Resistance to PARP inhibitors

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Disclosures

Receives and/or has received research funding from: AstraZeneca, Merck KGaA, Artios.

Received consultancy, SAB membership or honoraria payments from: Syncona, Sun Pharma, Gerson Lehrman Group, Merck KGaA, Vertex, AstraZeneca, Tango, 3rd Rock, Ono Pharma, Artios, Abingworth, Tesselate, Dark Blue Therapeutics, Neophore, Pontifax.

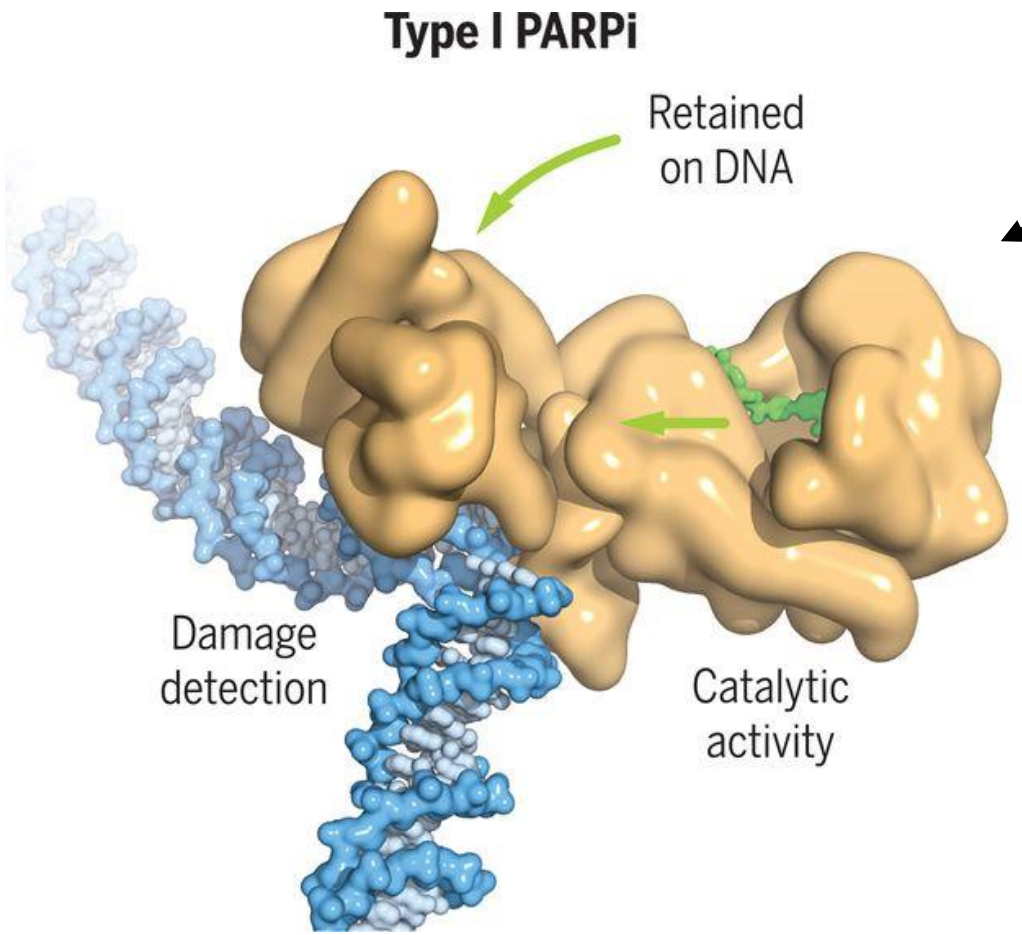
Has stock in: Tango, Ovibio, Enedra Tx., Hysplex, Tesselate.

Named inventor on patents describing the use of DNA repair inhibitors and stands to gain from their development and use as part of the ICR “Rewards to Inventors” scheme and also reports benefits from this scheme associated with patents for PARP inhibitors paid into CJL’s personal account and research accounts at the Institute of Cancer Research.

Summary

- Describe mechanisms of resistance to PARPi
- Real-world assessment of mechanisms of PARPi resistance in advanced breast cancer
- Targeting PARPi resistant disease

PARP1 is a DNA repair Poly ADP-Ribosylase



NAD+

Substrate protein

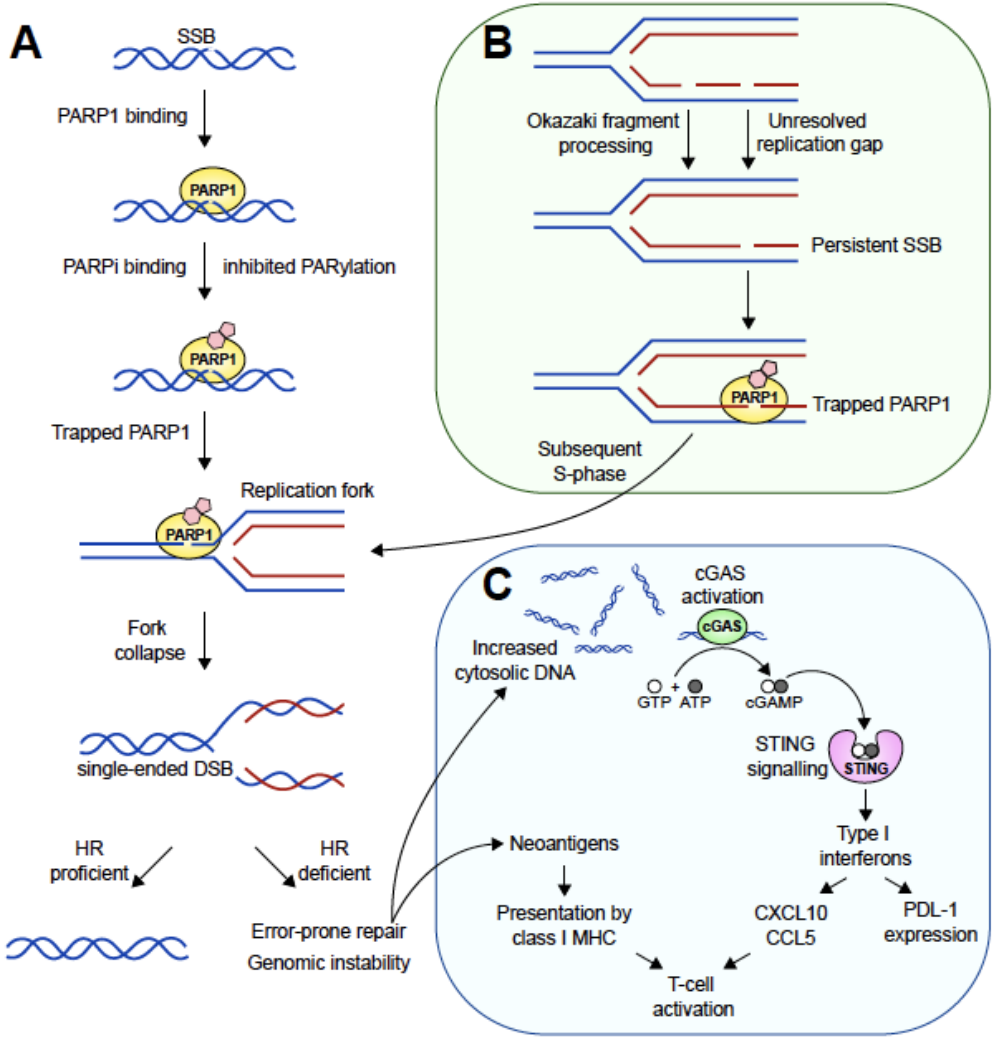
Nicotinamide

Poly (ADP-ribosyl)ated protein

Image from Zandarashvili et al Science. 2020 Apr 3;368(6486):eaax6367.

Image from Lord & Ashworth Science. 2017 Mar 17;355(6330):1152-1158..

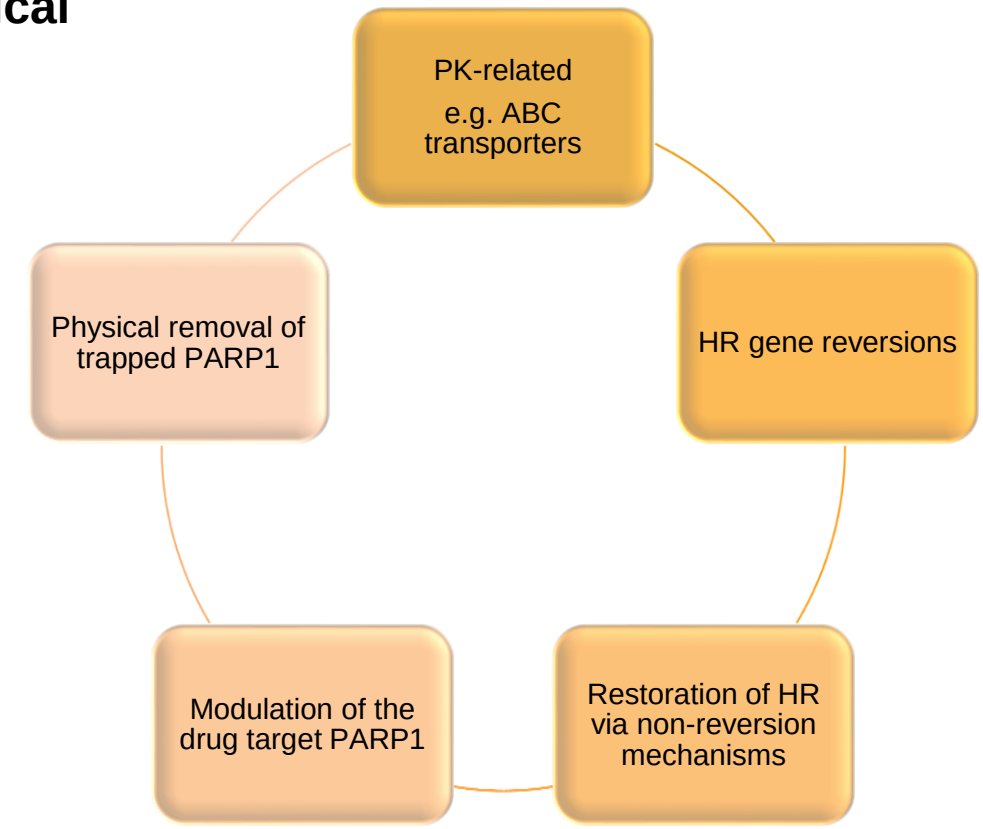
Small molecule PARPi kill BRCA1/BRCA2 mutant cells by synthetic lethality



Wicks AJ, Krastev DB, Pettitt SJ, Tutt ANJ, Lord CJ. Open Biol. 2022 Jul;12(7):220118.

