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EXPANDING AND TRANSLATING CANCER SYNTHETIC VULNERABILITIES
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AACR American Association for Cancer Research®

Targeting genome instability in cancer: Inhibition of PolΘ

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Disclosure Information

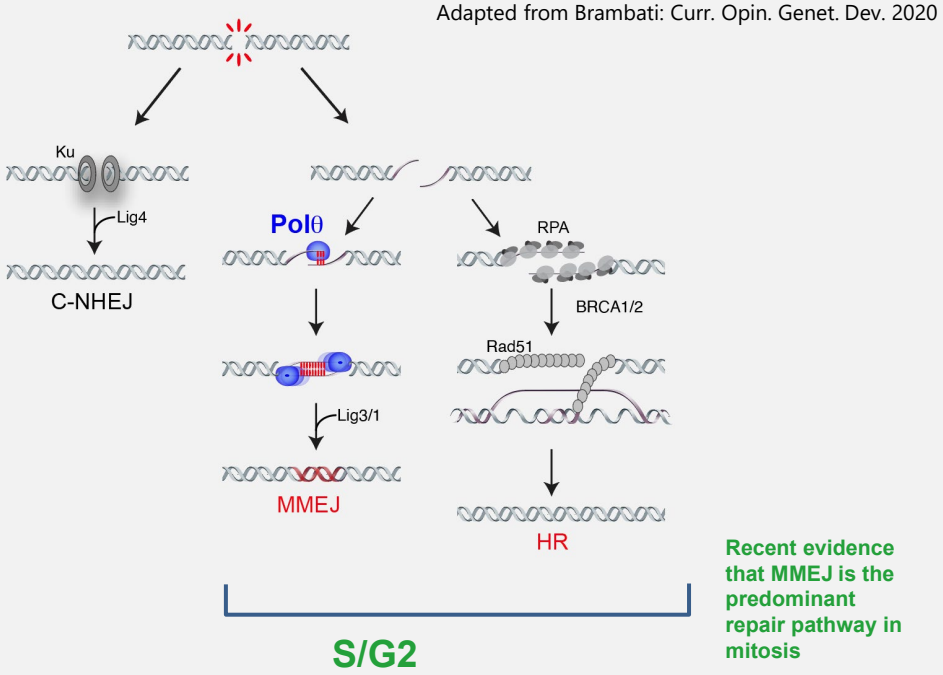
Michael Zinda

I have the following relevant financial relationships to disclose:

Employee of: **Repare Therapeutics**

Stockholder in: **Repare Therapeutics**

Polθ Background



- Unique, multifunctional DNA polymerase with ATP-dependent DNA helicase activity
- Central to microhomology-mediated end joining (MMEJ), a key mechanism of double-strand DNA break repair
- Uniquely active to repair double-strand DNA breaks during mitosis
- Minimally expressed in normal tissue and knockout animals are viable, fertile and exhibit some level of genome instability

MMEJ (Polθ) genomic signatures in BRCA/HRD tumors

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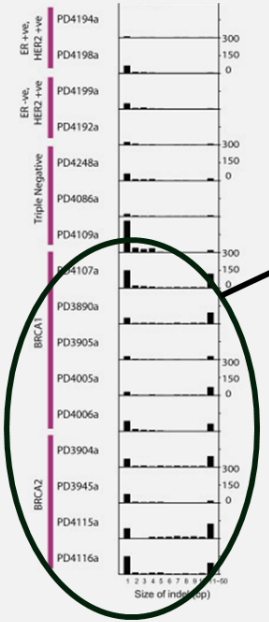
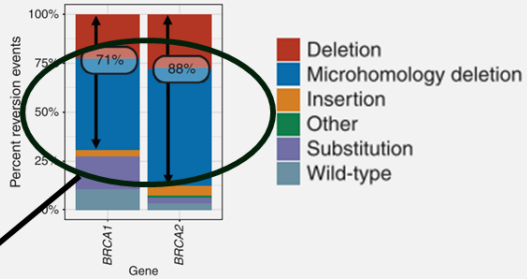
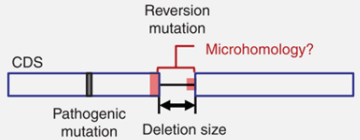


Nik-Zainal et al., 2012

MMEJ genomic signatures in HR-deficient tumors at diagnosis

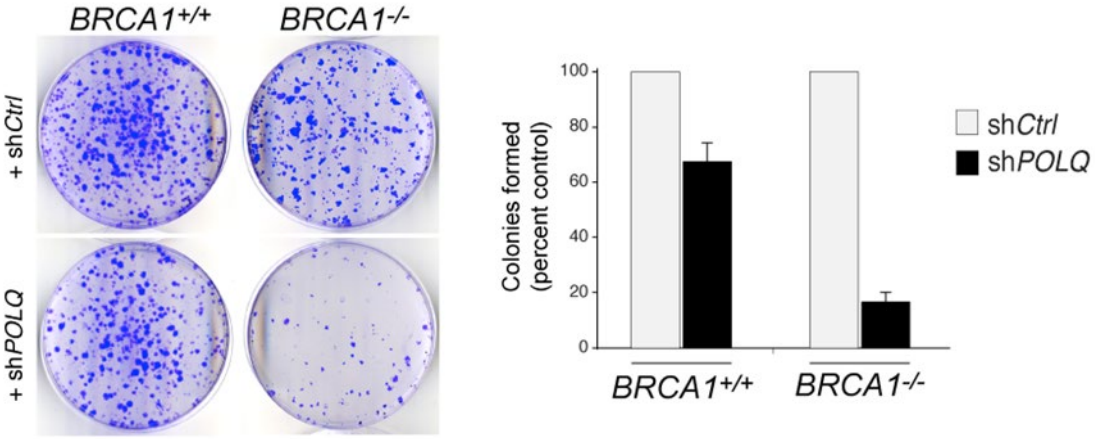
≥50% of BRCA1/2 clinical reversions are generated through MMEJ – highlighting role of Polθ in late-stage cancer





