GETTHI

Grupo Español de Oncología Transversal
y Tumores Huérfanos e Infrecuentes

VII SIMPOSIO GETTHI

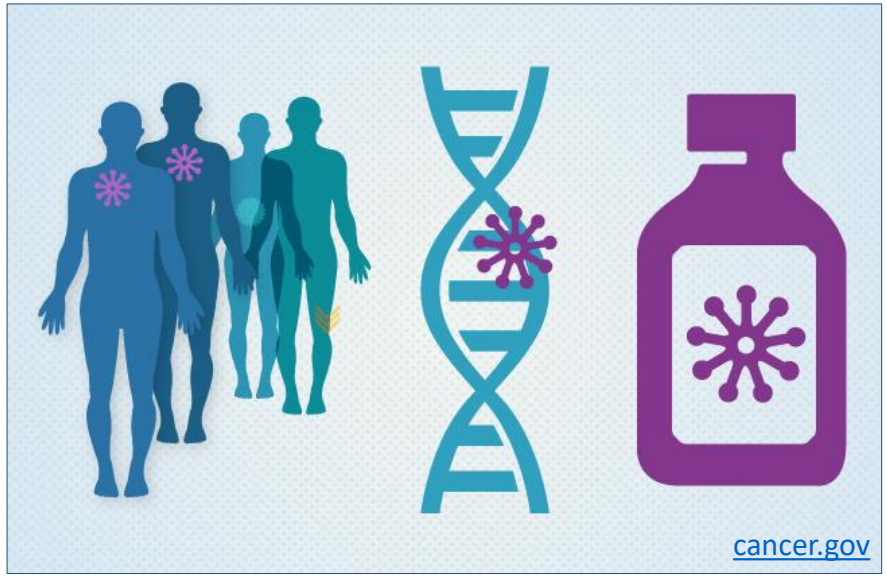
Sesión 3: Vías de desarrollo de la oncología transversal (I)

2 de diciembre de 2021 - *Formato virtual*

Letalidad sintética y la inhibición de PARP

Dr. Sergio Ruiz Llorente

Terapia personalizada

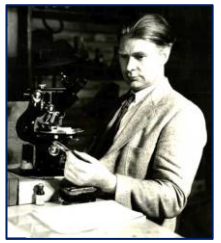


- Utilización de herramientas genómicas para la identificación de dianas terapéuticas es una **aproximación finita.**
- Específica de alteraciones de ganancia de función (GoF) → **Oncogenes** (↓ % de genes implicados en cáncer).
- **Limitaciones: mutaciones en genes supresores** que generan pérdida de función (LoF) o alteraciones "drivers" no tratables farmacologicamente.
- Células tumorales reconfiguran sus circuitos genéticos para **eliminar la dependencia genética de estas diana.**

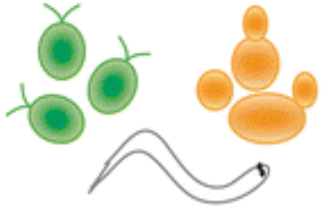
↓

Aproximaciones experimentales distintas para definir otros enfoques terapéuticos.

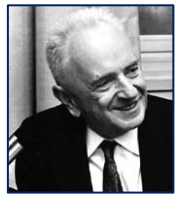
Letalidad sintética


Calvin Bridges


First description of synthetic lethality by Calvin Bridges based on observations made in fruit flies

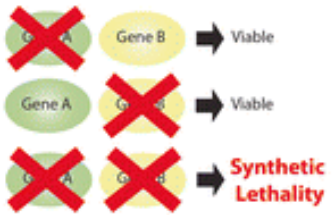

Synthetic lethal interactions studied in green algae, yeast, and *C. elegans*


Development of CRISPR-based screens


Theo Dobzhansky

1946

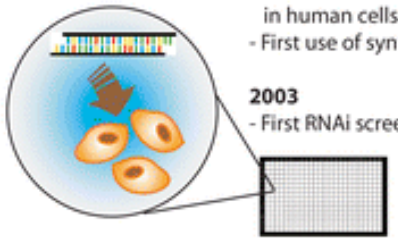
The term "synthetic lethality" was coined by Theodore Dobzhansky



2000s

2001
- First chemical synthetic lethal screen in human cells
- First use of synthetic siRNA