PARP inhibitor resistance can take multiple forms

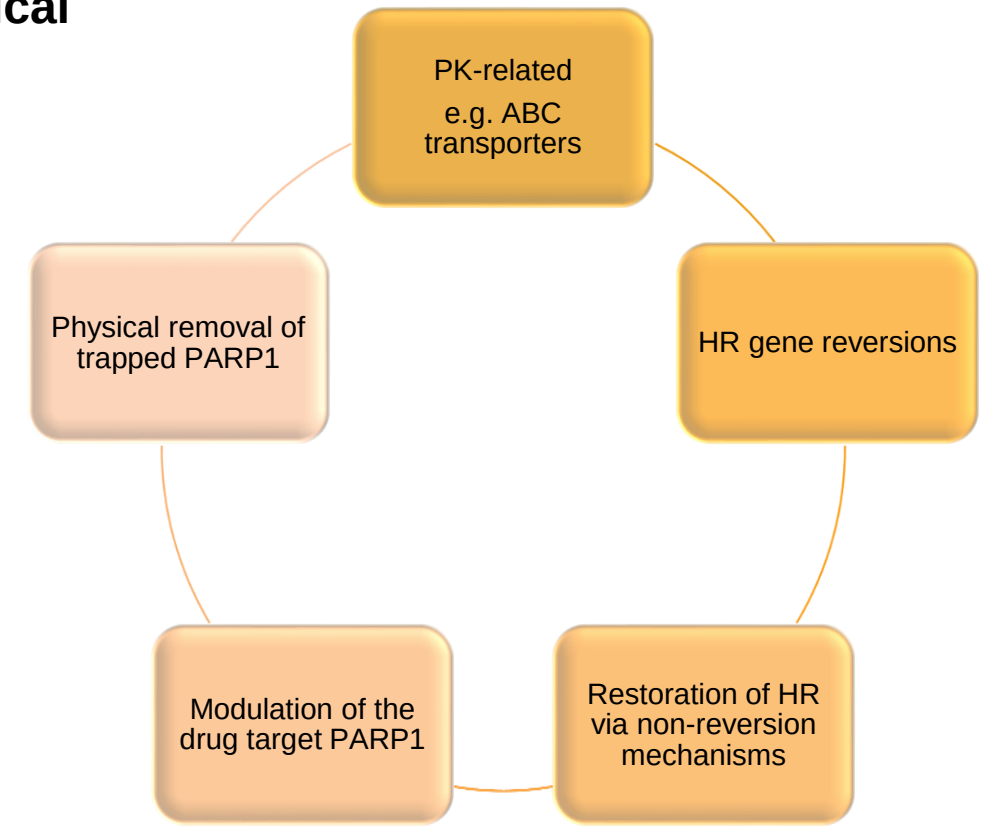
Pre-clinical



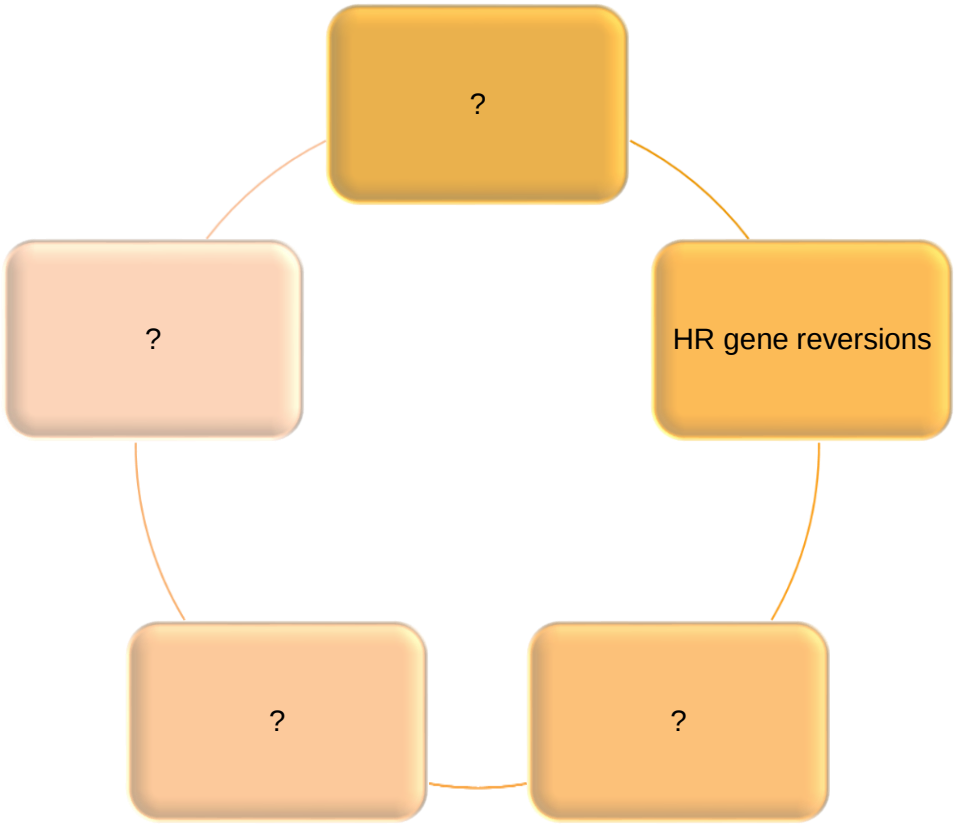
Baxter JS, Zatreanu D, Pettitt SJ, Lord CJ. Mol Oncol. 2022 Nov;16(21):3811-3827.

PARP inhibitor resistance can take multiple forms

Pre-clinical



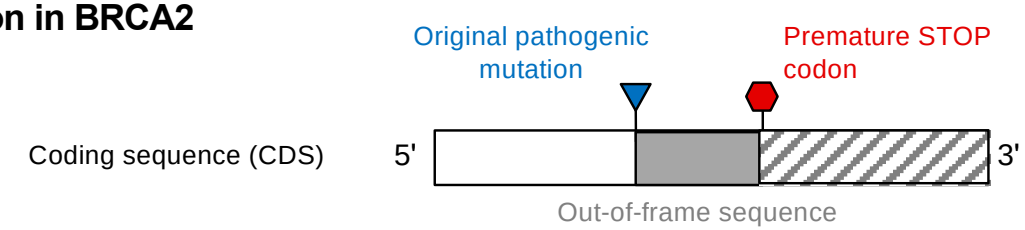
Clinical



Baxter JS, Zatreanu D, Pettitt SJ, Lord CJ. Mol Oncol. 2022 Nov;16(21):3811-3827.

Reversion mutations in BRCA1, BRCA2, PALB2, RAD51C, RAD51D

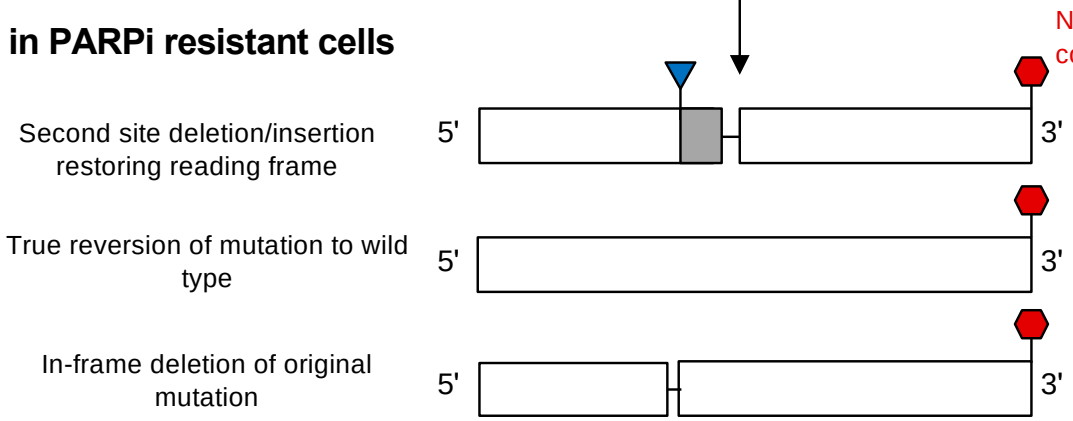
Frameshift mutation in BRCA2



- Truncated or absent protein
- Loss of HR function
- Platinum/PARPi sensitivitiy

Treatment with platinum or PARPi

Reverted alleles in PARPi resistant cells

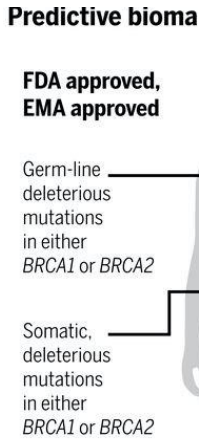


- Near full-length protein
- Reacquisition of HR function
- Platinum/PARPi resistance

Sakai A, et al. *Nature* 2008;28;451(7182):1116–20; 2. Edwards SL, et al. *Nature* 2008;28;451(7182):1111–5; 3. Pettitt SJ, et al. *Cancer Discov* 2020;10(10):1475-1488; 4. Lin KK, et al. *Cancer Discov* 2019;9(2):210–219

Reversion mutations in high-grade serous ovarian cancer

ARIEL2
(Lin Cancer Discov 2019)



Breast
Pancreas
Prostate
Ovary

1-3 prior chemotherapy (platinum) regimens

↓

classified as platinum refractory, resistant or sensitive

↓

relapsed disease

↓

cfDNA

↓

Rucaparib

Post platinum/pre-PARP inhibitor *BRCA1/2* reversions in:

18% of platinum-refractory

13% of platinum-resistant cancers

2% platinum-sensitive cancers

Reversion mutations in high-grade serous ovarian cancer

ARIEL2
(Lin Cancer Discov 2019)