Pettitt et al., Cancer Discovery 2020

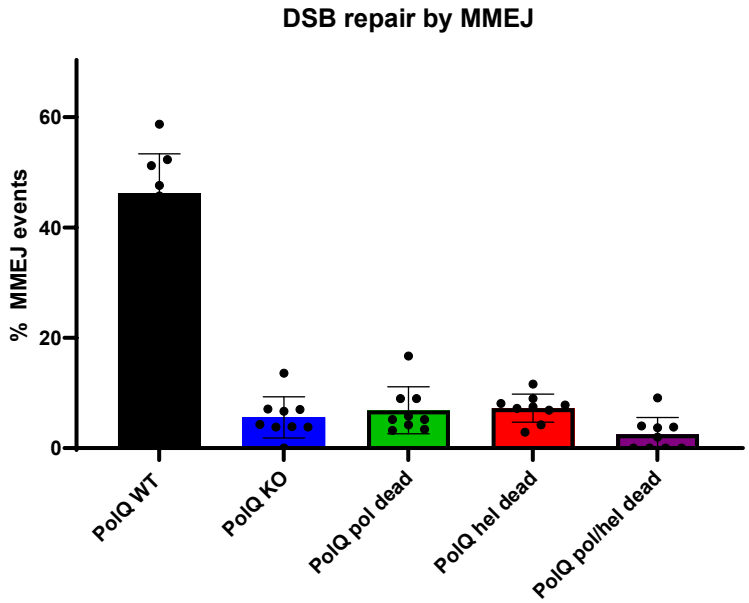
Polθ promotes survival of BRCA deficient cells



PolQ deficiency synthetic with:
ATM *(Shima et al. 2004)*
53BP1 *(Wyatt et al. 2016)*
Ku70/80 *(Wyatt et al. 2016)*

Mateos-Gomez et al. 2015 (HCC1937 cells)
Similar results in FANCD2 model *(Ceccaldi et al. 2015)*

Helicase and polymerase domains are both essential for Polθ cellular activity



PolQ Polymerase dead: D2540A, E2541A (Yoon et al. 2014)
PolQ Helicase dead: K121M (Ozdemir et al. 2018)
PolQ double-mutant: K121M, D2540A,E2541A

- **Knock-in of helicase or polymerase dead mutations equivalently impair MMEJ repair of an engineered DSB**

Protein structures enabled discovery of polymerase and helicase inhibitors

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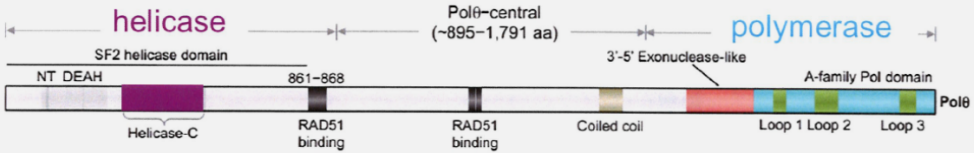
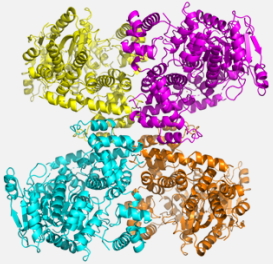
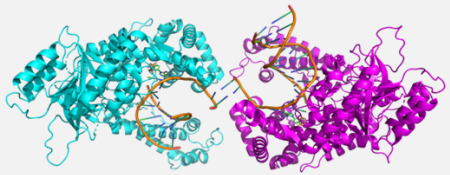
Adapted from Black: Genes 2016





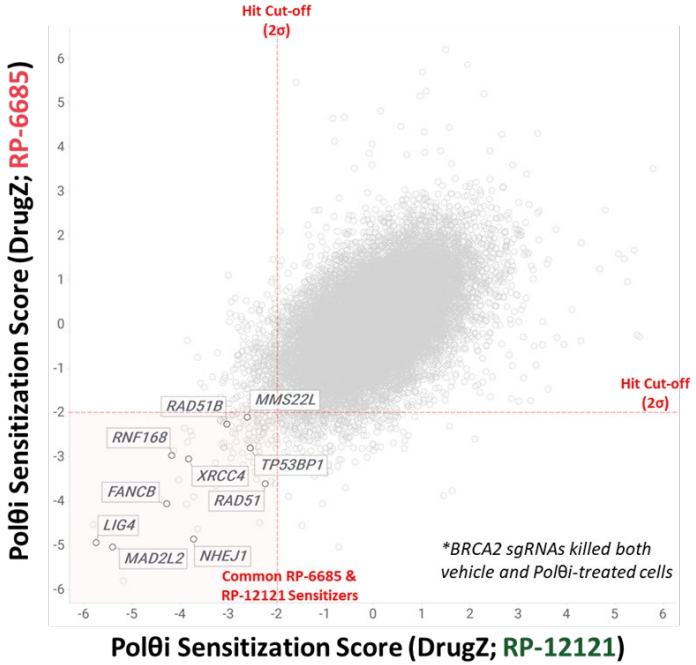
N-Terminal Helicase-Like Domain
Single-Particle Cryo-EM: **2.4 Angstroms**

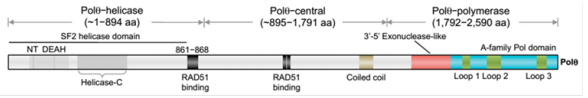


C-Terminal DNA Polymerase Domain
X-ray Crystallography: **2.0 Angstroms**

- **We generated potent and selective Polθ inhibitors against both the helicase and polymerase (requires co-crystals with DNA) domains**

Chemogenomic screens reveal equivalent effects of Helicase and Polymerase inhibitors





ATPase inhibitor
RP-12121

Polymerase inhibitor
RP-6685

- **Polθ polymerase and Helicase inhibitors reveal identical SL interactions in chemogenomic screens – both domains appear to be equivalent**