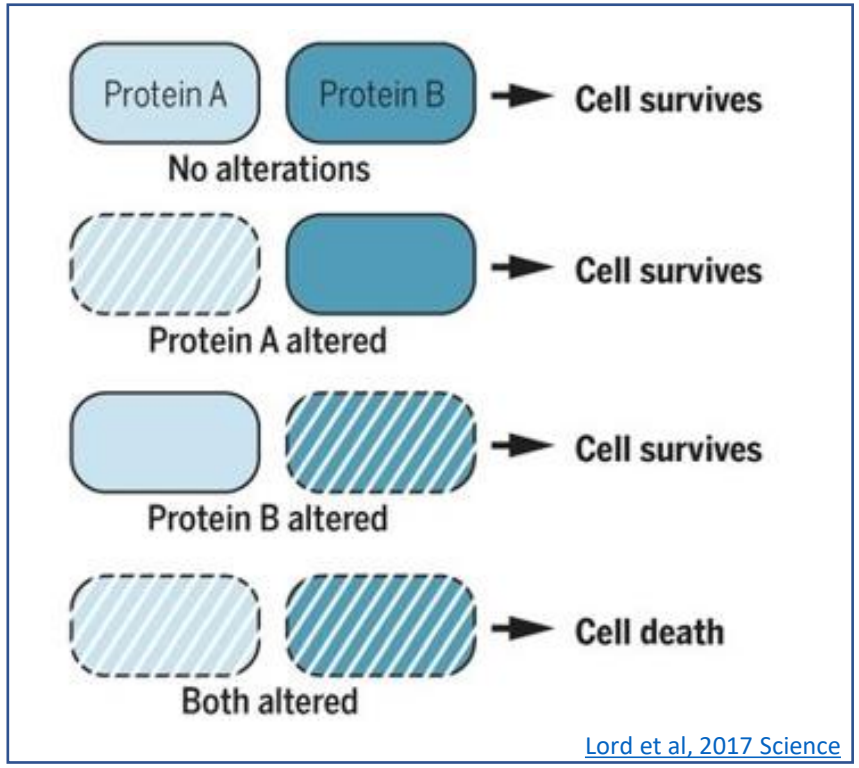
2003
- First RNAi screen in human cells



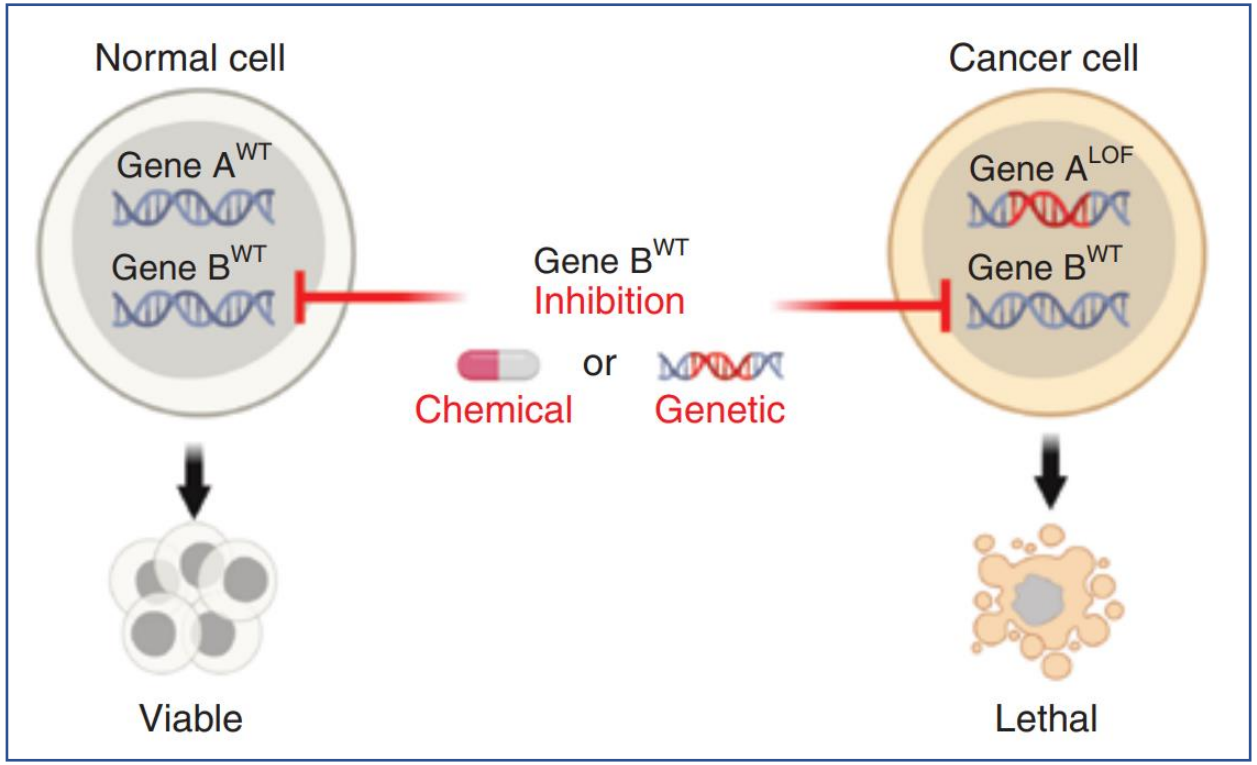
Leung et al, 2016

Letalidad sintética

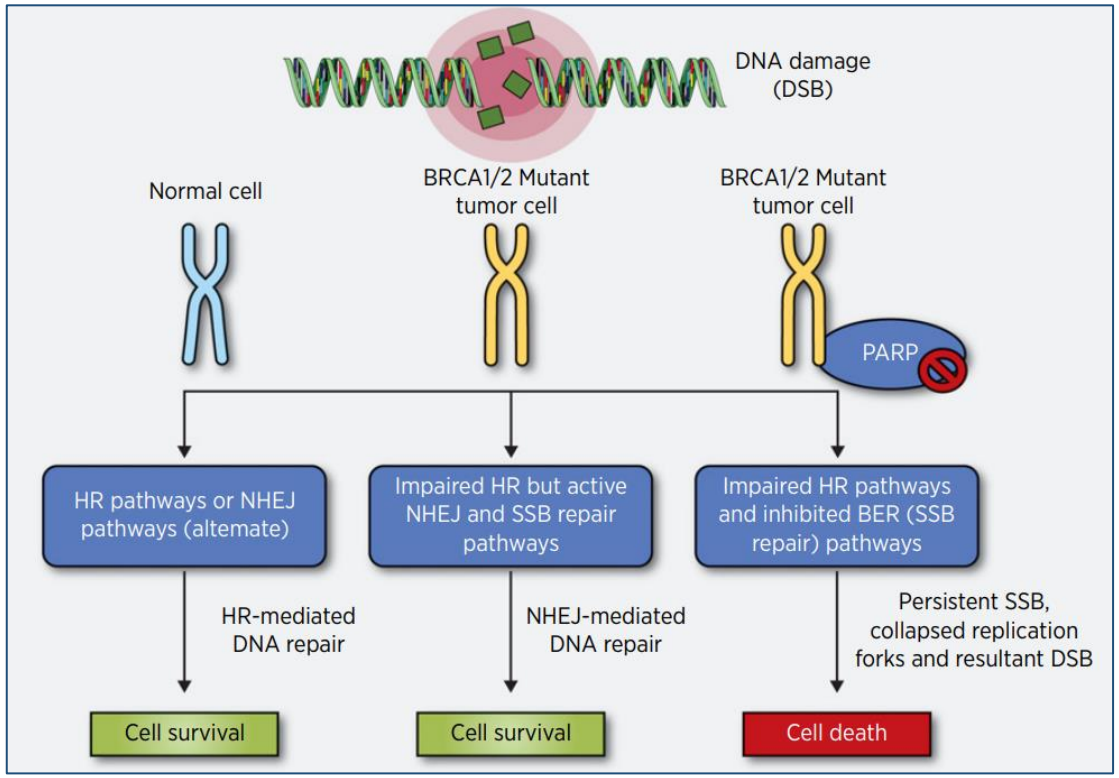
"Phenomenon in which non-lethal genetic mutations