Studying radiotherapy resistance of BRCA1-mutated mammary tumors

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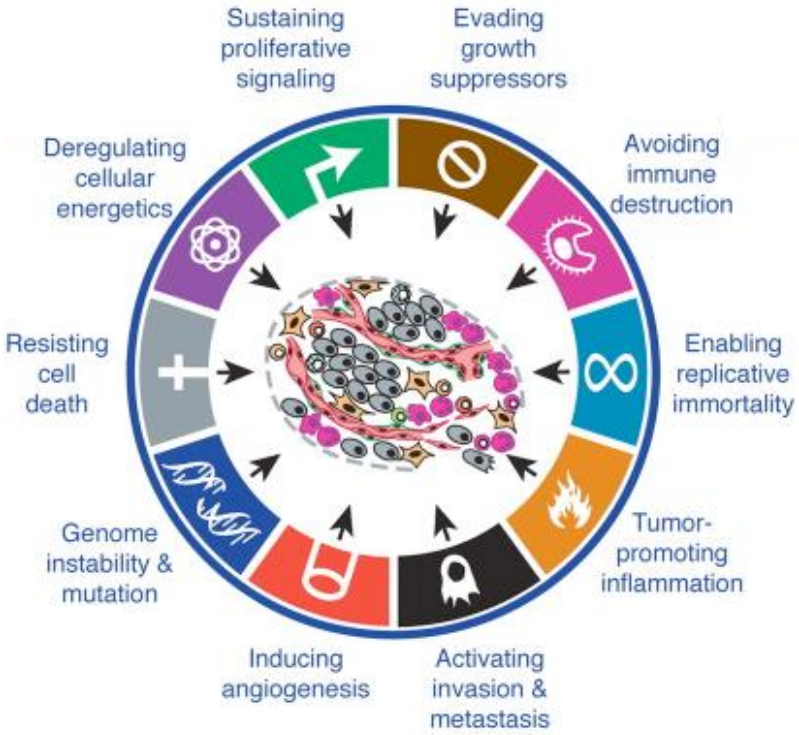
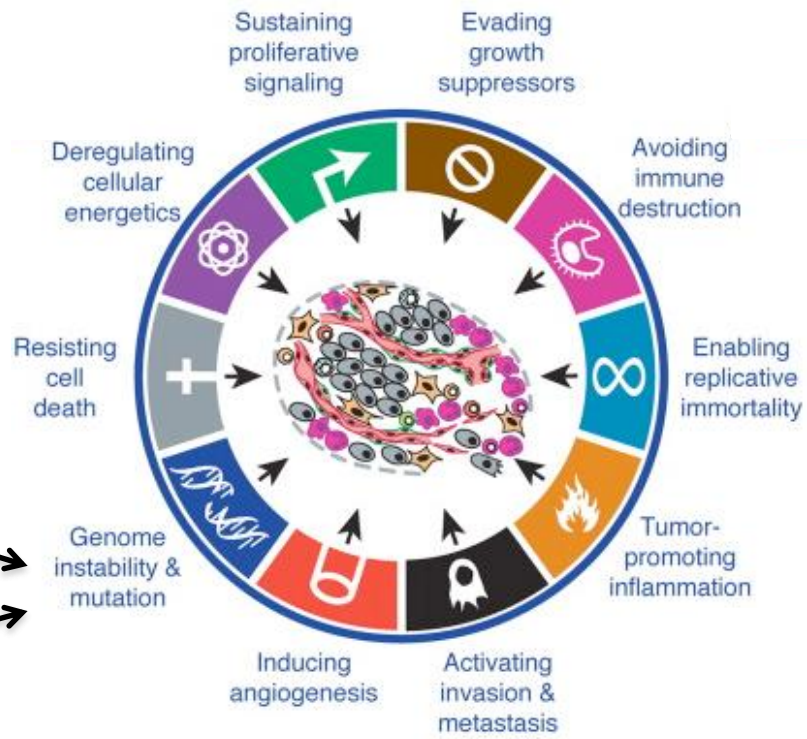
ANTONI VAN LEEUWENHOEK

