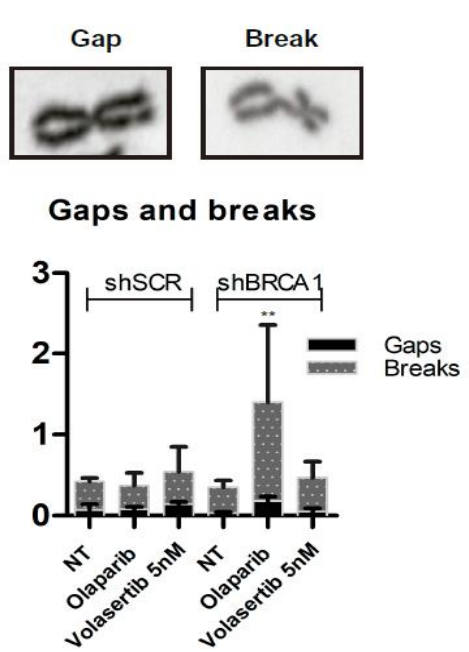
PLK1 inhibition does not trigger **genomic instability** at SL doses

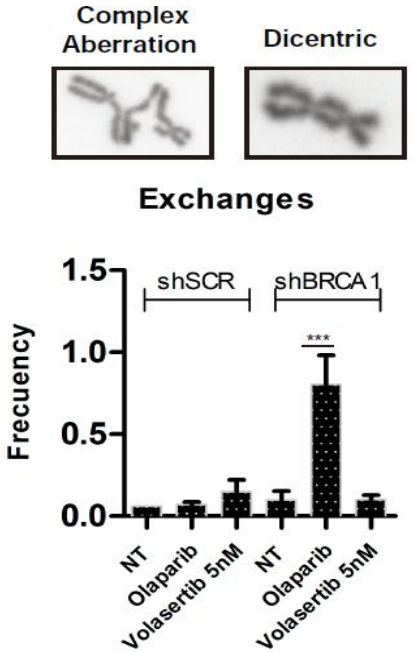


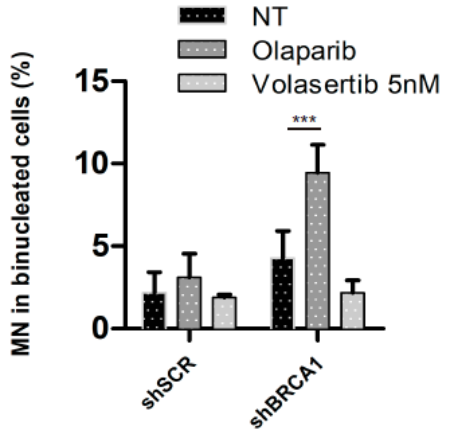


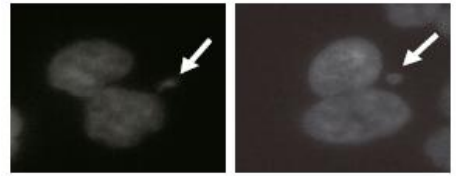












(Gottiftredi´s Lab)

Chemoproteomic profiling *for Target-ID*

cellzome
A GSK Company