are innocous when they occur individually, but which result in lethality to a cell in combination" (Dobzhansky T, 1946. Genetics)

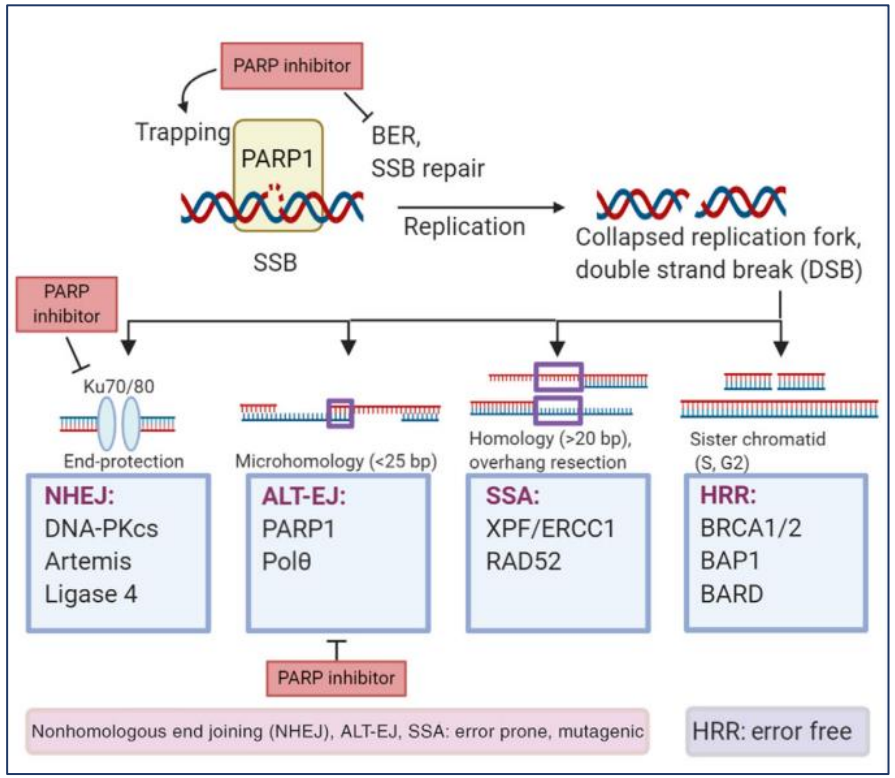


Lord et al, 2017 Science



PARPi en tumores defectuosos para “DNA damage repair”