Predictive bioma

FDA approved, EMA approved

Germ-line deleterious mutations in either *BRCA1* or *BRCA2*

Somatic, deleterious mutations in either *BRCA1* or *BRCA2*

Breast
Pancreas
Prostate
Ovary

1-3 prior chemotherapy (platinum) regimens

↓

classified as platinum refractory, resistant or sensitive

↓

relapsed disease

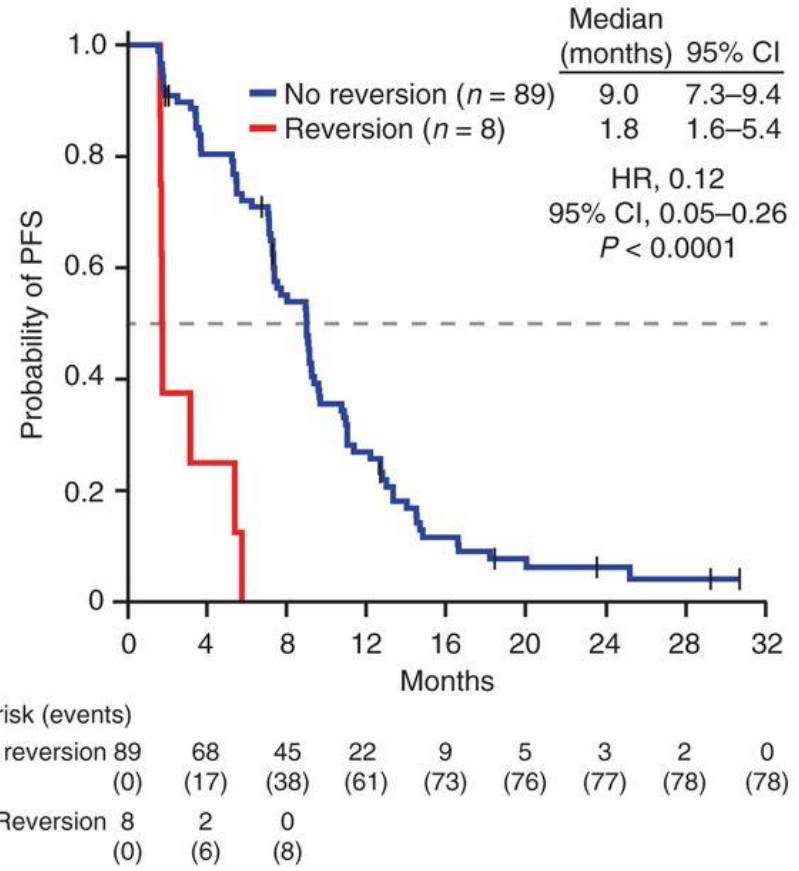
↓

cfDNA

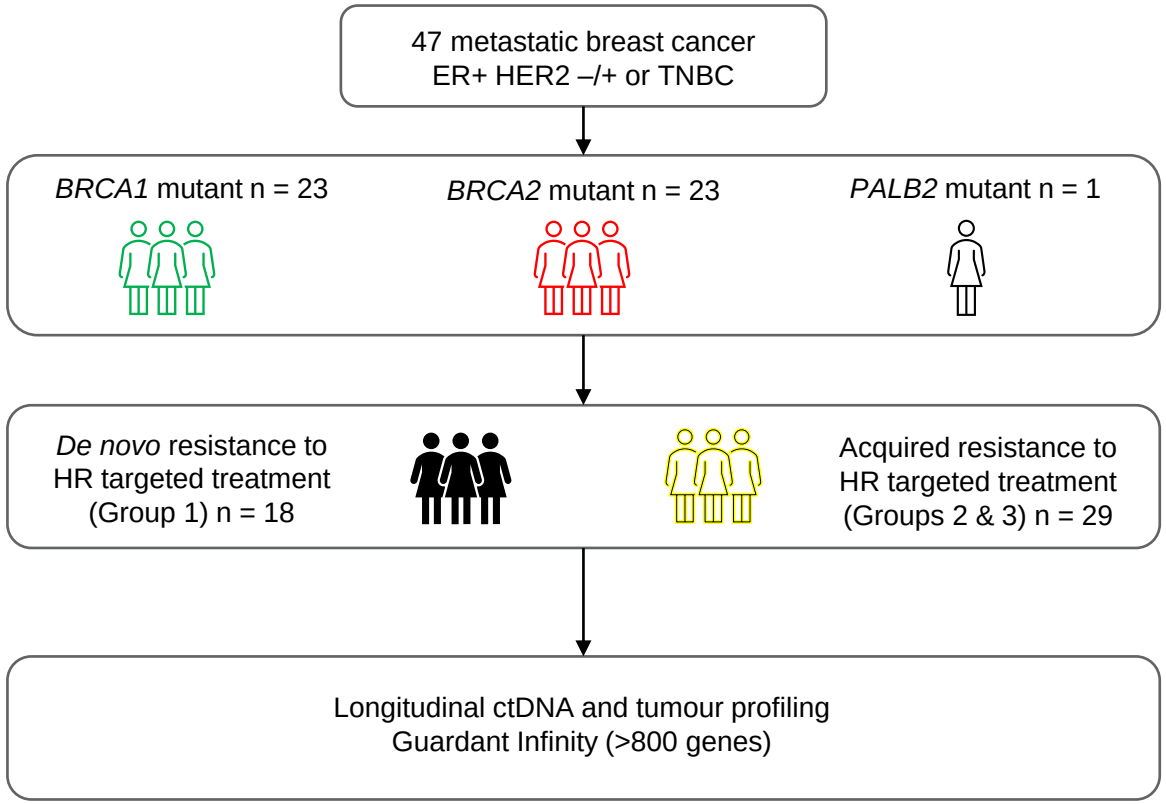
↓

Rucaparib

BRCA1/2 reversion mutations in pre-treatment cfDNA = shorter rucaparib PFS

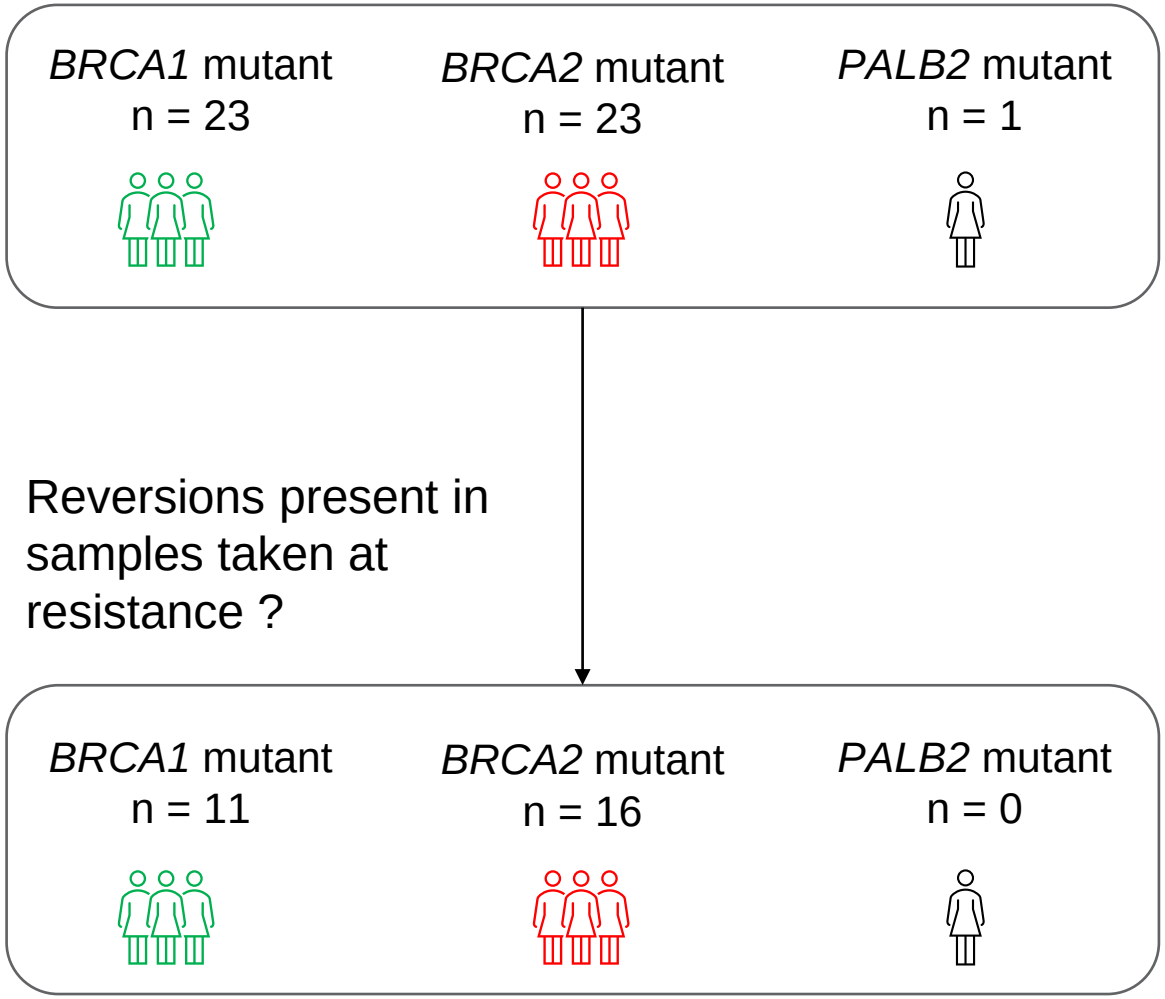


Real-world analysis of resistance in breast cancer



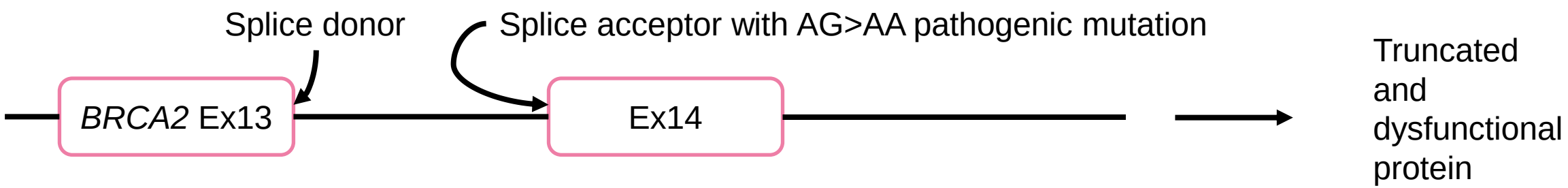
Elizabeth Harvey-Jones
Maya Raghunandan
Luisa Robbez-Masson
Alaguthurai Thanussuyah
Roberta Liccardo
Arielle Yablonovitch
Mingyang Cai
Leylah Drusbosky
Michael Dorschner
Lorena Magraner Pardo
Jason Yeung
Rebecca Marlow
Asha Konde
Jennifer Trendell
Thomas Savy
Hui Xiao
John Alexander
Syed Haider
Chris Starling
Ioannis Roxanis
Jennifer Yen
Stephen Pettitt
Christopher J Lord
Andrew NJ Tutt

BRCA1/2 reversions are common

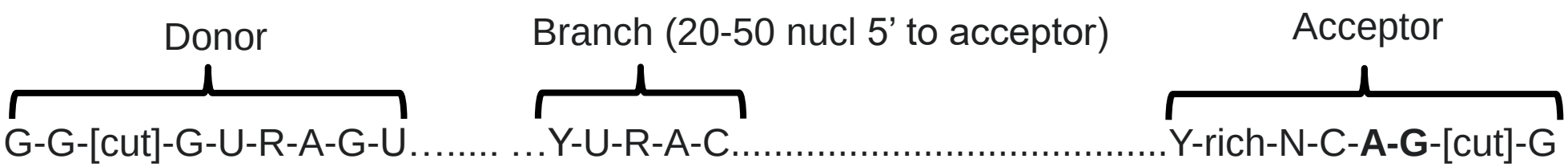


- Up to 14 different reversion mutations per patient detected.
- Most > secondary deletion mutations, often with evidence of microhomology use at the deletion.
- Some large genomic rearrangements (LGRs).

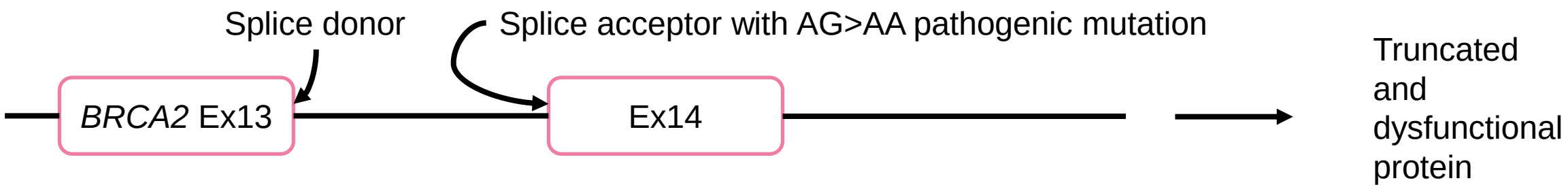
Reversion of splice site pathogenic mutations



Consensus sequence for introns:



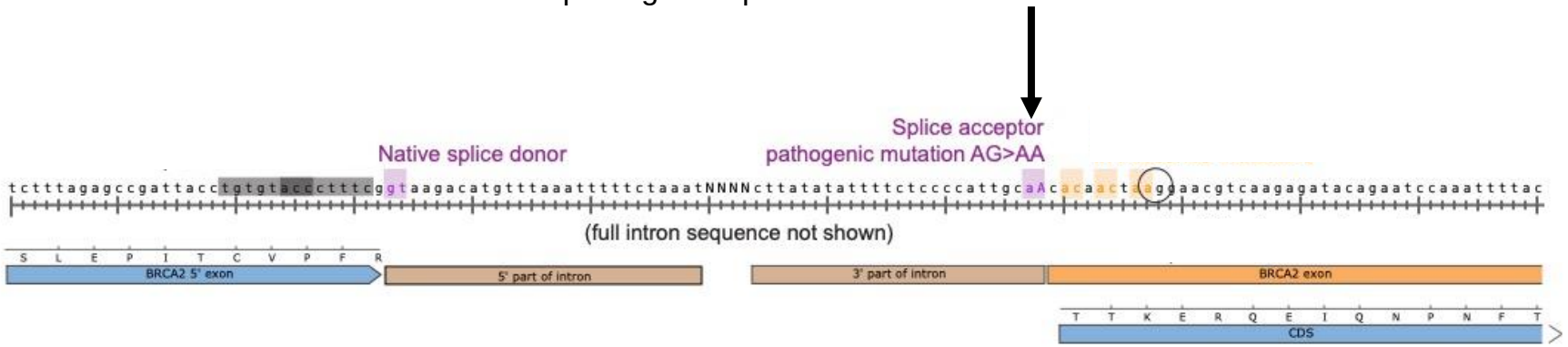
Reversion of splice site pathogenic mutations



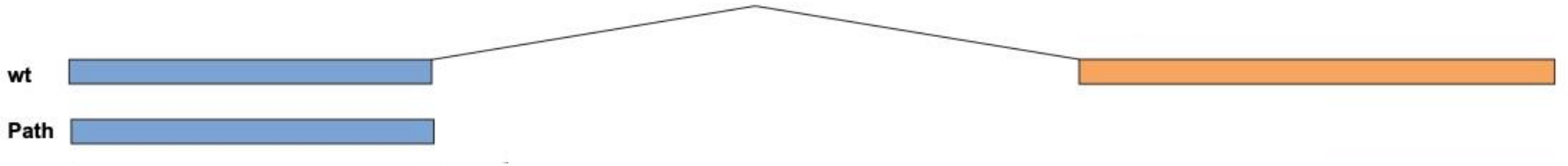
- Previous data suggested splice site pathogenic mutations always revert to wild type DNA sequence
- Assumed that sequence constraints (i.e. to encode functional gene) mean fewer ways of reverting splice sites
- Now know this is not the case….

KCL15 - AG > AA acceptor mutation BRCA2 5’ exon 14

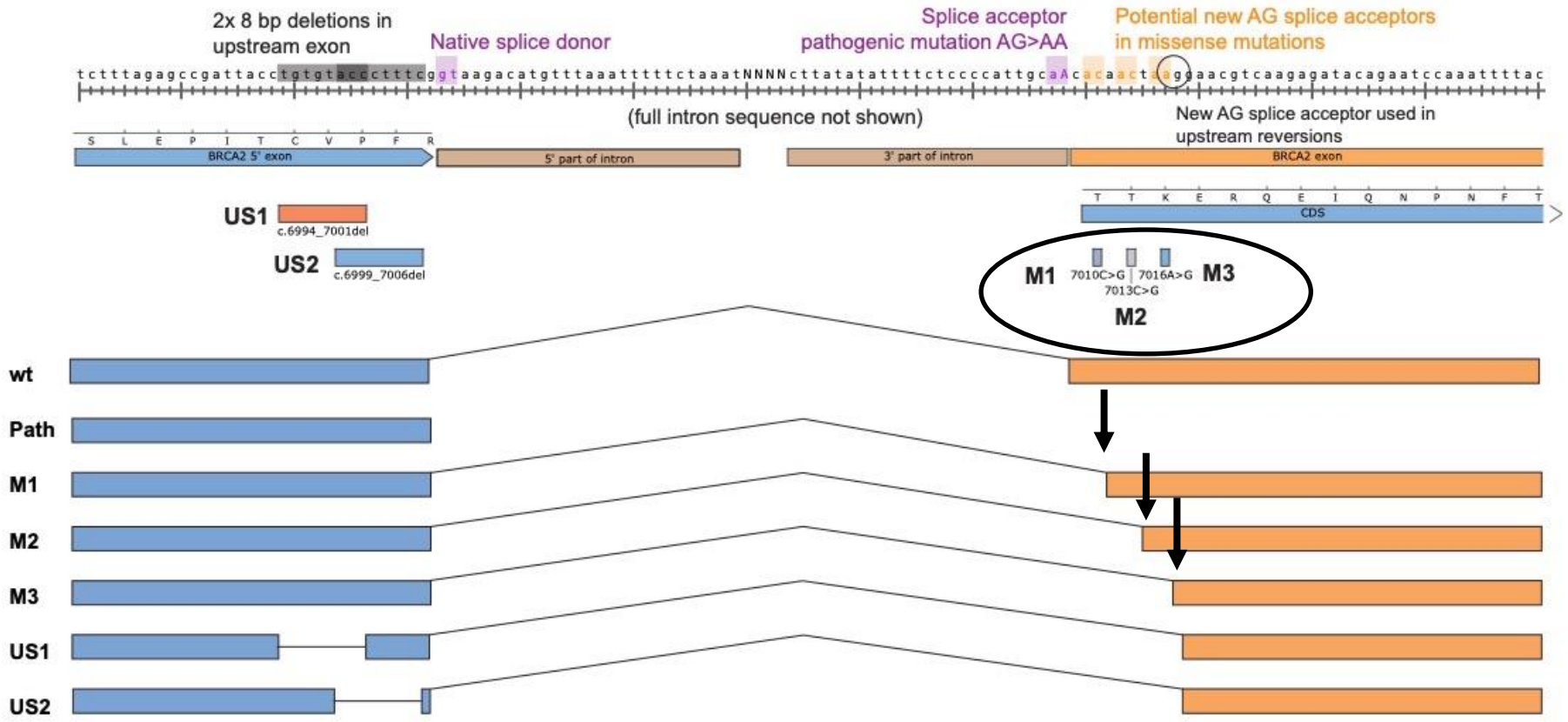
Structure of *BRCA2* locus around KCL015 pathogenic splice site mutation