Repare Polθ helicase inhibitors demonstrate superior cell potency

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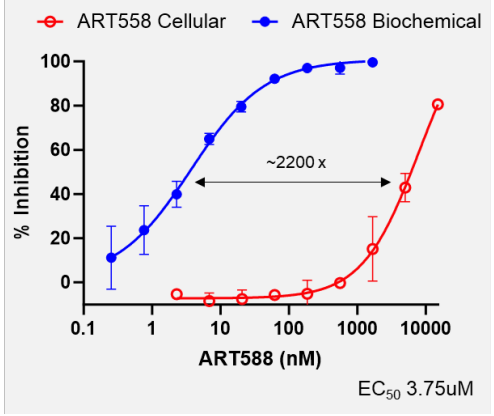
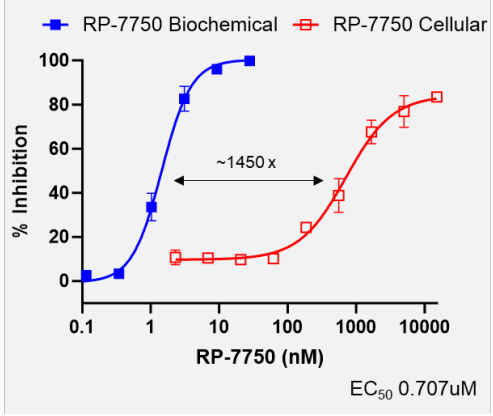
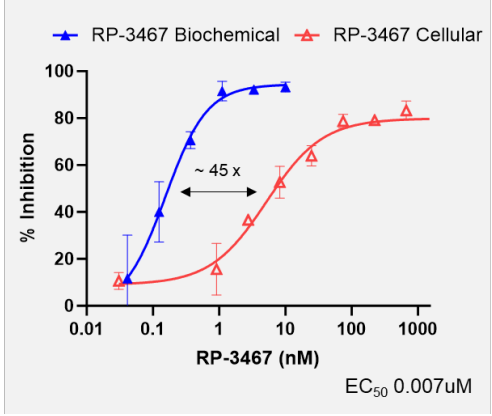
Published Polymerase Inhibitor



Repare Polymerase Inhibitor

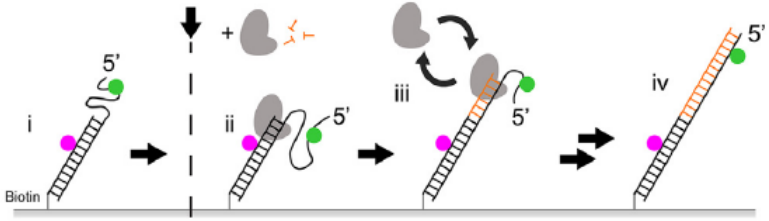


Repare Helicase Inhibitor (RP-3467)

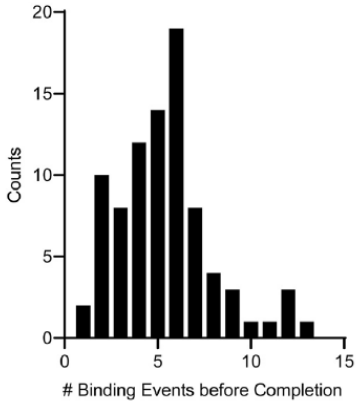


- **Helicase inhibitors demonstrated 100-1000X fold better cellular potency than could be achieved with polymerase-class inhibitors**

Analysis of DNA synthesis in real time at the single-molecule level reveals low processivity



Fijen et al. Mol. Cell 2024



- **Gap-filling DNA synthesis from annealed microhomology involves multiple cycles of Polθ binding and release**
- **Short duration of DNA binding may explain the weak potency of inhibitors acting only on DNA bound Polθ**

RP-3467: A Highly potent, selective and orally bioavailable Polθ helicase inhibitor

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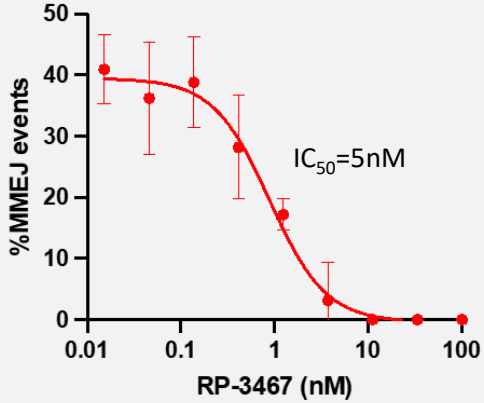
	Parameter	RP-3467
In vitro	Polθ ATPase Enzyme IC_{50}	<0.25 nM
	CETSA cellular target engagement IC_{50}	5 nM
	Cell proliferation DLD1 / HCT116 (BRCA2mt) EC_{50}	4 / 7 nM
	Off-target ATPase (HELQ, WRN, BLM) IC_{50}	> 10 µM
	Off-target Polθ polymerase domain IC_{50}	> 100 µM
ADME	Human Hepatocyte Clearance (µL/min/10^6 cells)	2.1
	Rat PK (%F, $t_{1/2}$)	90%, 13h
	Monkey PK (%F, $t_{1/2}$)	60%, 3h

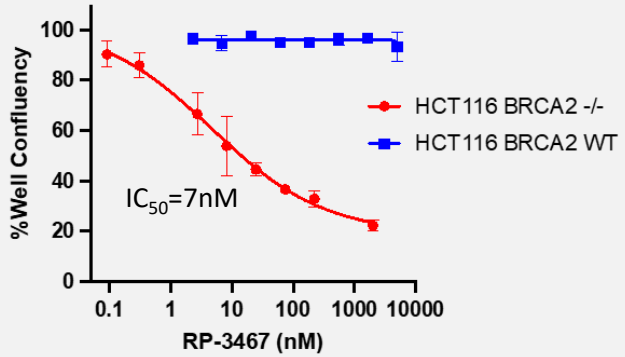
- Clean on PanLabs safety pharmacology screen

Inhibits DNA repair and is synthetic lethal with BRCA2 loss

Inhibition of DNA Repair

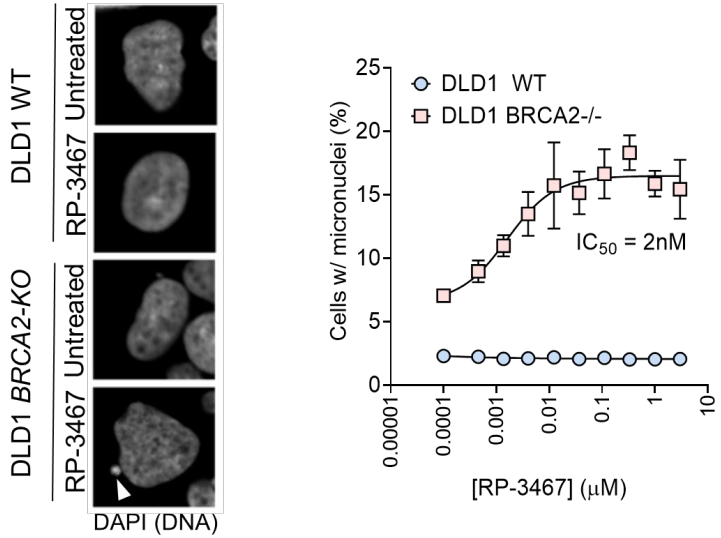


Cell Proliferation HCT116 Isogenic



- Demonstrates potent *in vitro* cellular target engagement and activity
- Huge synthetic lethal window – no effect on BRCA2 WT cells