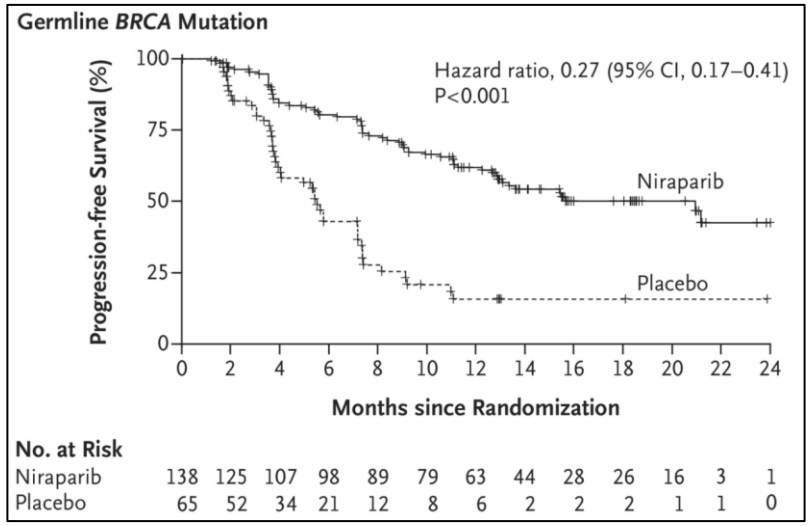




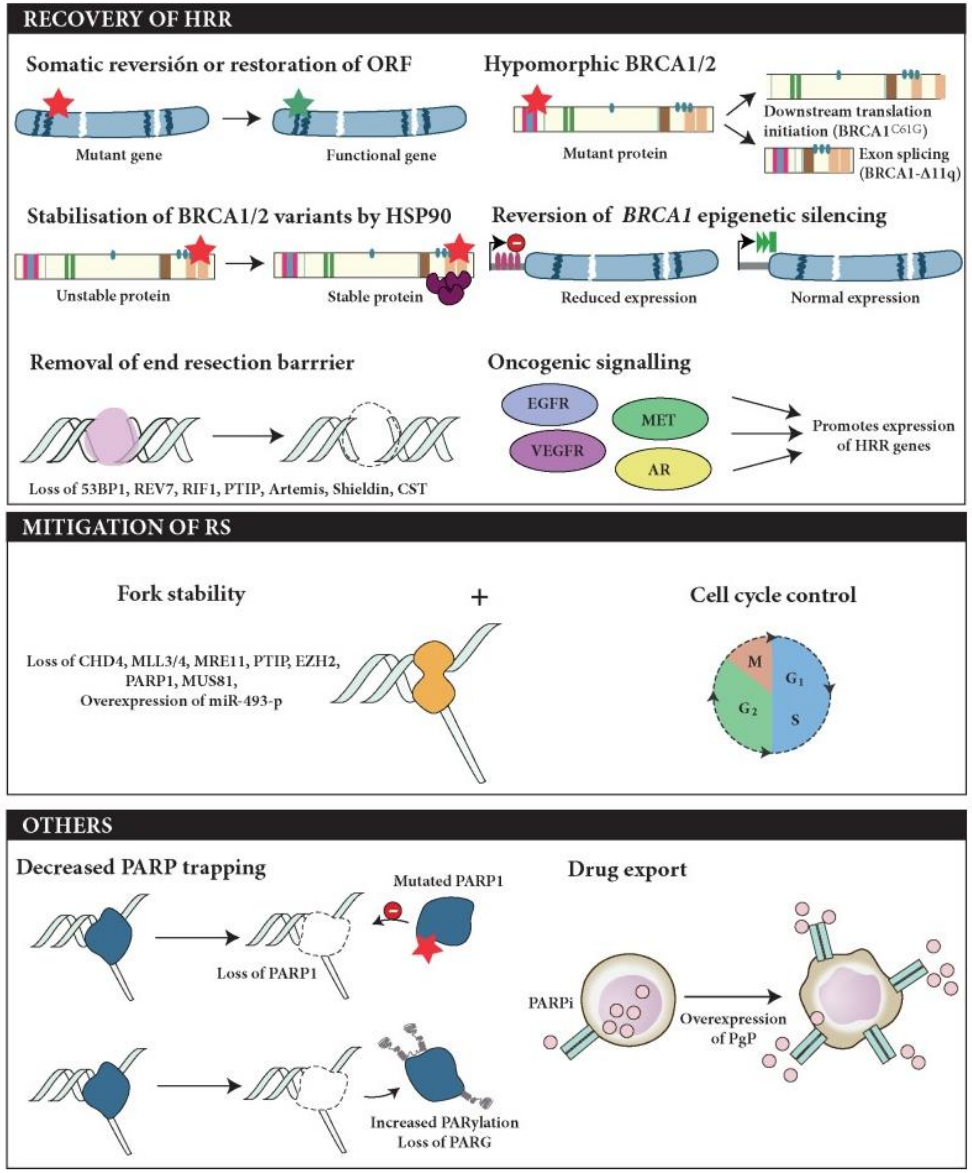
- Inhibición de la actividad catalítica (rutas BER, NHEJ y ALT-EJ)
- Inmovilización del complejo al DNA (trapping).

Lord et al, 2017 Science
Chandrasekaran A et al, 2020 Mol Cancer Ther

Resistencias terapéuticas a PARPi



Mecanismos de resistencia



Mirza MR et al, 2016 NEJM
Mateo J et al, 2019 Ann Oncol

Carcinoma ovárico: letalidades sintéticas distintas de PARP

Target	Trial	Phase	Design
ATR	NCT02487095	I/II	Trial of topotecan with ATRi VX-970 (M6620) in OC
	NCT04616534	I	Gemcitabine combined with the BAY 1895344 ATRi with expansion cohorts in advanced OC
	NCT02627443	I	Dose escalation and expansion cohort of carboplatin and gemcitabine with or without ATRi berzosertib M6620 (VX-970) in first or second recurrence platinum-sensitive EOC
Chk1/2	NCT02203513	II	Study of the Chk1/2 inhibitor (LY2606368) In *BRCA1/2m* HGSOC
	NCT02797964	I/II	Chk1 inhibitor (SRA737) administered orally in *BRCA1/2m* advanced OC
WEE1	NCT02101775	II	Randomized trial comparing gemcitabine monotherapy to gemcitabine in combination with WEE1 inhibitor (MK-1775) in recurrent platinum-resistant EOC
PI3K	NCT04711161	I/Ib	Evaluation of the safety, pharmacokinetics and efficacy of GRN-300, a salt-inducible kinase inhibitor, alone and in combination with paclitaxel, in recurrent OC
	NCT03719326	I/Ib	Dose-escalation, and dose-expansion study to evaluate the safety, tolerability, pharmacokinetics and clinical activity of etrumadenant (AB928) in combination with pegylated liposomal doxorubicin (PLD) with or without PI3K inhibitor IPI-549 in OC
Akt	NCT04374630 PROFECTA II	II	Study to assess the efficacy and safety of Akt inhibitor afuresertib plus paclitaxel vs. paclitaxel in platinum-resistant OC
p53	NCT03113487	II	Modified vaccinia virus ankara vaccine expressing p53 and pembrolizumab in recurrent OC
	NCT02272790	II	Study of adavosertib plus chemotherapy in p53 mutated platinum-resistant OC
	NCT04489706	N/A	Trial to evaluate the efficacy, safety, and tolerability of arsenic trioxide in recurrent metastatic OC with P53 mutation
ARID1A	NCT04493619	I/II	Study of BET inhibitor PLX2853 monotherapy in ARID1A-mutated advanced gynecologic malignancies and study of PLX2853/carboplatin combination in platinum-resistant EOC

Abbreviations: bid, twice daily; HRD, homologous recombination deficiency; IP, intraperitoneal; IV, intravenous; OC, ovarian cancer; po, orally; qd, once daily.