We have substantial knowledge about what causes cancer



Hanahan & Weinberg
Cell 2011

…and we have powerful weapons to target cancer



radiotherapy

PARP inhibitors

Hanahan & Weinberg
Cell 2011

The painful truth

- Local radiotherapy resistance and the subsequent emergence of distant metastasis remain major obstacles in the clinic

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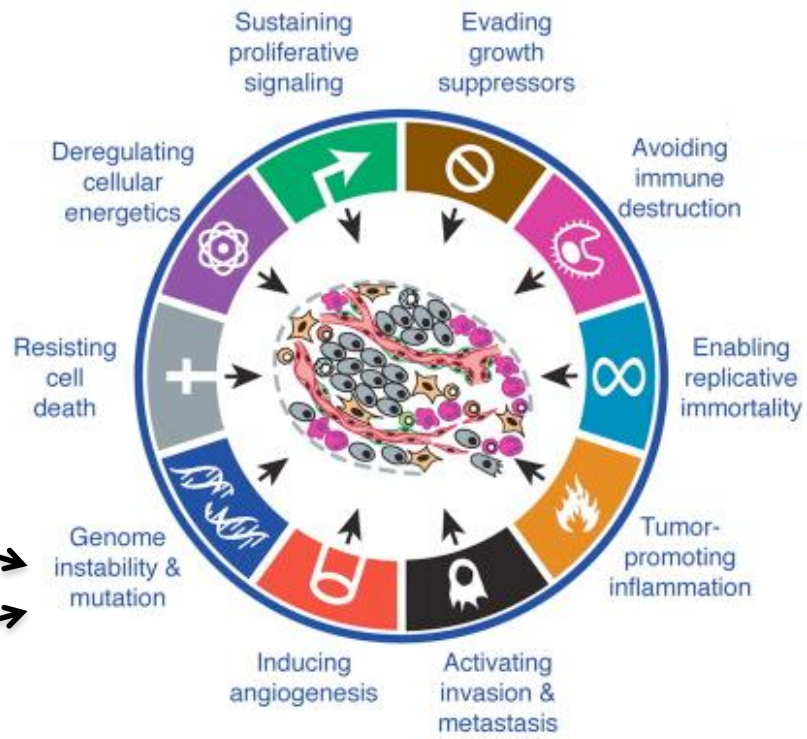
- Most cancer patients who have disseminated cancers cannot be cured and cancer is not easily turned into a chronic disease

- The metastatic tumors are eventually resistant to all anti-cancer therapy available

→ Therapy resistance remains <u>the</u> major handicap in cancer treatment

…and we have powerful weapons to target cancer



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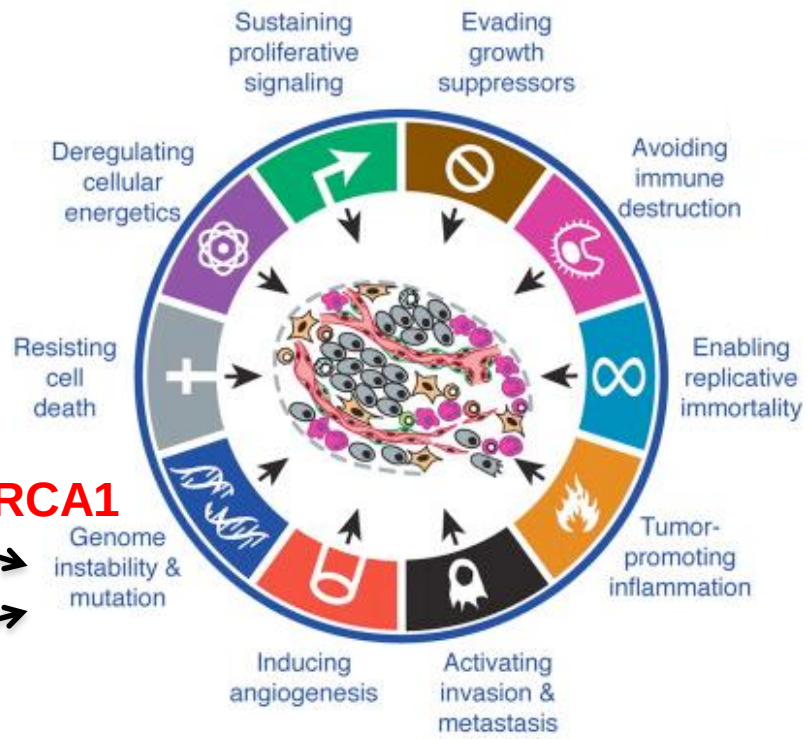