RP-3467 induces micronuclei in BRCA2-/- cells



- Polθ inhibition induces micronuclei formation in HRD cells
- Micronuclei formation are a biomarker for Polθ inhibition

Monotherapy activity against BRCA2 -/- tumors

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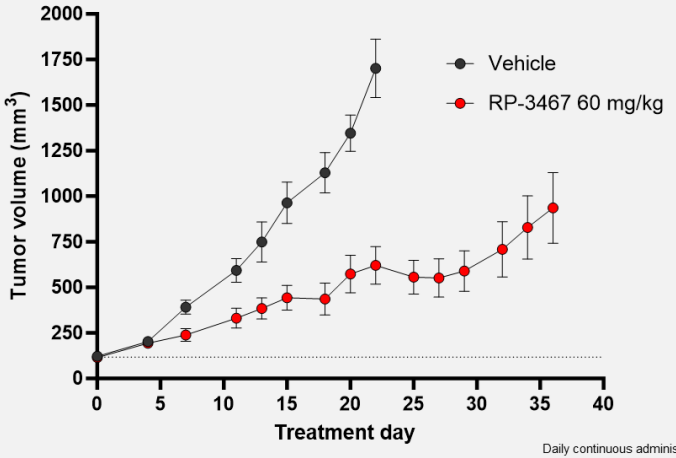
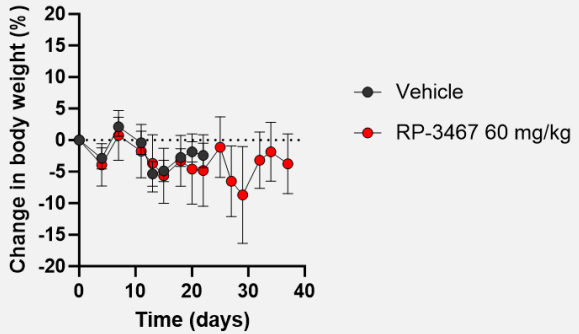
HCT116 BRCA2 -/- tumors

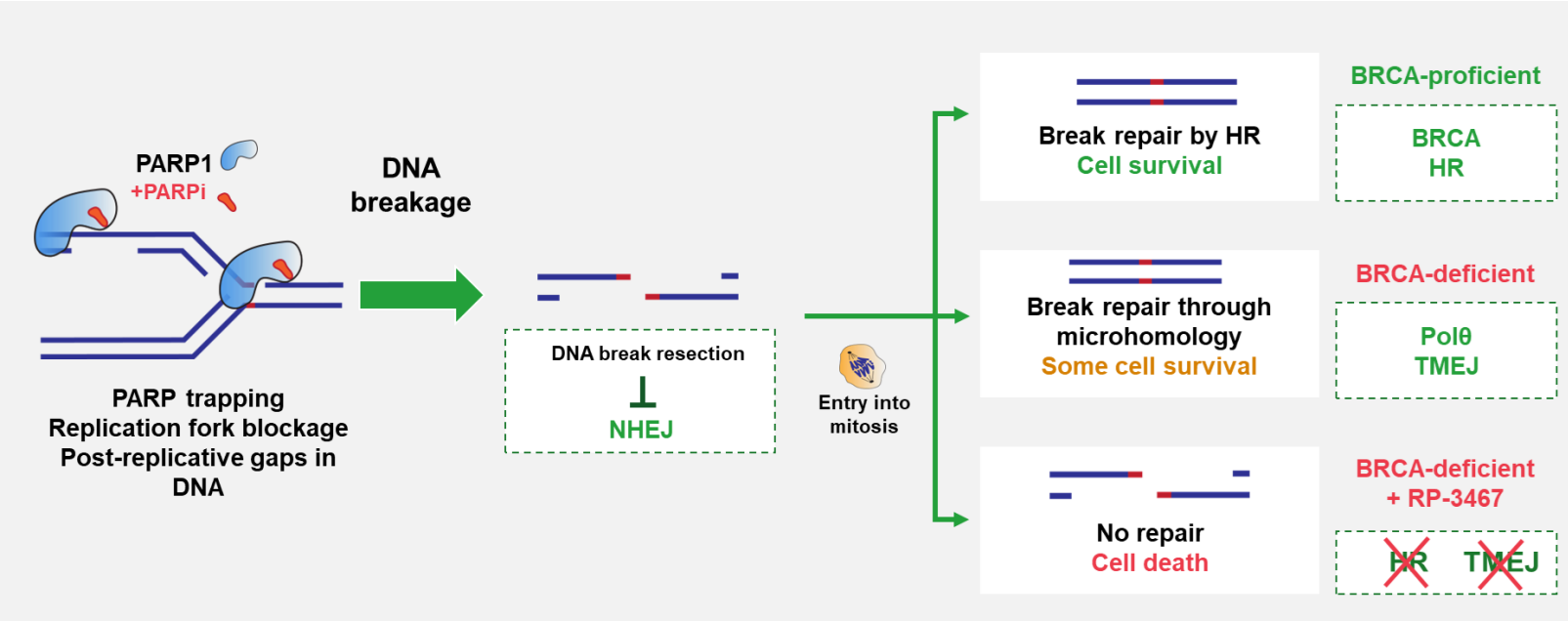


Body Weight



- **Monotherapy tumor growth suppression at a well-tolerated dose of RP-3467**

Rationale for synergy between Polθi and PARPi



- **PARPi + Polθi combination synergizes to kill homologous recombination deficient tumor cells**