Chandrasekaran A et al, 2020 Mol Cancer Ther

Optimización de aproximaciones terapéuticas basadas en LS.

Evaluación prospectiva de inactivación bialélica
(*BRCA1/2, RB1, MTAP*)
LOH frecuente en largos respondedores

Significado biológico varía en función del linaje del tumor y del contexto genético
Neutralidad vs. Patogenicidad

Iniciación ⟺ **Mantenimiento**
BRCA1/2 — *KRAS, MYC, ABL* (Oncog) / *TP53, APC, ATM** (TSGs)

Setton J et al, 2021 Cancer Discov

Aproximaciones para la identificación de letalidad sintética

Table 1. Summary of common synthetic lethal screening approaches and their advantages and limitations

Synthetic lethal screening method	Advantage	Disadvantage
Yeast screens	• Simple genome and easy genetic manipulation	• Inability to reflect the complexity of the mammalian/cancer genome
Drug screens	• Easily translated to clinical practice	• Variable drug specificity • Drug target sometimes unknown • Limited to 'druggable' genes
RNAi screens	• May be transcript specific • Ability to target any gene within the genome • Possibility of being performed in vivo	• Difficult to achieve complete gene knockdown • Potential toxicity of siRNA knockdown • Less specific than CRISPR (off-targets)
CRISPR screens	• Possibility of achieving complete genetic knockout • Ability to target both transcribed and untranscribed regions • Possibility of being performed in vivo	• Off-target effects • Possibility of poor guide efficiency at inducing knockout • Failure of gene knockout to recapitulate drug inhibition of the target
Bioinformatic approaches	• Able to utilize data from a wide range of sources, both from experiments and sequencing data	• Generates long lists of potential SL pairs requiring extensive experimental validation

Thompson N et al, 2017

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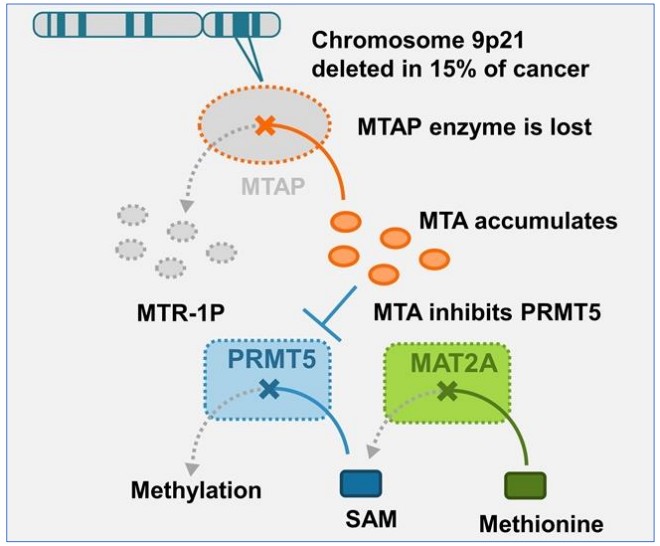
Sesión 3: Vías de desarrollo de la oncología transversal (I)