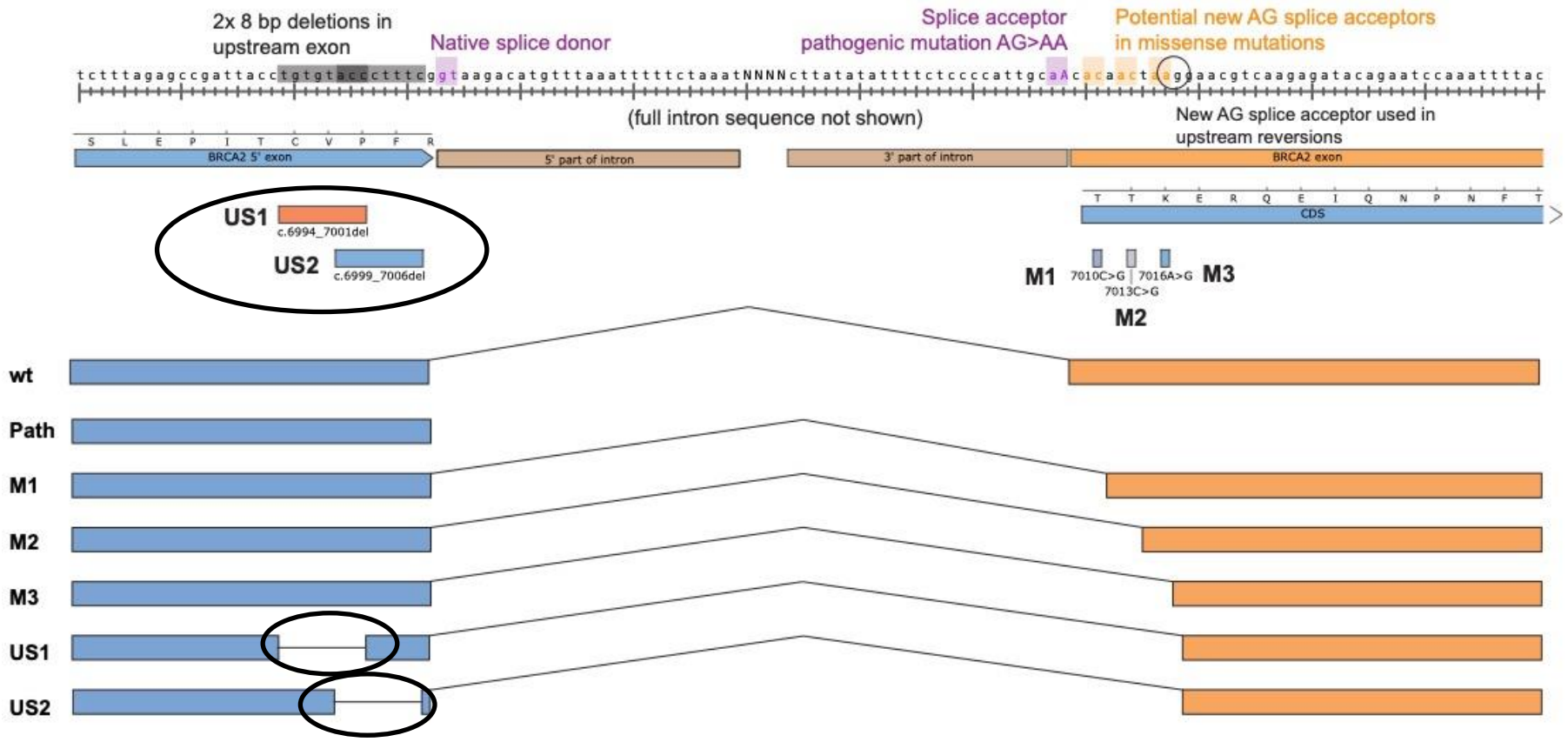
Predicted proteins



3 mutations at resistance that create new AG splice acceptor within exon 14



Two 8 bp deletions in exon 13 that allow use of AG site within exon 14

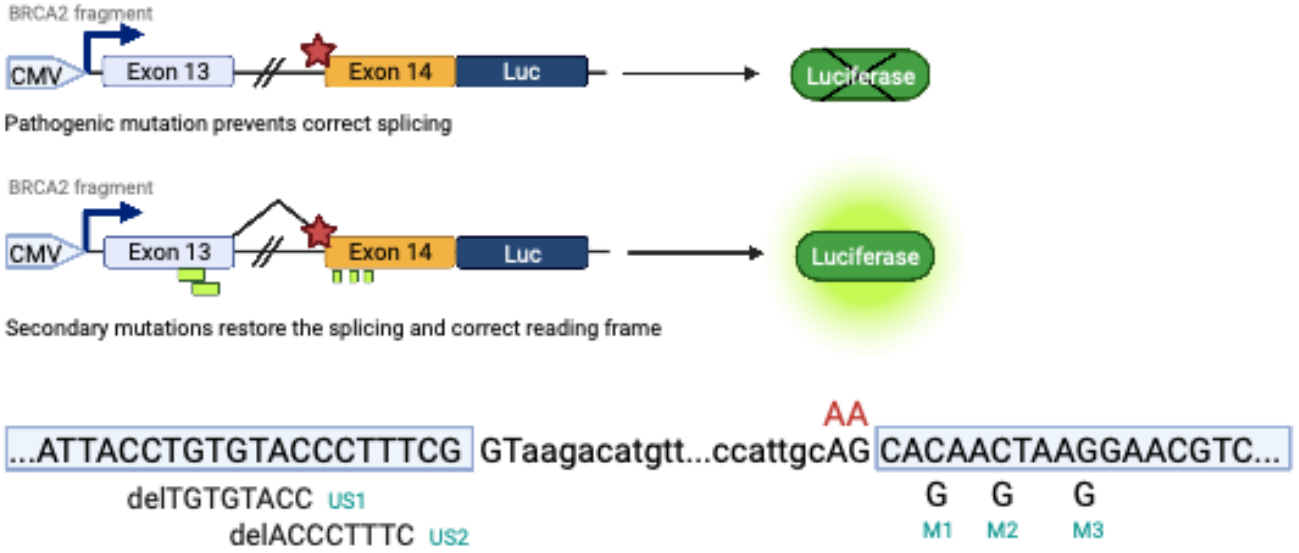


Predicted proteins

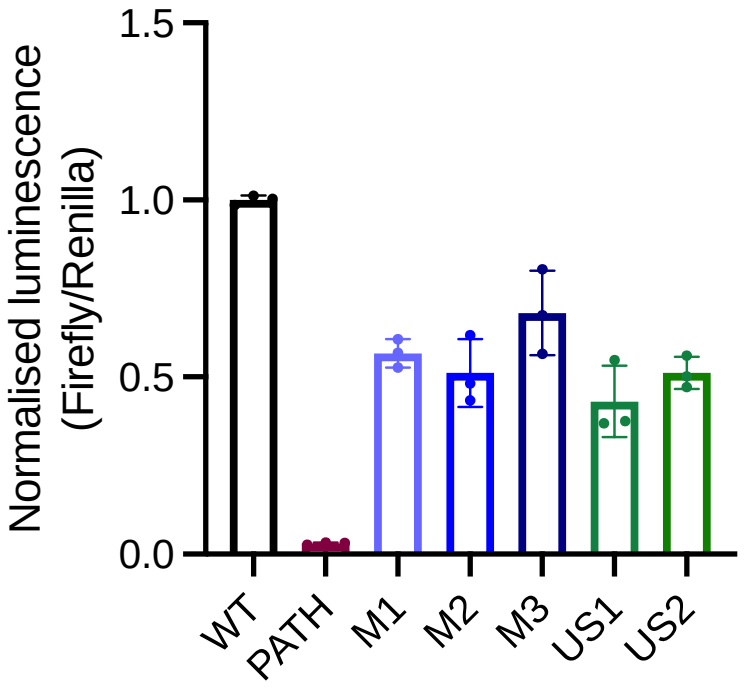
Predicted proteins in resistant ctDNA

New splice acceptor mutations are functional

Minigene reporter assay to test possible effects on splicing revealed the use of novel splice acceptors in the downstream exon



Results of luciferase reporter assay (HEK293 cells)

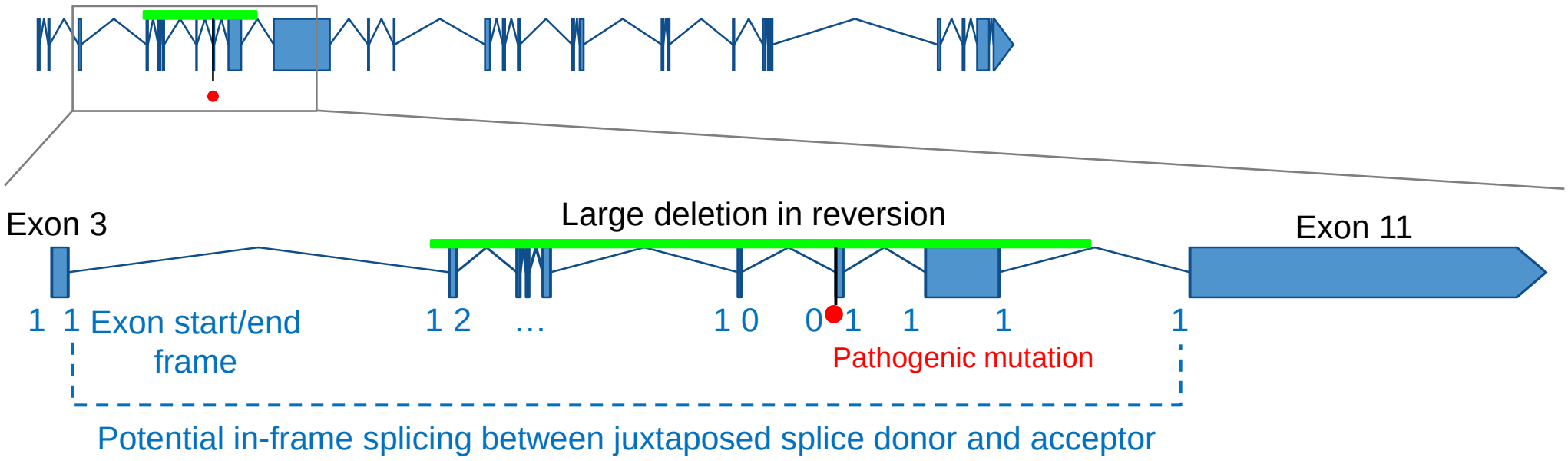


KCL757: Bypass of pathogenic mutation via splice site deletion and non-canonical splicing

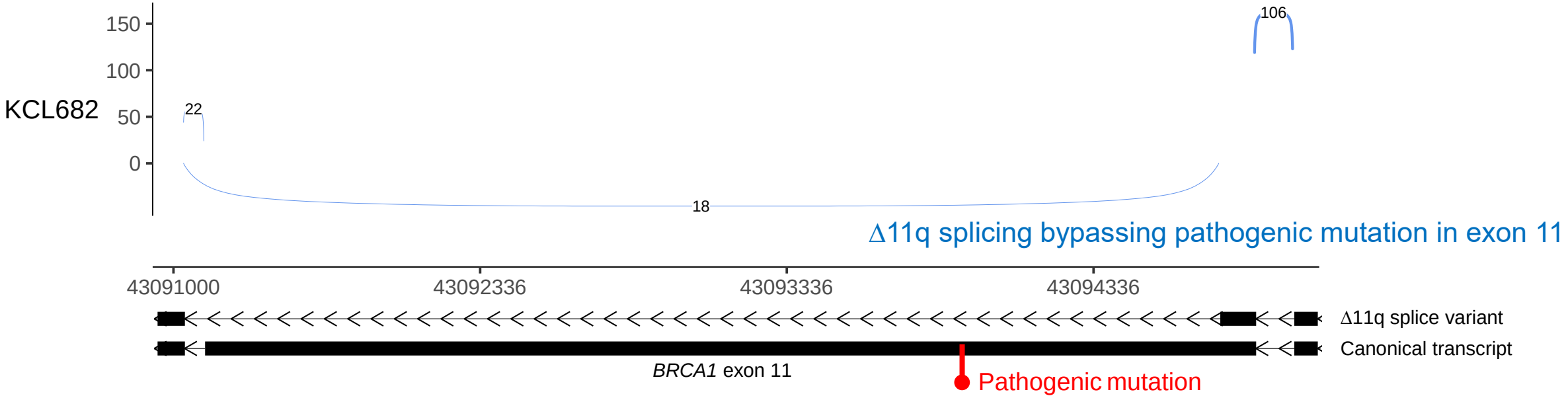
KCL757

BRCA2: c.682-1G>C

Splice acceptor pathogenic mutation

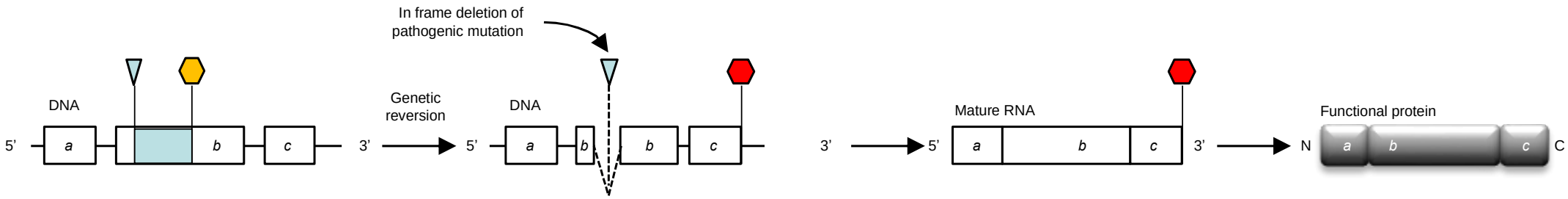


KCL682: Evidence for Δ11q splice variant expression in tumour RNAseq

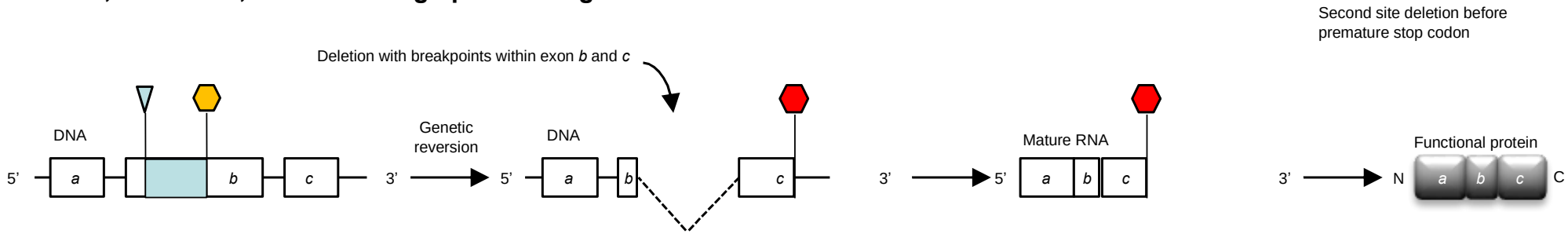


Previously known types of reversions

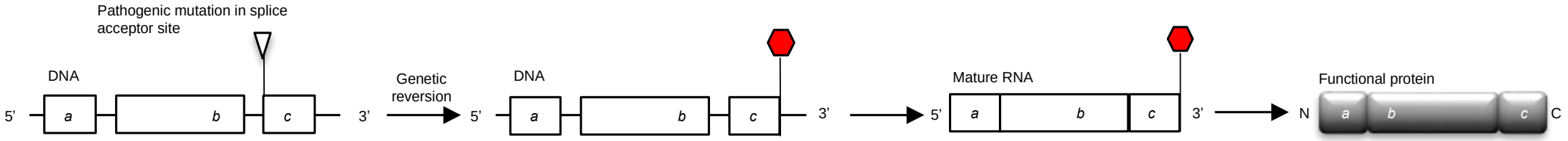
In frame, intra exon, deletion of pathogenic mutation