RP-3467 drives complete regressions in combo with full-dose olaparib

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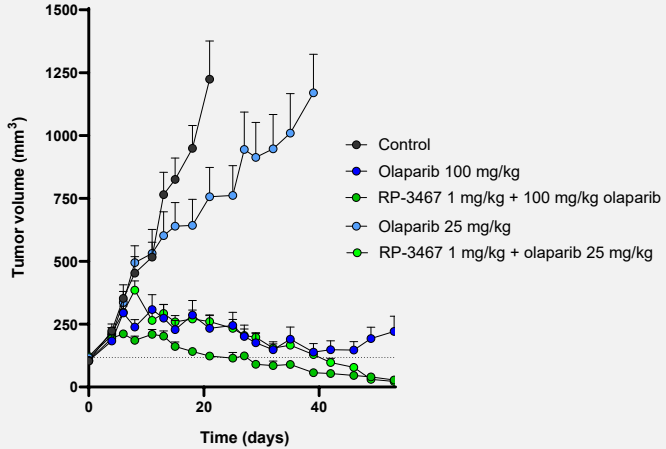
Efficacy HCT116 BRCA2 -/-



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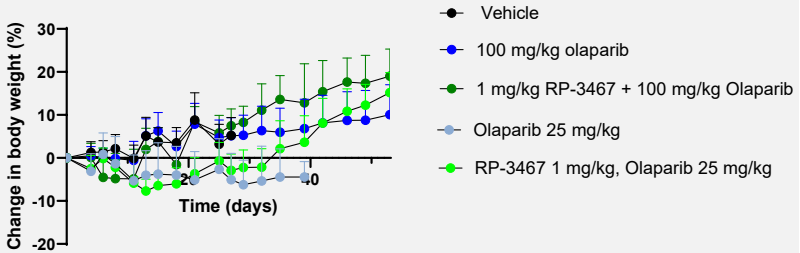

- **Complete regressions with high and low dose Olaparib suggest that RP-3467 will allow PARPi dose reductions**

RP-3467 does not potentiate PARPi in BRCA WT cells

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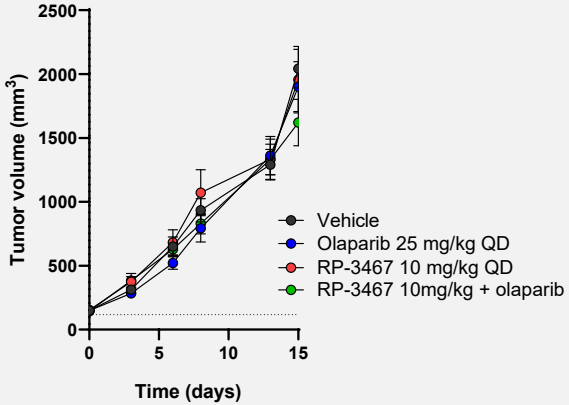
HCT116 WT



- **Lack of effect in HR competent cells supports safety in normal tissues**

Profound, durable synergy with PARP1/2 inhibition

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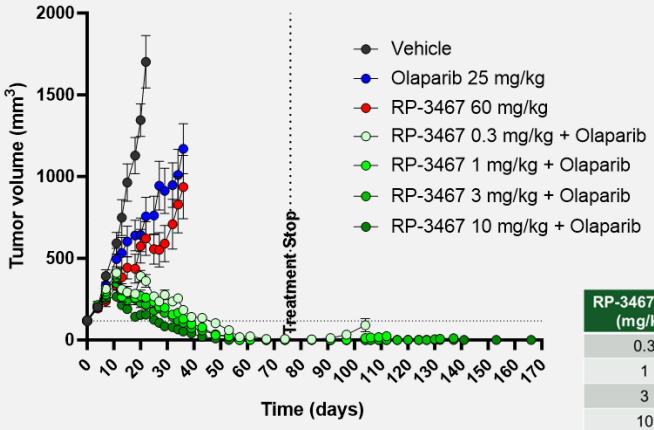
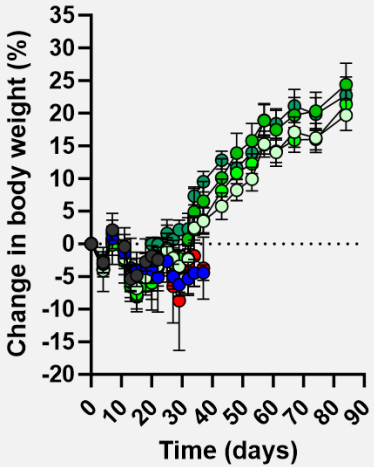
HCT116 BRCA2 -/-



RP-3467 Dose (mg/kg)	Cures
0.3	4/10
1	7/10
3	10/10
10	10/10

Body Weight



- **Deep/durable complete regressions across a wide dose range and extremely well tolerated**