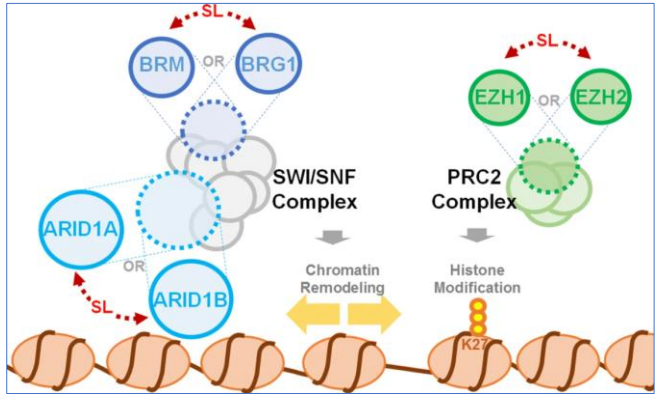
Gene	Chromosome	Cellular process and mechanism	In vitro	In vivo	Cancer type	Reference
ARID1A	1p36.11	Target SWI/SNF complexes, which regulate chromatin remodeling. SWI/SNF complexes are involved in controlling the cell cycle, DNA replication, and repairing DNA damage.	H1299, H2023, H2030	Smarca4-deficient genetically engineered mouse	Lung cancer	[34]
ATM	11q22.3	Activates cell cycle checkpoints; recognizes damaged DNA and triggers ATM-mediated DNA damage response pathway to repair damaged DNA strands.	KC (850, 6059, 8878), AKC (995, 5615, 5980, 5982)	AKC, KC, and SCID mouse	Pancreatic cancer	[35]
ATR	3q23	Cell cycle checkpoint signaling activation upon DNA stress and triggers ATR-mediated DNA damage sensing.	Human-derived CLL and Mec1 cell line	Primary CLL xenograft mouse	Leukemia	[36, 37]
BRCA1	17q21.31	Repair DNA double-strand breaks via ubiquitination, transcriptional regulation, and homologous recombination.	A2780, HEK293, SUM149PT	N/A	Ovarian cancer	[38]
BRCA2	13q13.1	Repair DNA double-strand breaks via ubiquitination, transcriptional regulation, and homologous recombination.	PL2F7, Y3308Y	BRCA-deficient mouse	N/A	[39]
CDC6	17q21.2	Initiation of DNA replication; regulates cell cycle.	HCT-116, HKE-3	KRAS-induced lung cancer mouse	Lung cancer	[40, 41]
CDK1	10q21.2	Regulate cell cycle (G1/S and G2/M phase transitions).	LIM1215, SW48	KRAS-mutated mouse	N/A	[42]
CDK2	12q13.2	Regulate cell cycle (G1/S phase transition).	HACAT	N/A	N/A	[43, 44]
CDK17	12q23.1	Serine-threonine protein kinase; regulate G2/M phase transition.	HeLa, K562, MCF10A, MDA-MB-231, RPE1	N/A	Breast cancer	[45]
CHEK1	11q24.2	Serine-threonine protein kinase; triggers cell cycle arrest in response to DNA damage; integrate signals from *ATR* and *ATM*; phosphorylation of *CDC25A* to delay cell cycle progression following DNA double-strand breaks.	PEO14, PEO23, SKOV3	SKOV3 xenograft mouse	Ovarian cancer	[46]
CHEK2	22q12.1	Serine-threonine protein kinase; triggers cell cycle arrest in response to DNA damage; integrate signals from *ATR* and *ATM*; phosphorylation of *CDC25A* to delay cell cycle progression following DNA double-strand breaks.	Cal27, HN30, HN31, SCC61, UMSCC17A	N/A	Head and neck cancer	[47]
GATA2	3q21.3	Zinc-finger transcription factor; regulate transcription genes.	A549, H226, HL7702	A549 xenograft mouse	Lung cancer	[48]
KRAS	12p12.1	Transcriptional activator that regulates endothelial cells endothelin-1 gene expression.	A549, H441	A549 xenograft mouse	Lung cancer	[49, 50]
MRE11	11q21	MRN complex component; DNA double-strand breaks repair via nonhomologous end-joining and homologous recombination activation in ATM-mediated checkpoint.	V-C8	N/A	N/A	[51]
MYC	8q24.21	Regulate cell cycle progression, transcription, and apoptosis.	Kelly, BE-2C, NLF, SK-N-AS, SHEP, MYCN-ER	BALB/c nude mouse	Neuroblastoma	[52, 53]
NBN	8q21.3	MRN complex component; DNA double-strand breaks repair via nonhomologous end-joining and homologous recombination activation in ATM-mediated checkpoint.	B220, Gr-1, Mac-1	Nbn-mutated mouse	Leukemia	[54]
PAK3	Xq23	Serine-threonine protein kinase; regulates cell cycle, cell migration, and apoptosis.	CaSki, HeLa, HFK, SiHa	N/A	Cervical cancer	[55]
PARP1	1q41.42	Regulate cell proliferation and differentiation; repair DNA single- and double-strand breaks.	DLD-1, HEK293FT, KB1P-G3, KB2P, SUM149PT, U2OS	BRCA2-mutated mouse	Breast and ovarian cancer	[56]
PLK1	16p12.2	Serine-threonine protein kinase; regulate cell proliferation and apoptosis; triggers G2/M transition.	A549, H441, H522, T29	BALB/c and C57BL/6 nude mouse	Lung cancer	[57]
RAD50	5q31.1	MRN complex component; DNA double-strand breaks repair via nonhomologous end-joining and homologous recombination activation in ATM-mediated checkpoint.	D1241, L1240, Q1262, WT	N/A	Metastatic small cell cancer	[58]
RAD51	15q15.1	Repair DNA double-strand breaks via homologous recombination.	HeLa, K562, M059, U2OS	N/A	N/A	[59]
TP53	17p13.1	Major tumor suppressor; regulate cell cycle, senescence, and apoptosis.	C4-2, LNCaP, U2OS	NSG mouse	Prostate cancer	[60, 61]
53BP1	15q15.3	Repair DNA double-strand breaks by promoting non-homologous end-joining pathways while limiting homologous recombination.	DOHH2, G452, HCC1187 OCI-LY (1, 8, 19), SUDHL-6, U2932, VAL	NOD, NSG, and SCID mouse	Lymphoma	[62, 63]
WEE1	11p15.4	Serine-threonine protein kinase; regulates G2/M checkpoint via CDC2 inhibition.	MCF7, MDA-MB-231, T-47D, Zr-75-1	Breast cancer xenograft NSG mouse	Breast cancer	[64]



Deleciones en *MTAP* (proximal a CDKN2A)

- *15%* de los tumores y 50% en glioblastomas.