In frame, inter exon, indel restoring open reading frame

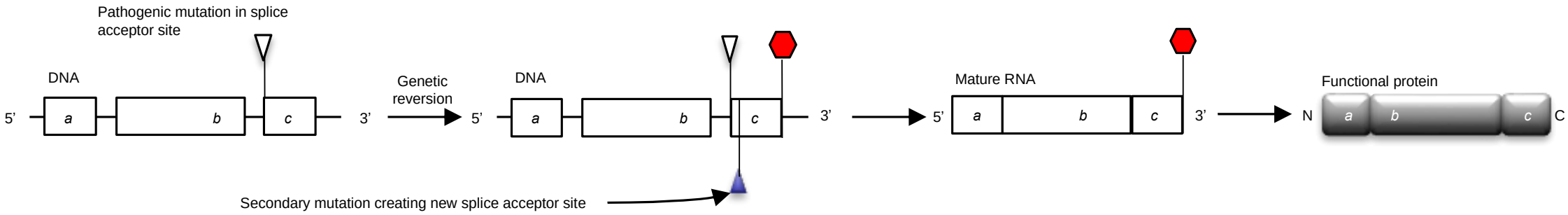


Reversion mutation restoring native splice acceptor site

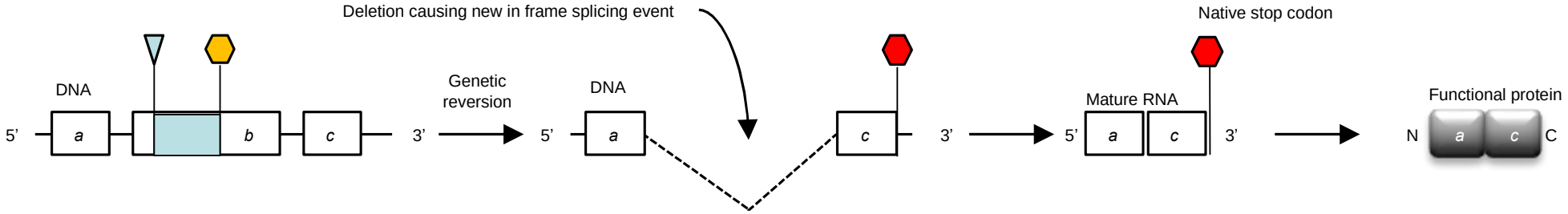


Novel reversion types

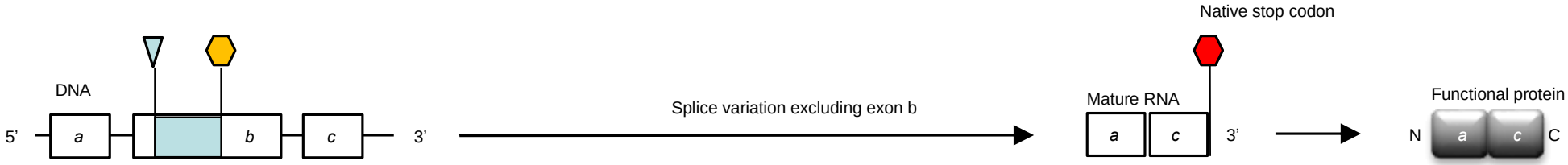
Reversion mutation generating new splice acceptor site



Deletion leading to new in frame splicing event

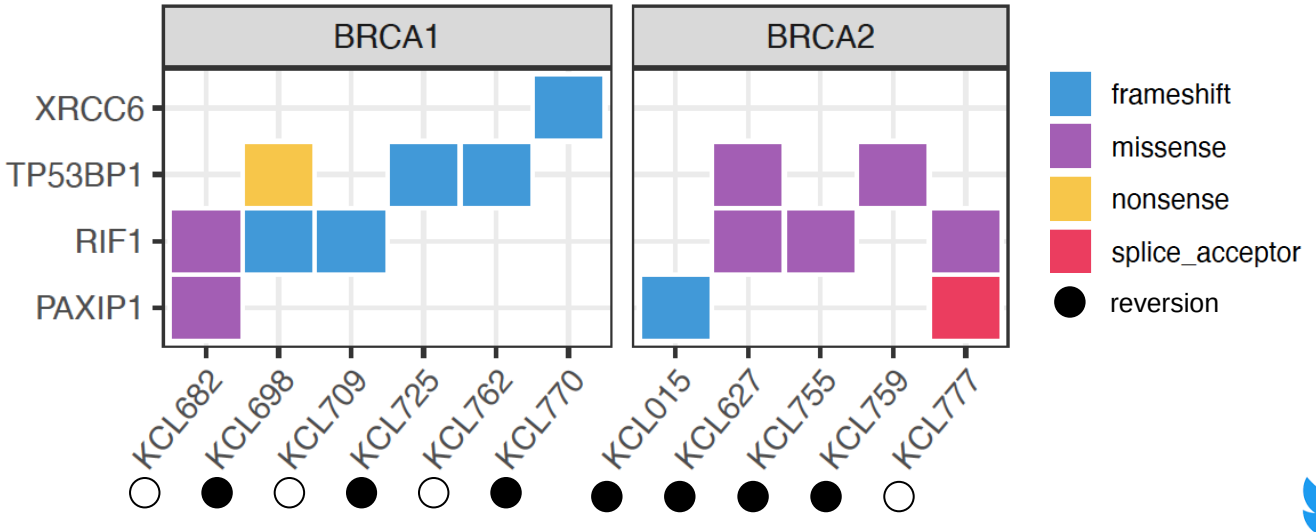


Splice variation without genetic reversion

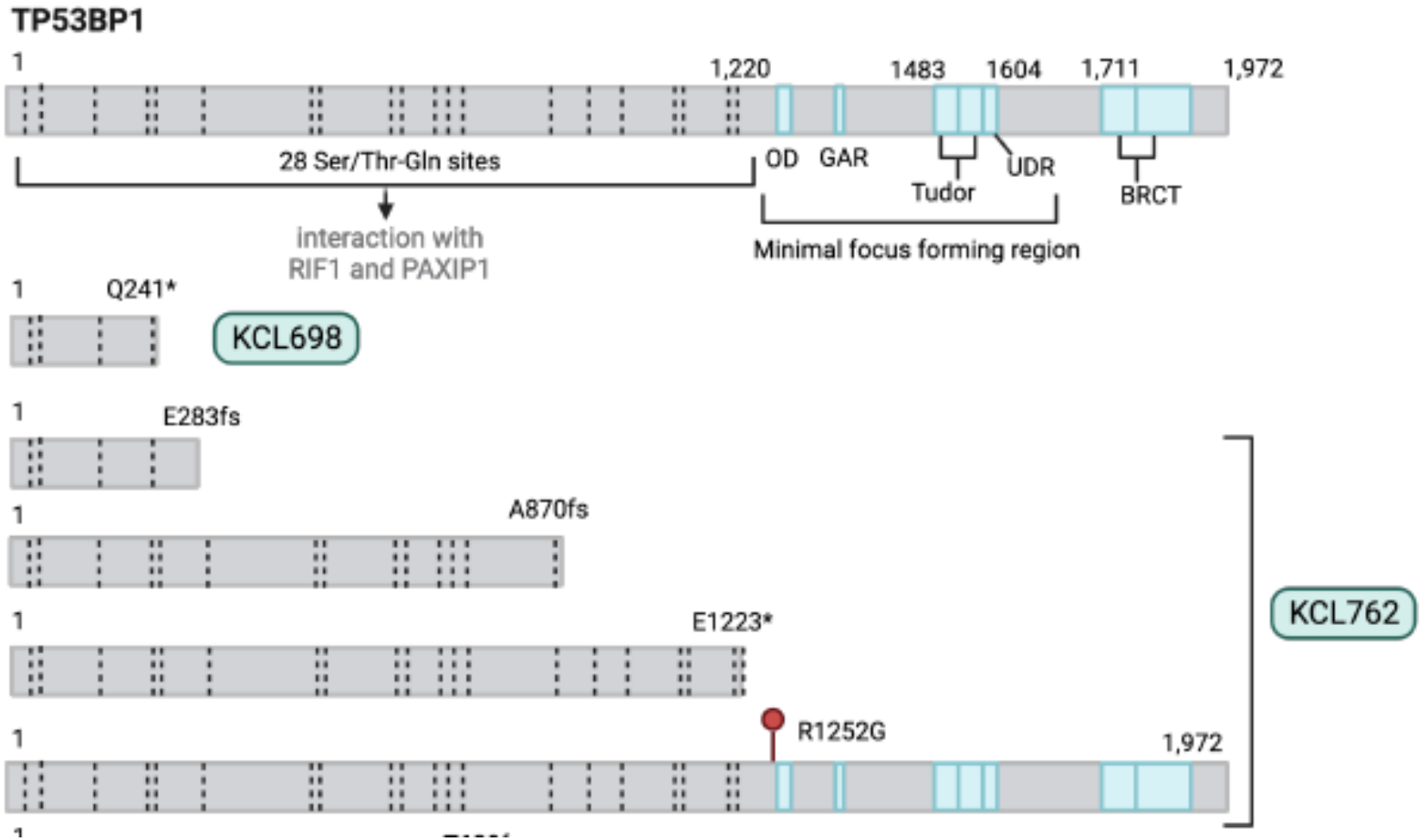


Co-occurrence of reversion mutations with other resistance mechanisms

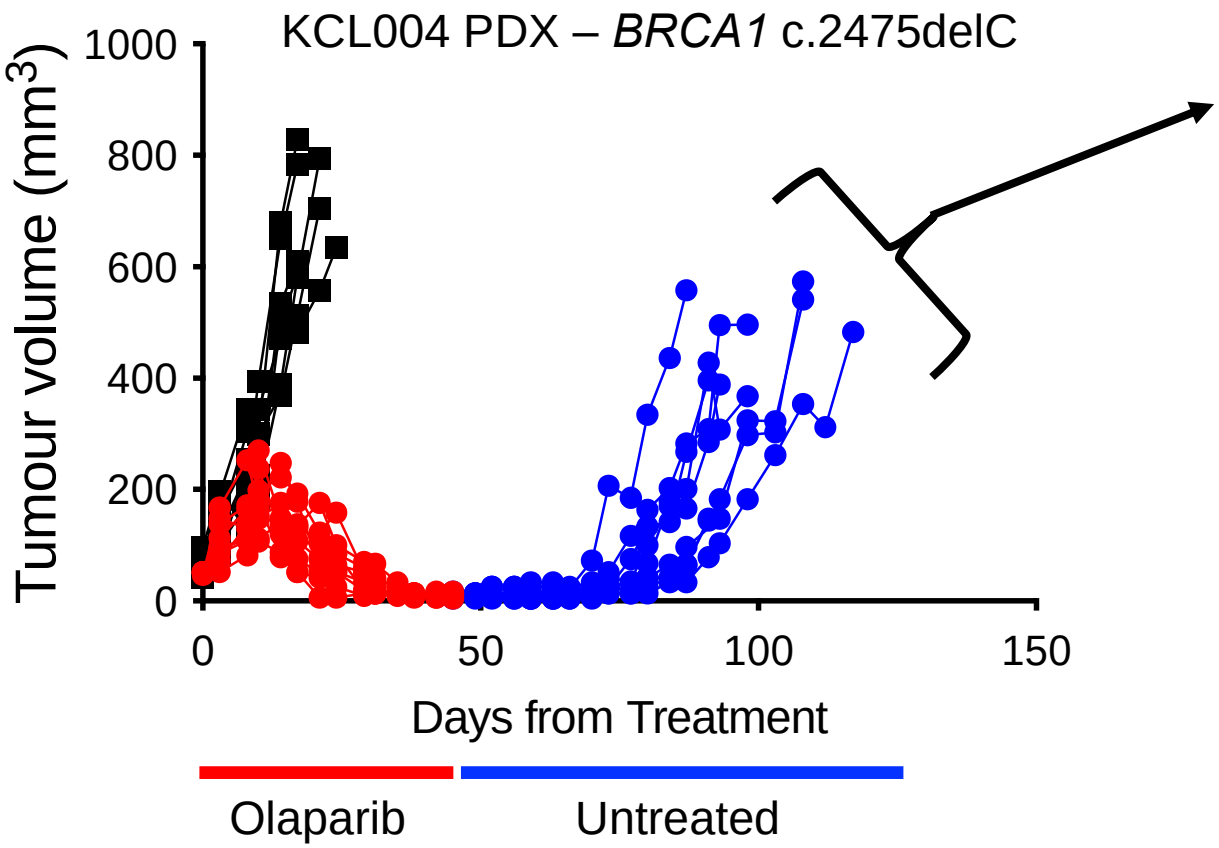
- Evidence in 11 / 72 patients
- 7 of these patients also have BRCA1/2 reversion mutations
- Non-reversion mutations alter with subsequent treatment



Truncating mutations in 53BP1

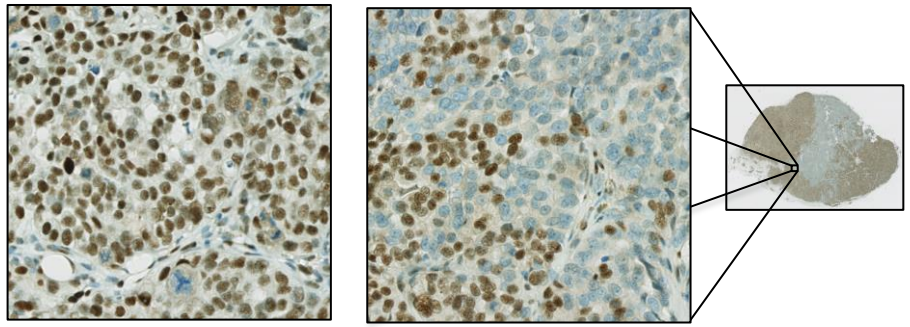


Polyclonal resistance in a PDX model of *BRCA1m* breast cancer



4 outgrowths with BRCA1 reversions

1 outgrowth with 53BP1 loss



Untreated Olaparib resistant

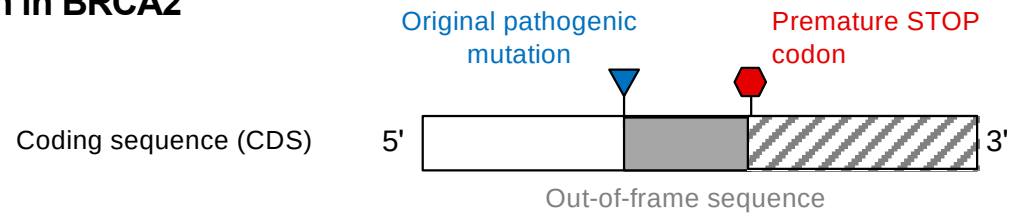
Conclusions

- Functional BRCA1/2 reversion is the main mechanism of PARPi resistance visible in advanced breast cancer
- Selective pressure clearly on functional BRCA1/2 – how the cell achieves this varies
- Other DNA repair-related resistance mechanisms such as mutation / loss of NHEJ and Shieldin pathway proteins are observed concurrently with reversions but are less common
- Some evidence of polyclonal resistance

How to target BRCA1/2 reversions ?