No added hematological toxicity in combination over PARP1/2i alone

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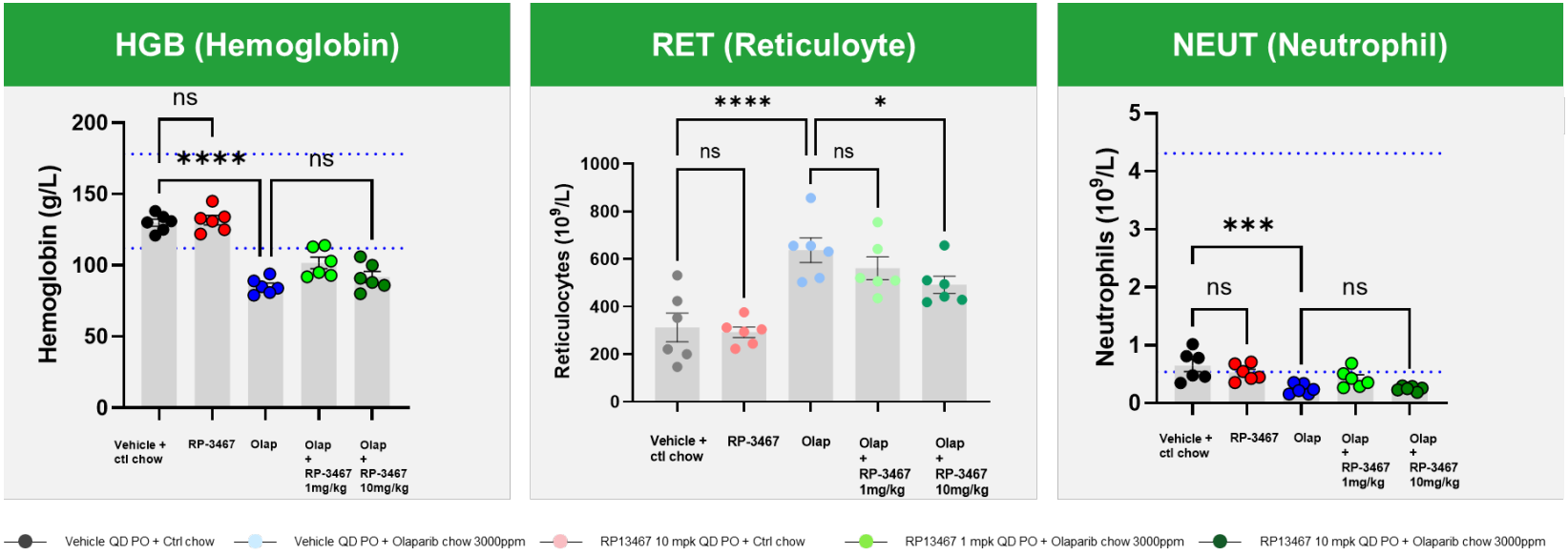
5 weeks co-administration of human clinical PK equivalent dose of olaparib with RP-3467 up to 10mg/kg in CD1 mice



- **Extremely well tolerated combination at relevant olaparib doses**

Synergy with PARPi combinations across BRCA2 null PDX models

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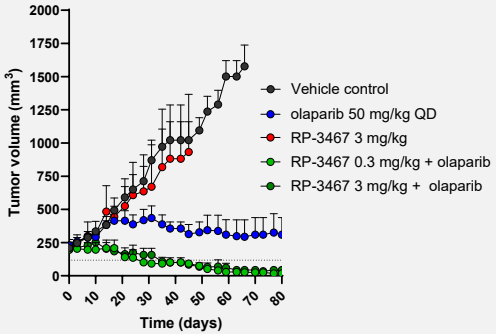
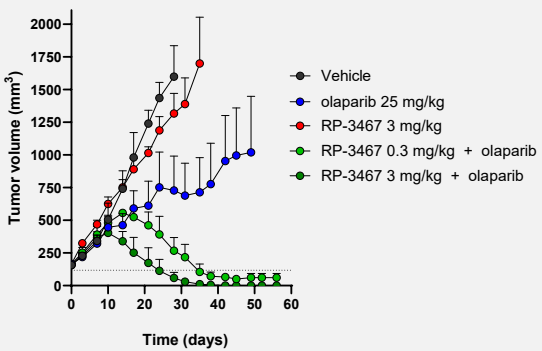
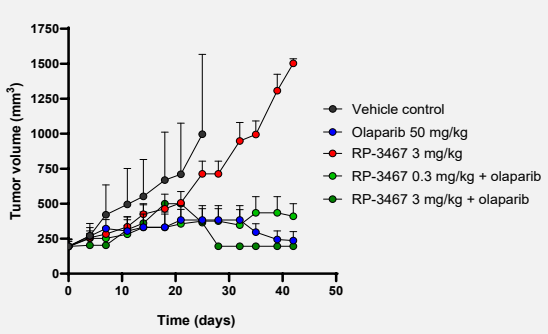
Breast ER+



Breast TNBC



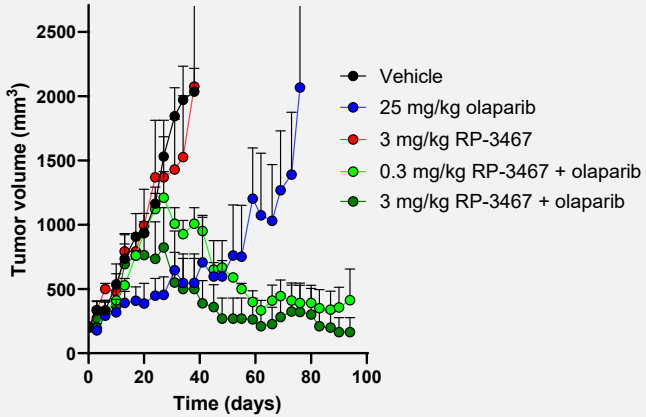
Breast ER+



- **Complete/partial regression in BRCA2 null PDX models**

Synergy with PARP1i combinations in a PALB2 null PDX model

Breast TNBC



- **Partial regression in a PALB2 null PDX models**

Synergy with PARPi combinations across BRCA1 null PDX models

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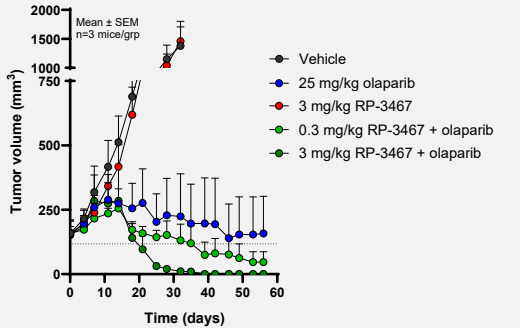
Breast TNBC