Vulnerabilidades: inhibición de *PRMT5, MAT2A y RIOK1*



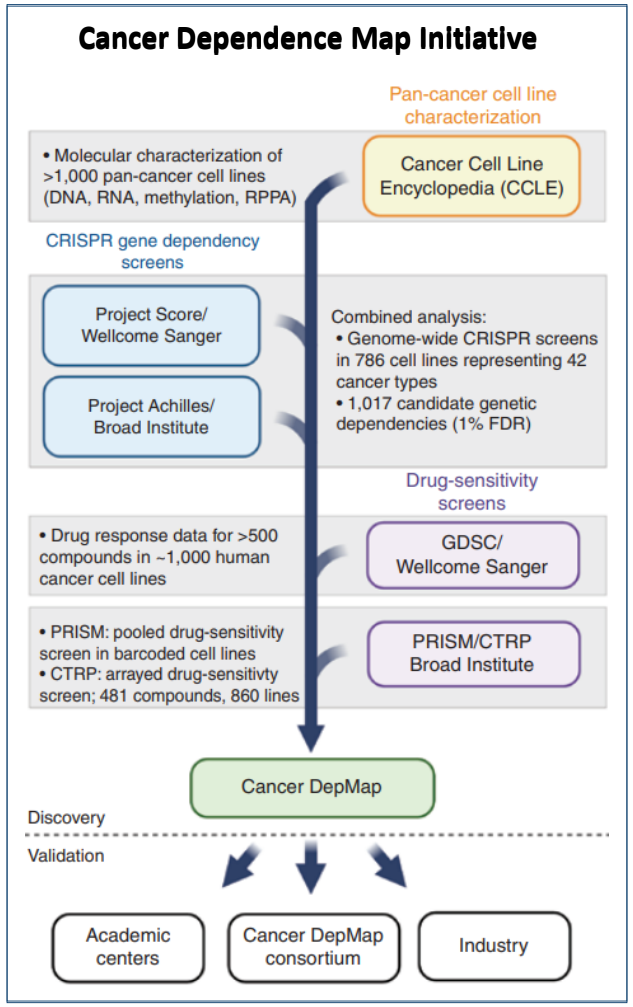
Remodeladores de cromatina

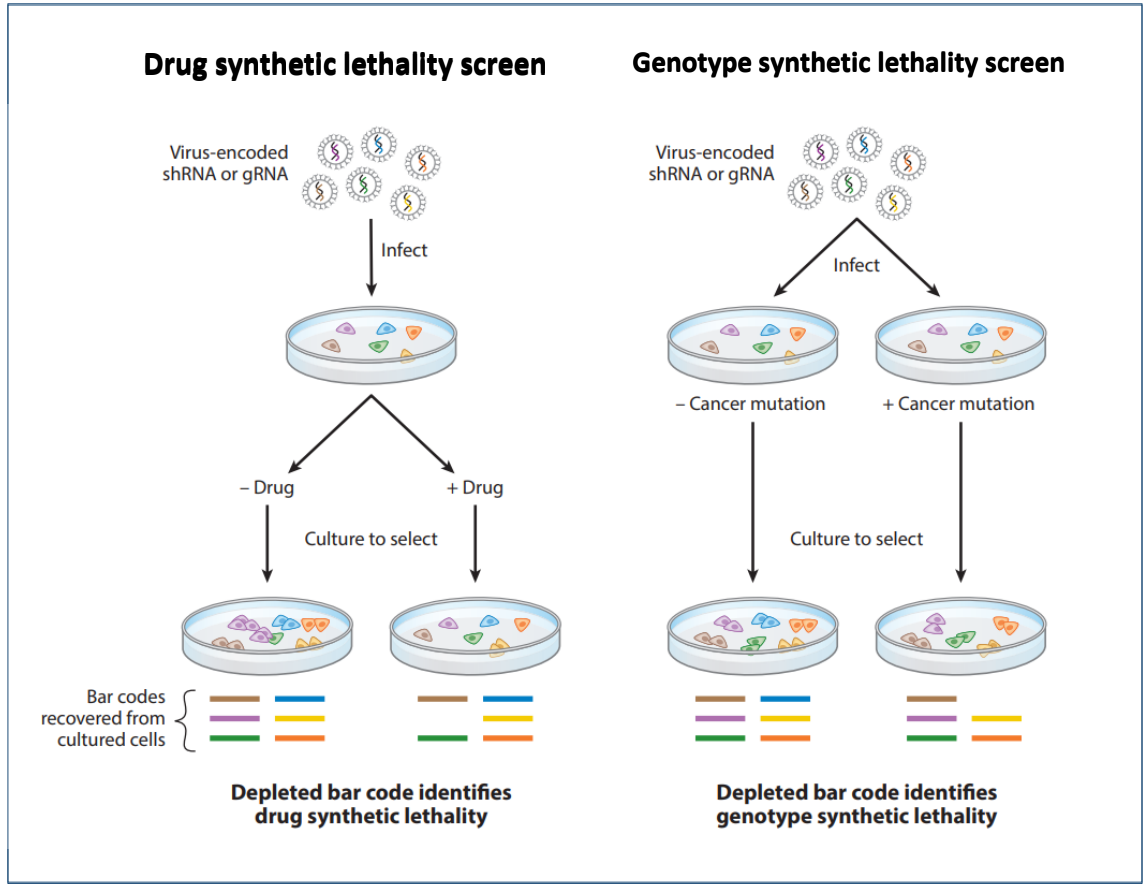
- *SMARCA4* (BRM) mutado en 20% de los tumores.
- *ARID1A*, mutado en 50% de los carcinomas ováricos.

