Target-ID by Synthetic Lethality Induction for Precision Oncology of BRCA-deficient Cancers
The Evolution of Cancer Therapeutics

ONE-FITS-ALL APPROACH

Molecular Profiling

1. Molecular Profiling

2. Prognostic Markers
   - Markers predictive of drug sensitivity/resistance
   - Markers predictive of adverse events

PRECISION ONCOLOGY
(STRATIFICATION – PERSONALIZED MEDICINE)
Functional assays based on the concept of *Synthetic Lethality (SL)*

Yeast and Drosophila historical studies

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

A successful SL therapeutic approach should provide:

• Selective cytotoxicity
• Attenuated adverse effects
Proof of concept of SL as a therapeutic strategy

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

Hannah Farmer1,2, Nuala McCabe3,4, Christopher J. Lord1,2, Andrew N. J. Tutt1,3, Damian A. Johnson2, Tobias B. Richardson2, Manuela Santarosa1, Krystyna J. Dillon1, Ian Hickson1, Charlotte Knights1, Niall M. B. Martin1, Stephen P. Jackson1,5, Graeme C. M. Smith1 & Alan Ashworth1

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. Bryant1, Niklas Schultz2, Huw D. Thomas3, Kayan M. Parker1, Dan Flower1, Elena Lopez1, Suzanne Kyle1, Mark Meuth1, Nicola J. Curlin1 & Thomas Helleday1,5

230 clinical trials – 4 PARPi approved 14 years after its preclinical discovery
WHICH ARE THE **MAIN GOALS** OF OUR GROUP?

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

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

*Synthetic Lethality in Cancer Lab*
Screening assays and platforms developed in our Lab

- Miniaturized Western Blot
- High Content Imaging
- Virtual Screening

Translesion DNA Synthesis (TLS) Inhibitors

In Cell Western

- Mutant p53 downregulation (Girardini)

Automated Flow cytometry

- 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*

**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

**BRCAness** is much more widespread in human cancers than anticipated

![Pie charts showing BRCAness in different cancers](chart.png)

- **Breast Cancer**: 22% BRCAness
- **Ovarian Cancer**: 63% BRCAness
- **Pancreatic Cancer**: 11% BRCAness
- **mProstate Cancer**: 19% BRCAness

*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

**shRNAs lentiviral transduction**

1st stable cell line
- Parental cell line (HCT116 (B21-1))
  - CFP
  - iRFP 713
  - mcherry

2nd stable cell line
- 4 rounds of cell sorting
  - CFP
  - mCherry
  - IRFP

≥ 95% purity

Robotic Arm for cell seeding and high-throughput processing

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

<table>
<thead>
<tr>
<th>DISCARDED</th>
<th>SYNTHEtic LETHAL</th>
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<tbody>
<tr>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
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**Non-toxic**
- shBRCA1: 31,539%
- shSCR: 32,384%
- shBRCA2: 37,470%
- shBRCA1 + shBRCA2: 34,503%

**Toxic treatment (non selective)**
- shBRCA1: 27,825%
- shSCR: 35,123%
- shBRCA2: 46,112%
- shBRCA1 + shBRCA2: 45,656%

**BRCA1 single hit**
- 7,228%

**BRCA2 single hit**
- 9,826%

**Double hit**
- 51%

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

**Convergent** functions of BRCA1 and BRCA2
- Mechanisms 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.

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

4) An open source collection of 688 Kinase inhibitors.
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

<table>
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<td>GSK978744A</td>
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PLK1 inhibition triggers **strong SL** in BRCA1-deficient cells.
PLK1 inhibition triggers strong SL in BRCA1-deficient cells

A PLK1 inhibitor in Phase III clinical trials from Boehringer Ingelheim

![Chemical structure of PLK1 inhibitor]

![Bar graph showing survival rates for Volasertib with and without shSCR or shBRCA1]

**Survival (%)**

- **nM:** NT, 2.5, 5, 7.5, 10, 12.5
- **Volasertib**
  - shSCR
  - shBRCA1

*** p-values indicate statistical significance.
Validation of SL induction in other cellular models

**ISOGENIC VALIDATION MODELS**

- **Breast Cancer**
  - HCC1937
  - BRCA1<sup>−/−</sup>
  - HCC1937
  - BRCA1<sup>−/−</sup>

- **Ovarian Cancer**
  - SKOV-3
  - shSCR
  - SKOV-3
  - shBRCA1

**NON-ISOGENIC VALIDATION MODEL**

- **Breast Cancer**
  - MDA-MB-231 (BRCA1 WT)
  - MDA-MB-436 (BRCA1 KO)

**NON-TUMORAL ISOGENIC MODEL**

- **MEFs**
  - MEF shSCR
  - MEF shBRCA1

**Volasertib (nM)**
- **Survival (%)**
  - NT
  - 1
  - 10
  - 15
  - 20
  - 30

- **Survival (%)**
  - NT
  - 3.3
  - 5
  - 7.5
  - 11
  - 17
  - 25.3
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 \textit{in vivo}

![Diagram showing the process of developing a model to study SL induction in vivo.](Image)

- **CFP shSCR**
- **iRFP 713 shBRCA1**
- **Weekly oral administration of Volasertib**
- **Tumor growth normalized within the same animal**
- **Measurable tumor \((\approx 50 \text{ mm}^3)\) (Day 0)**
- **End of experiment (Day 21)**

(In collaboration with Gil’s Lab)
Validation of Volasertib SL-inducing activity using *dual tumor xenografts*
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

85% Triple negative
15% Triple negative

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

Synthetic Lethal Team
Virginia Agniolini, Laura Guantay, Sofía Cabajosa, María Florencia Pansa
Florencia Villafañez, Alejandra García, Candelaria Llorens
5 - 8 November 2019

SALTA-ARGENTINA
CONVENTION CENTER

1ST WORKSHOP ON DRUG DISCOVERY

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-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**

- Aspirate media
- MTT solution
- Yellow MTT
- DMSO solubilization
- Absorbance 570 nm
- Purple Formazan

**Protein conc : cell number?**

- Measures ATP Content
- Based on ATP dependent luciferase reaction
- CellTiter-Glo® Assay is lytic

- ViewLux Plate reader tower

- Clonogenic assay – Cristal violet

- Sulforhodamine B (SRB)
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 SUCCESSFUL INDUCTION OF SYNTHETIC LETHALITY**

Gate BRCA1

Gate BRCA2

<table>
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<th>NT</th>
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<th>Type 2 Compound</th>
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**Fold of SL (Intra-well)**

**Survival shBRCA1 (%)**

**Survival shSCR (%)**

**Survival difference (%)**

(intra-plate)

88 drugs screened / 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