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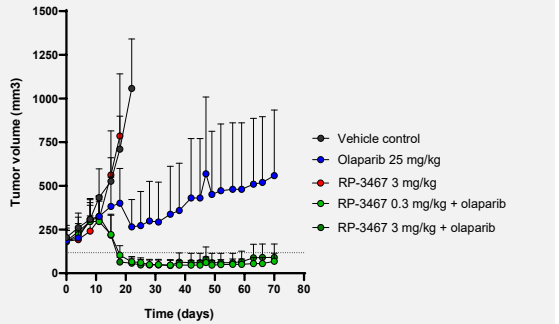
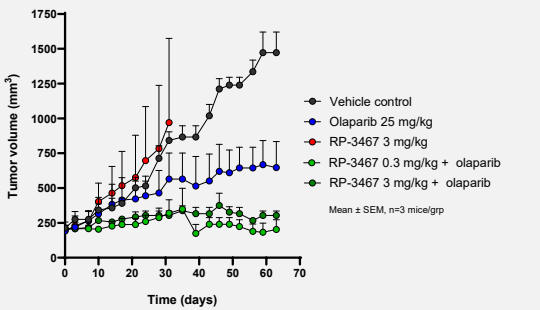
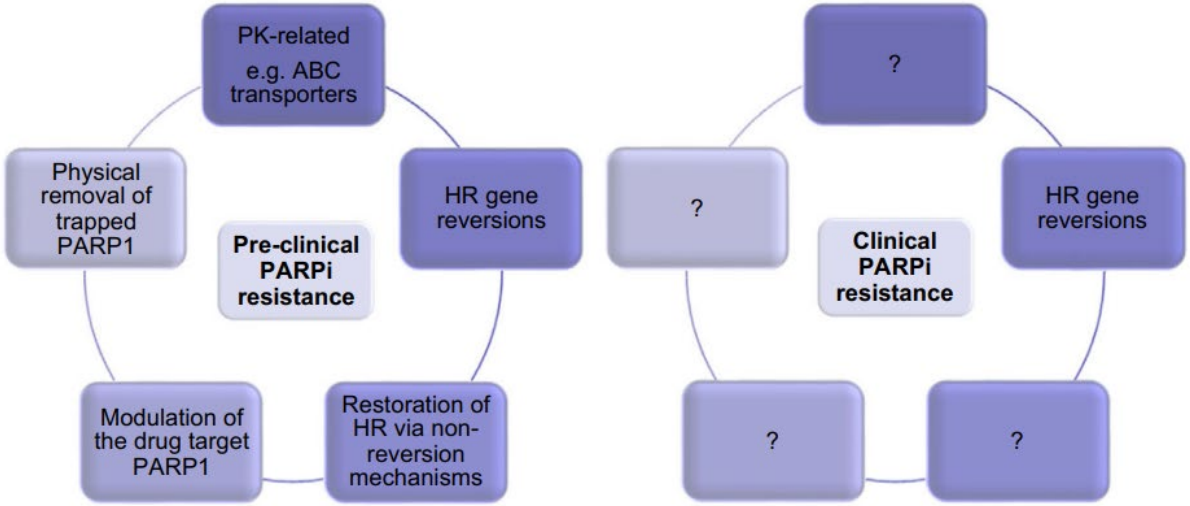


Breast TNBC



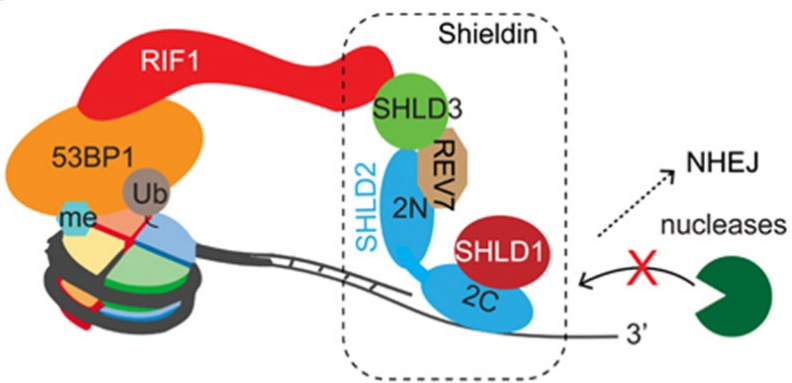
- **Complete/partial regression in BRCA1 null PDX models**

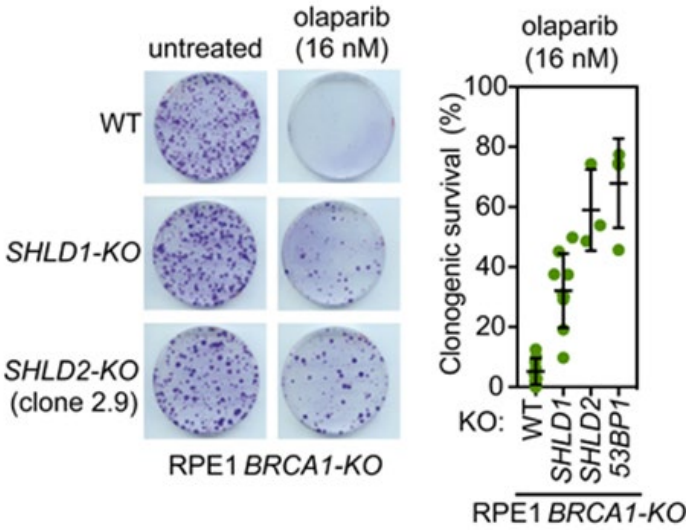
Mechanisms of resistance to PARPi



J. S. Baxter et al. Molecular Oncology (2022)

53BP1/Shieldin Loss: A potential mechanism of PARPi resistance



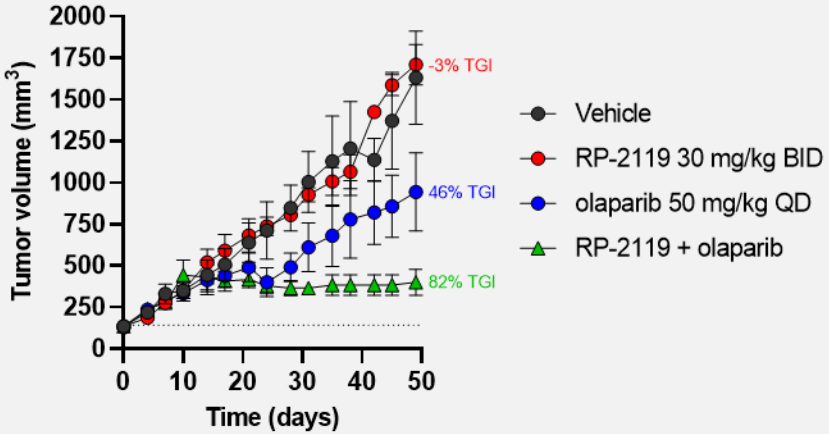


Noordermeer et al. (2018) Nature

- **Loss of components of the 53BP1 pathway results in PARPi resistance**

Polθi is active in PARPi resistant PDX model

Breast TNBC (BRCA1mt, SHLD2 LOF)