Targeting Reversion Mutations

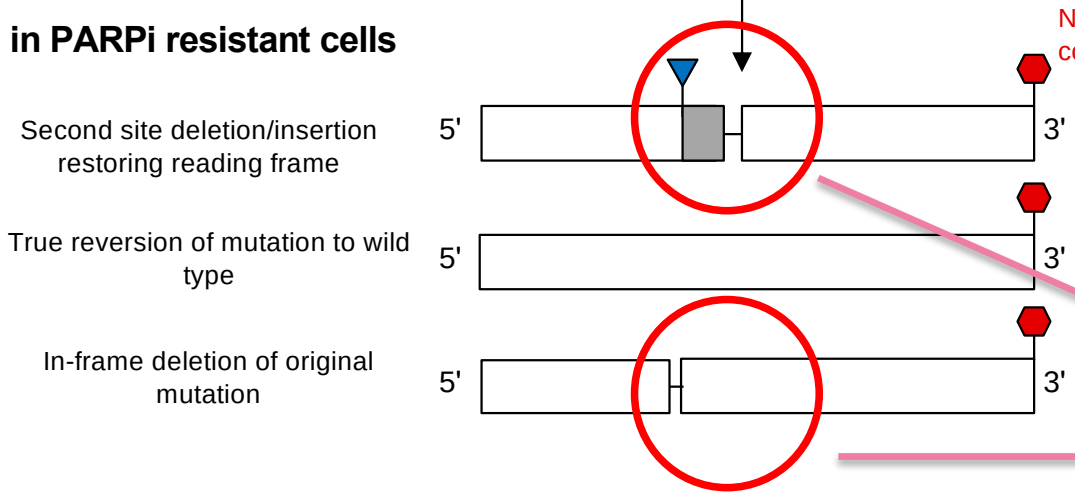
Frameshift mutation in BRCA2



- Truncated or absent protein
- Loss of HR function
- Platinum/PARPi sensitivitiy

Treatment with platinum or PARPi

Reverted alleles in PARPi resistant cells



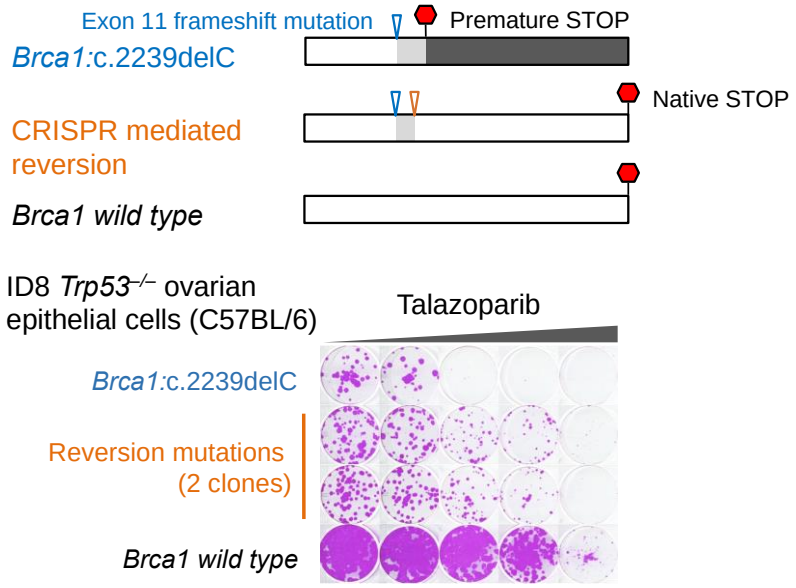
- Near full-length protein
- Reacquisition of HR function
- Platinum/PARPi resistance

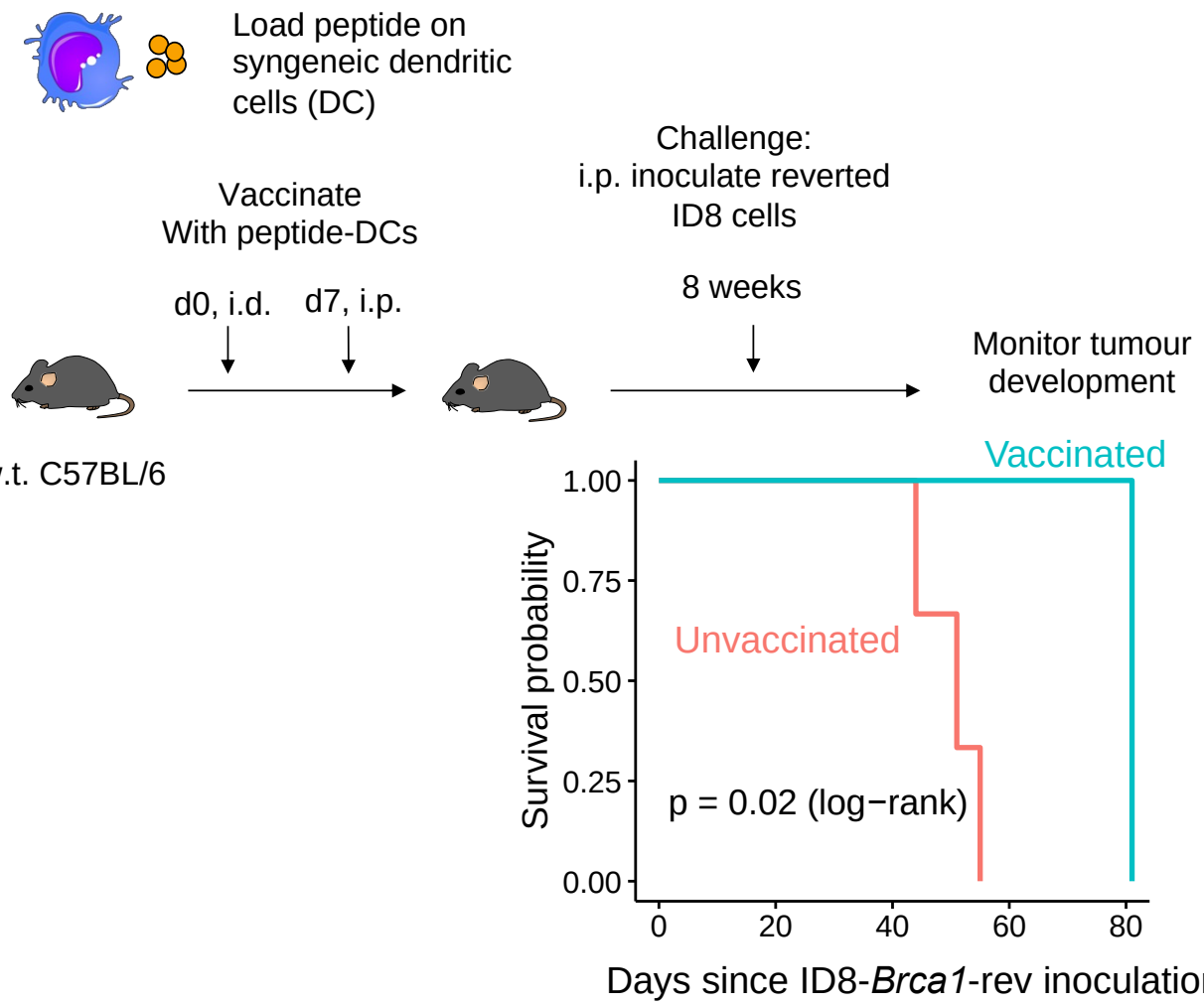
Neopeptides

Pettitt et al Cancer Discov. 2020 Oct;10(10):1475-1488.

Neopeptide vaccination delays tumour formation in mice

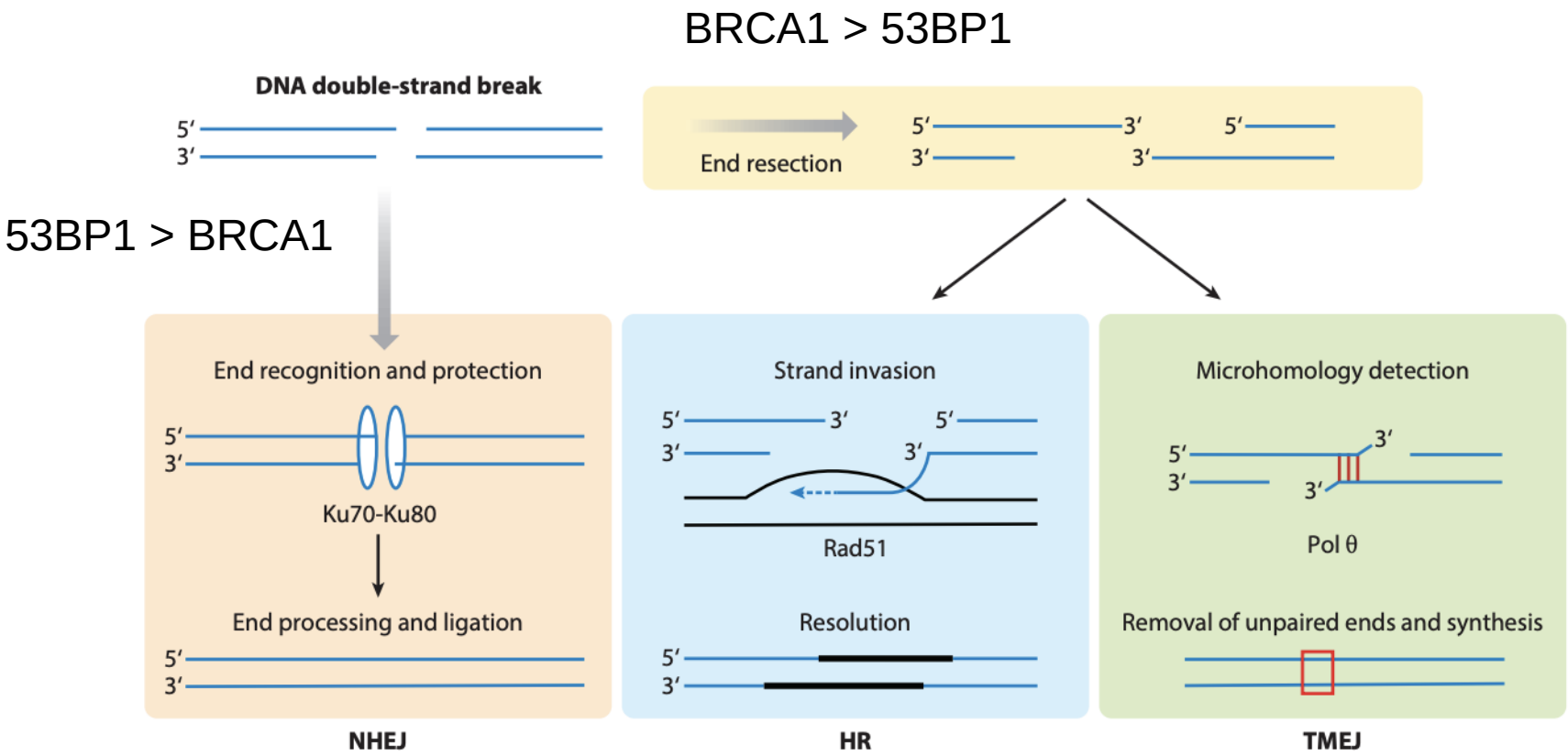
Engineered cell line model of *Brca1* reversion