Vulnerabilidades: *SMARC2 (BRG1), ARID1B* o *EZH2.*

Topatana W et al, 2020 J. Hematol. Oncol.
Chen ES et al, 2018 Cell. Mol. Life Sci.
Marjon K et al, Cell Rep 2016

Screenings genómicos basados en CRISPR





Setton J et al, 2021 Cancer Discov
Beijersbergen Rl et al, Annu. Rev. Cancer Biol. 2017

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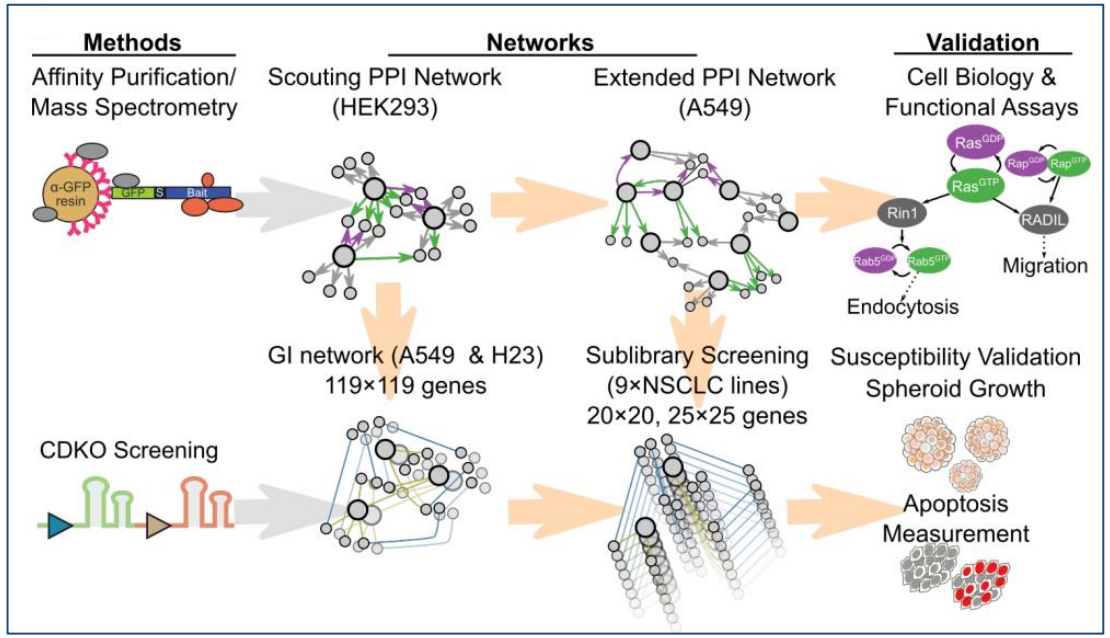
Sesión 3: Vías de desarrollo de la oncología transversal (I)

Screenings de CRISPR knockout acoplados a técnicas –ómicas (mapas protéicos).

***KRAS* en carcinomas de pulmón**

(Kelly MR et al, Cancer Discov 2020)

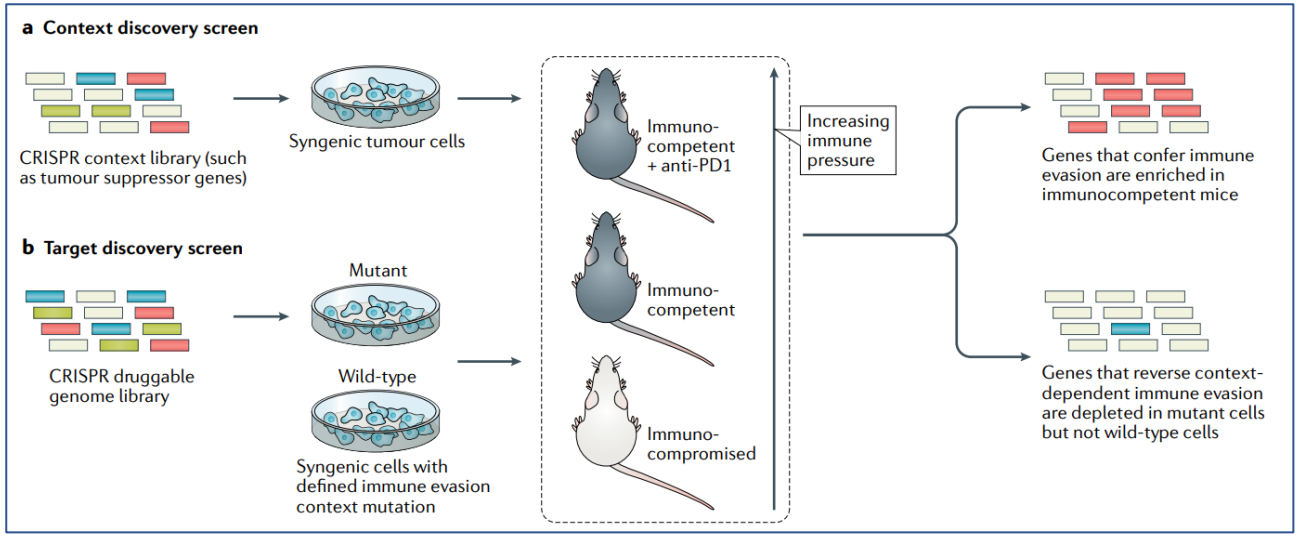
Vulnerabilidades: *RADIL* y *RIN1*



Definición de reguladores de la respuesta inmune en tumores.

- Melanoma, *PTPN2* (Manguso RT et al, Nature 2017)

- CD8+ T cells (Dong MB et al, Cell 2019)



- Aunque el descubrimiento de fenómenos de letalidad sintética se ha acelerado, la aplicación clínica en la actualidad es limitada.

- Necesario aclarar cómo las dianas definidas y su contexto genético condicionan la respuesta clínica, así como identificar marcadores propios de inactivación bialélica en TSGs.

- La tecnología CRISPR, y las herramientas de análisis “single-cell”, presentan suficiente potencial para abordar la heterogeneidad tumoral propia causante de resistencias terapéuticas primarias.