- PARPi + Polθi synergize in tumors with alterations of the Shieldin complex (a mechanism of PARPi resistance)

Durable synergy with PARPi inhibition in a BRCA1 null CDX

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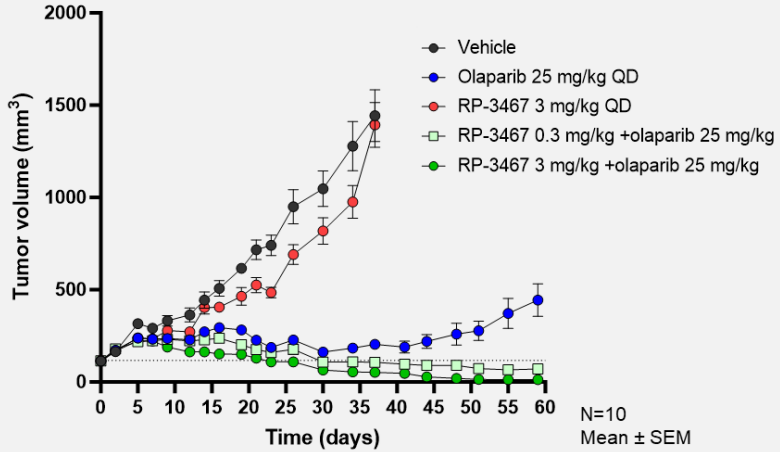
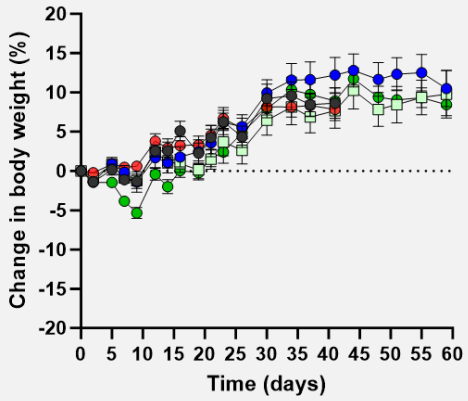
MDA-MB-436



Body Weight



- **RP-3467 and olaparib co-treatment results in tumor regressions in a BRCA1 deficient model**

Tumors regrowing on Olaparib are sensitive to RP-3467 + PARPi combo

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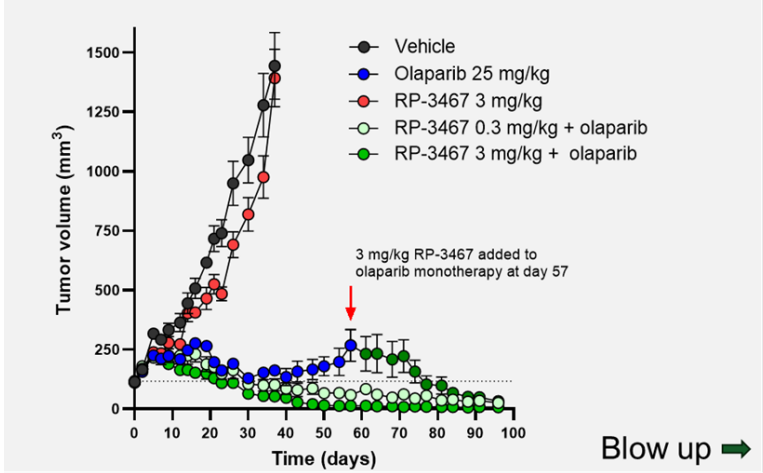
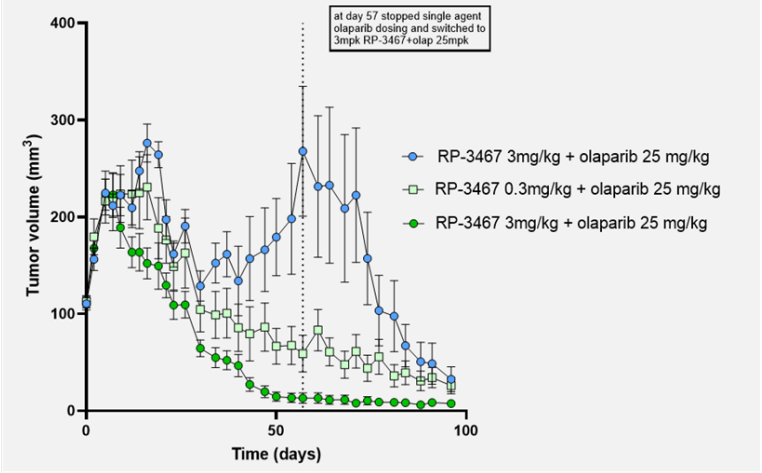
RP-3467- causes regression in MDA-MB-436 BRCA1 deficient tumors



Combination with RP-3467 provides regression in tumors that escape olaparib single agent treatment



- **Tumors that escape single-agent therapy can be successfully retreated with the combination**

Polθ Inhibition: Summary

- **Phase 1 clinical trial initiation expected in 2H 2024**
- **Primary Goal: PK, safety and recommended Phase 2 dose**

Synthetic lethal opportunity – homologous recombination deficient (HRD) genetic alterations

Exciting combination opportunity – Polθ inhibition is extremely well tolerated preclinically, with no expected overlapping toxicities

PARPi combinations – Upfront in HRD driven prostate, ovarian, breast and pancreatic cancer, innate/acquired PARPi resistance

Radioligand Therapy (RLT) – Potential for *unselected* RLT combinations and external beam irradiation

Chemotherapy/ADCs – Combinations with dsDNA break inducing chemo therapies (e.g. first line ovarian (CarboTaxol), ADC therapies with topoisomerase payloads)

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Danielle Henry
David Bendahan
Eisha Jain
Evelyne Dietrich
Frank Sicheri
Gino Ferraro
Helen Burston
Hyeyeon Kim
Jesse Leblanc
Jessica Desjardins
Jordan Young
Julian Bowlan
Marie-Eve Leclaire
Michal Zimmermann
Michel Gallant
Mike Zinda
Pavel Mader
Philippe Mochirian
Robert Houle
Robert Papp
Sara Fournier
Shou Yun Yin
Simon Surprenant
Stephen Morris
Yael Mammane