How to target non-reversion based mechanisms of resistance

Resection of DSB is a prelude to HR



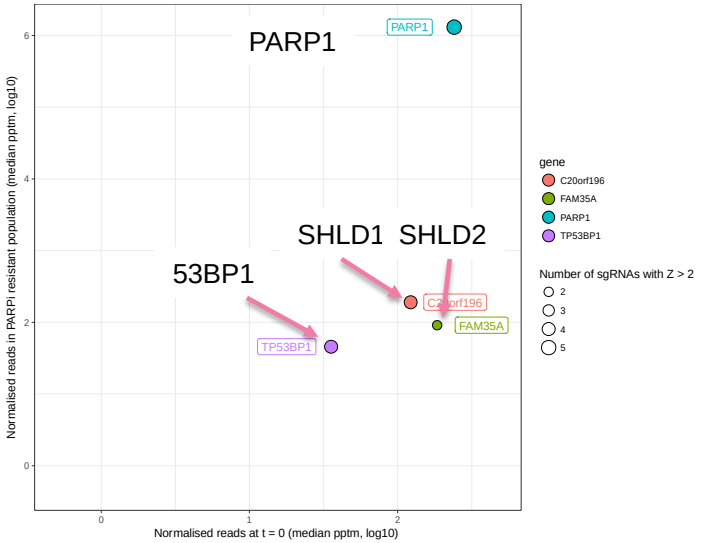
Wood and Doublie *Ann Rev Genet.* 2022

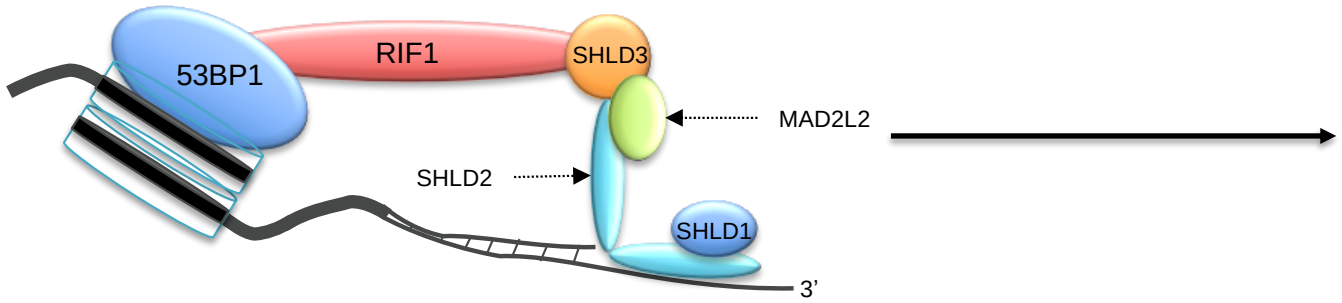
Restoring HR = PARP inhibitor resistance

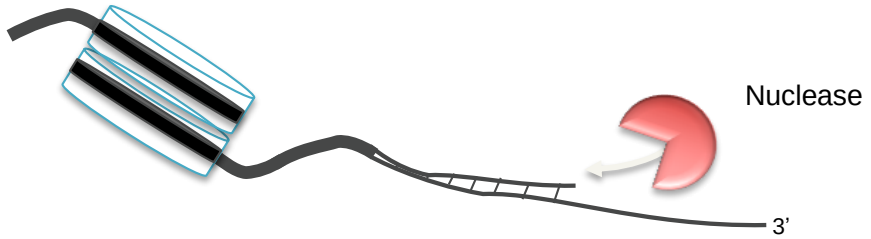
BRCA1 mutant SUM149

Human genome-wide lentiviral CRISPR library (Yusa, Sanger)

Selection screens in PARPi







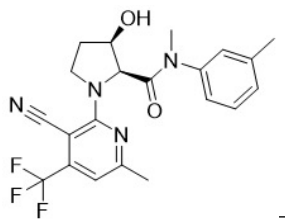
Noordermeer et al Nature. 2018 Aug;560(7716):117-121.
Mirman et al Nature. 2018 Aug;560(7716):112-116.
Ghezraoui et al Nature. 2018 Aug;560(7716):122-127.
Dev et al Nat Cell Biol. 2018 Aug;20(8):954-965.
Gupta et al Cell. 2018 May 3;173(4):972-988.e23.
Findlay et al EMBO J. 2018 Sep 14;37(18):e100158.

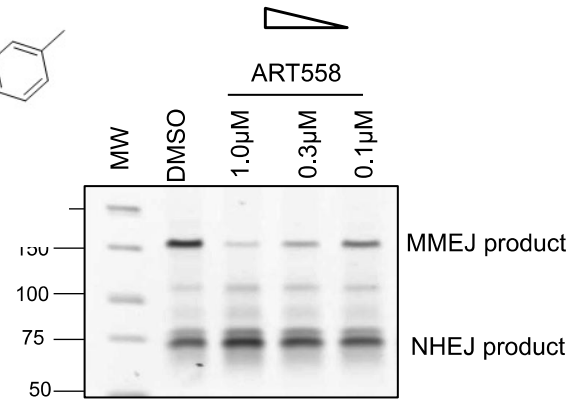
Targeting restoration of HR via Polθ inhibitors

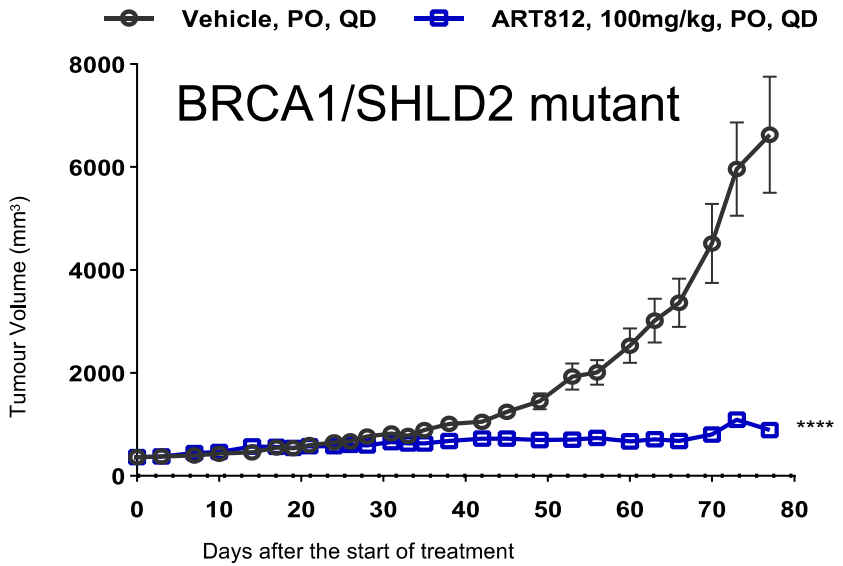
Target PARPi resistant BRCA1/53BP1, Shieldin defective cells

ART558

Polθ inhibitor IC_{50} 7.9 nM – **Inhibits MMEJ**

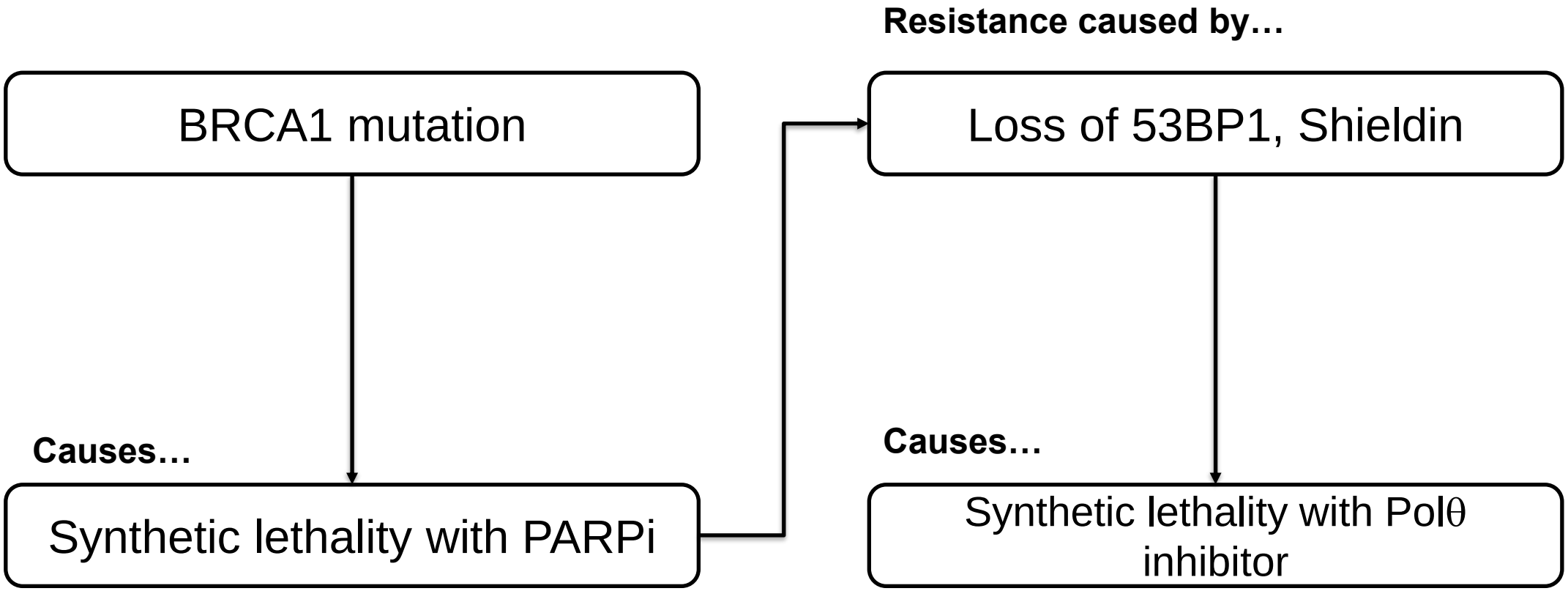






Zatreanu D, et al. *Nat Commun* 2021;17;12(1):3636

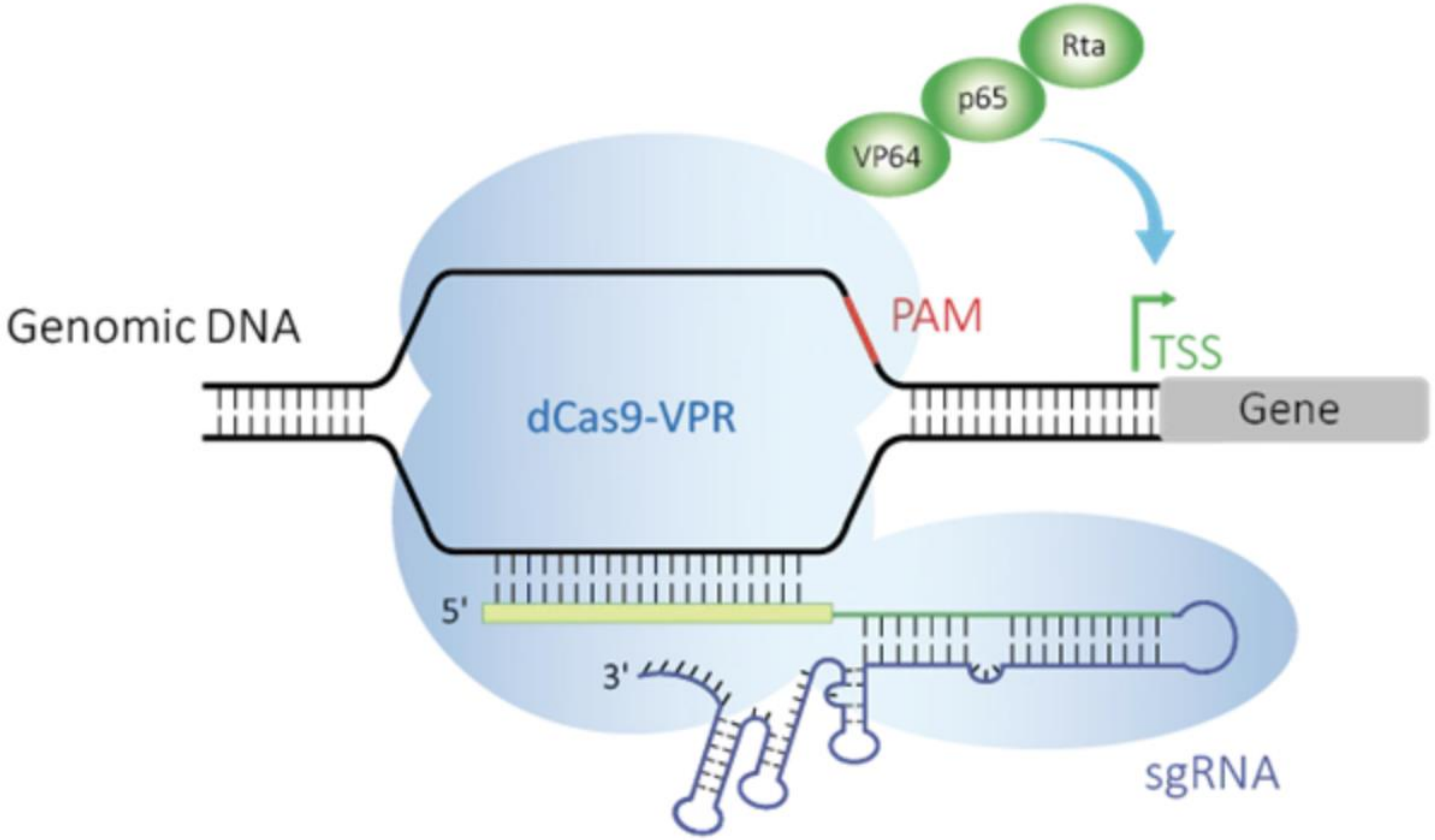
Double binds



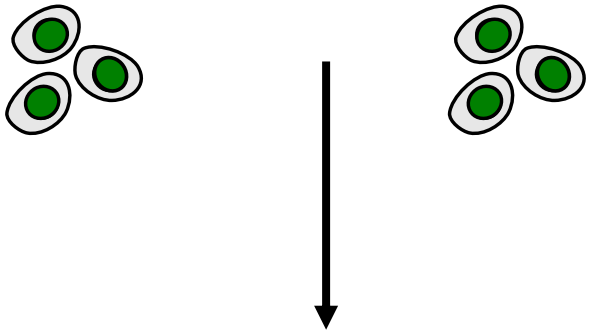
Identifying new mechanisms of PARPi resistance

Newly identified mechanisms of PARPi resistance

- Lots know about LoF = resistance
- Less known about GoF other than reversions



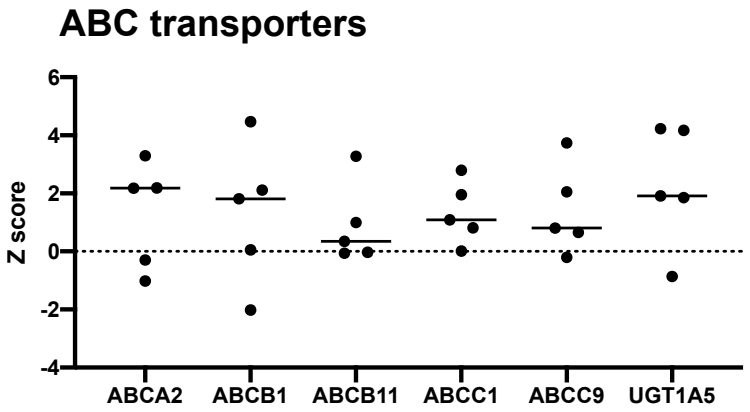
BRCA1m SUM149 BRCA1m COV362

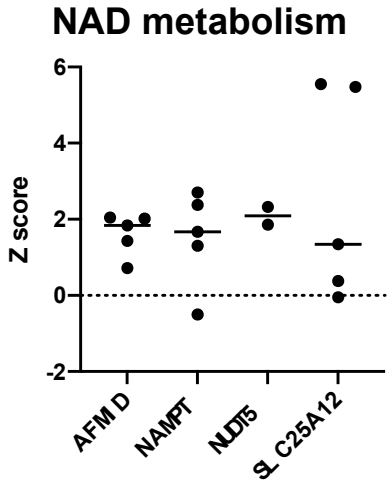


Parallel CRISPRn + CRISPRa

PARPi resistance

Newly identified mechanisms of PARPi resistance





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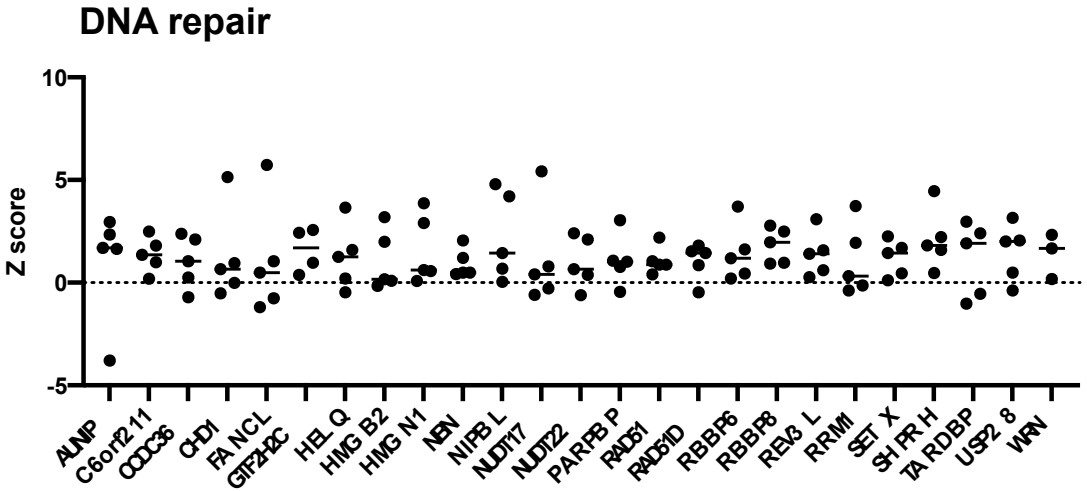
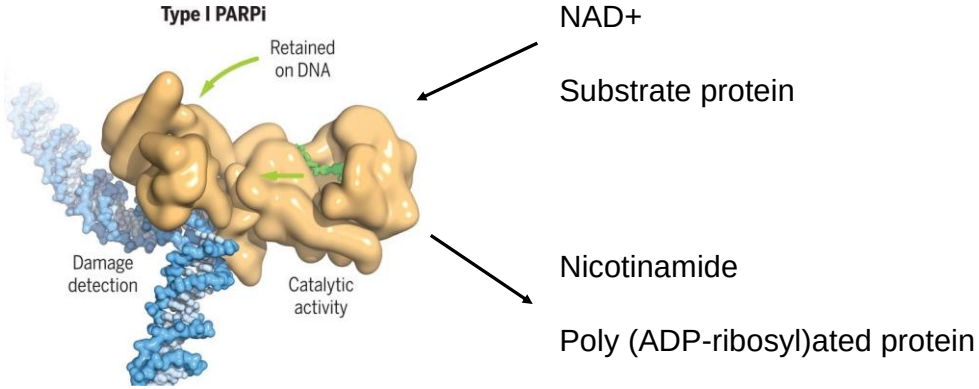
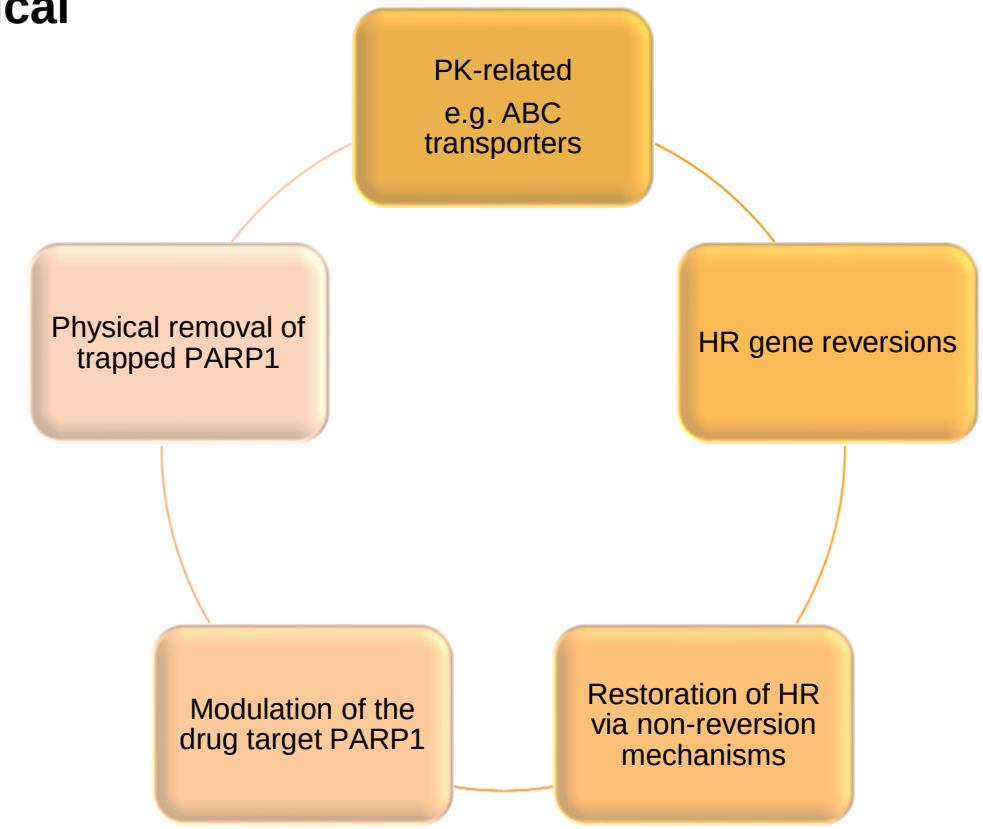




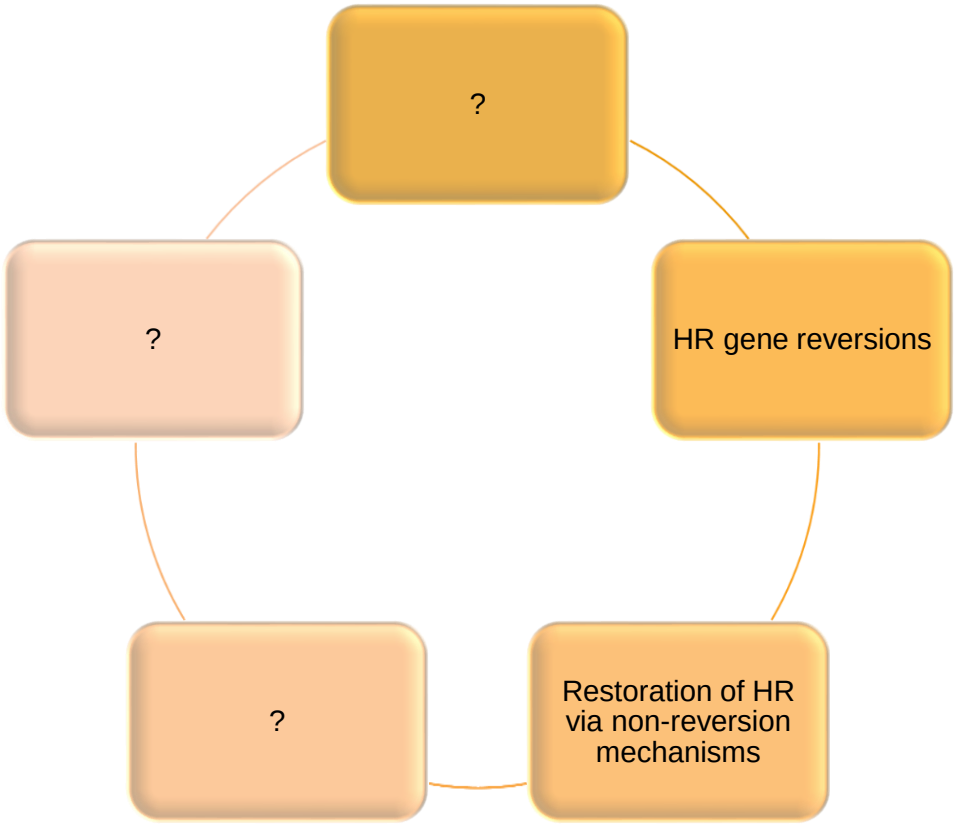
Remaining questions

Clinical vs. pre-clinical resistance

Pre-clinical



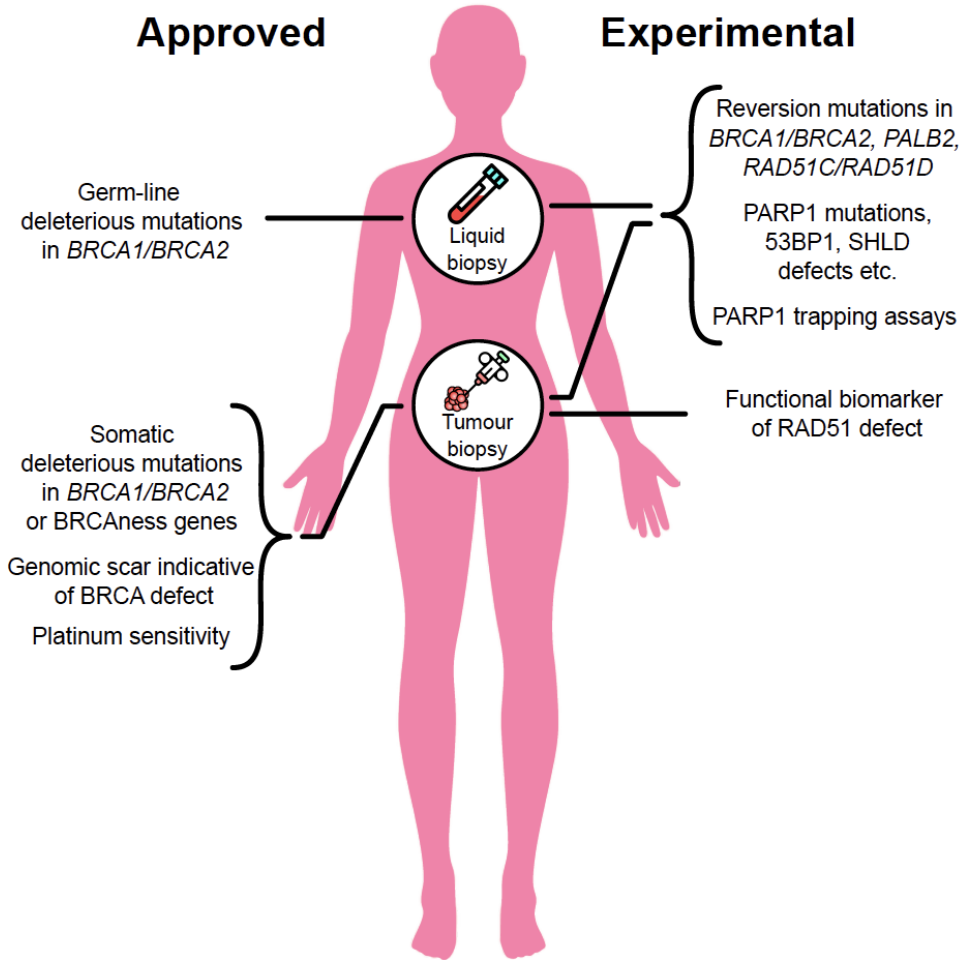
Clinical



Baxter JS, Zatreanu D, Pettitt SJ, Lord CJ. Mol Oncol. 2022 Nov;16(21):3811-3827.

Which biomarkers are required to refine the best use of PARPi?

- Making the distinction between different *BRCA1* and *BRCA2* mutations and different HR-associated genes
- Clinical-grade assays for identifying reversion mutations
- Biomarkers that detect non-reversion mechanisms of PARPi resistance
- Biomarkers of HR function
- Biomarkers that predict dose-limiting toxicity



Wicks AJ, Krastev DB, Pettitt SJ, Tutt ANJ, Lord CJ. Open Biol. 2022 Jul;12(7):220118.

How can we prevent or delay PARPi resistance?

- Targeting PARPi resistance when caused by reversion
- Targeting PARPi resistance when caused by non-reversion-based mechanisms
- Using drug combination approaches to target PARPi resistance

Wicks AJ, Krastev DB, Pettitt SJ, Tutt ANJ, Lord CJ. Open Biol. 2022 Jul;12(7):220118.

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