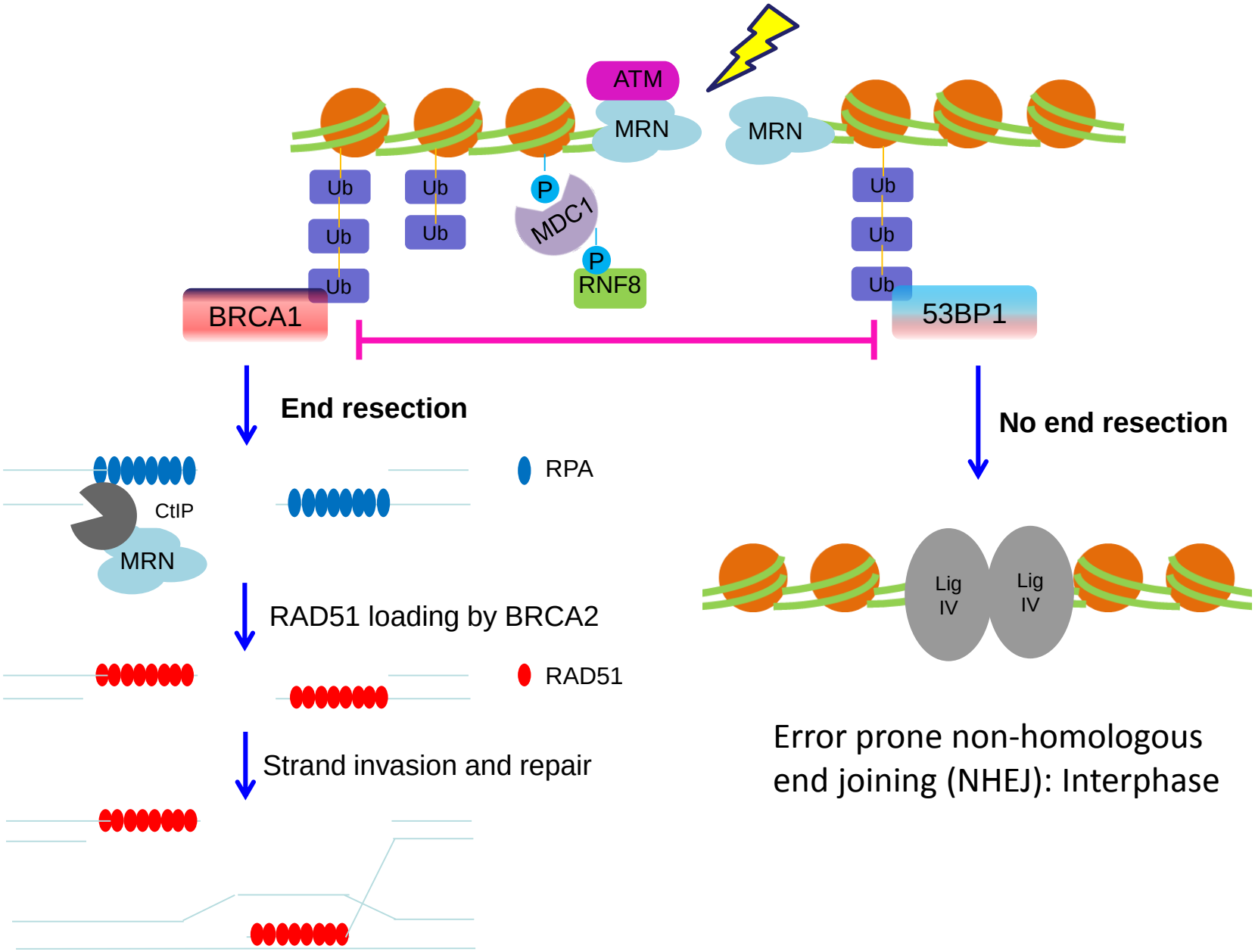
radiotherapy

PARP inhibitors

BRCA1

Hanahan & Weinberg
Cell 2011

BRCA1 plays a crucial role in homologous recombination (HR)



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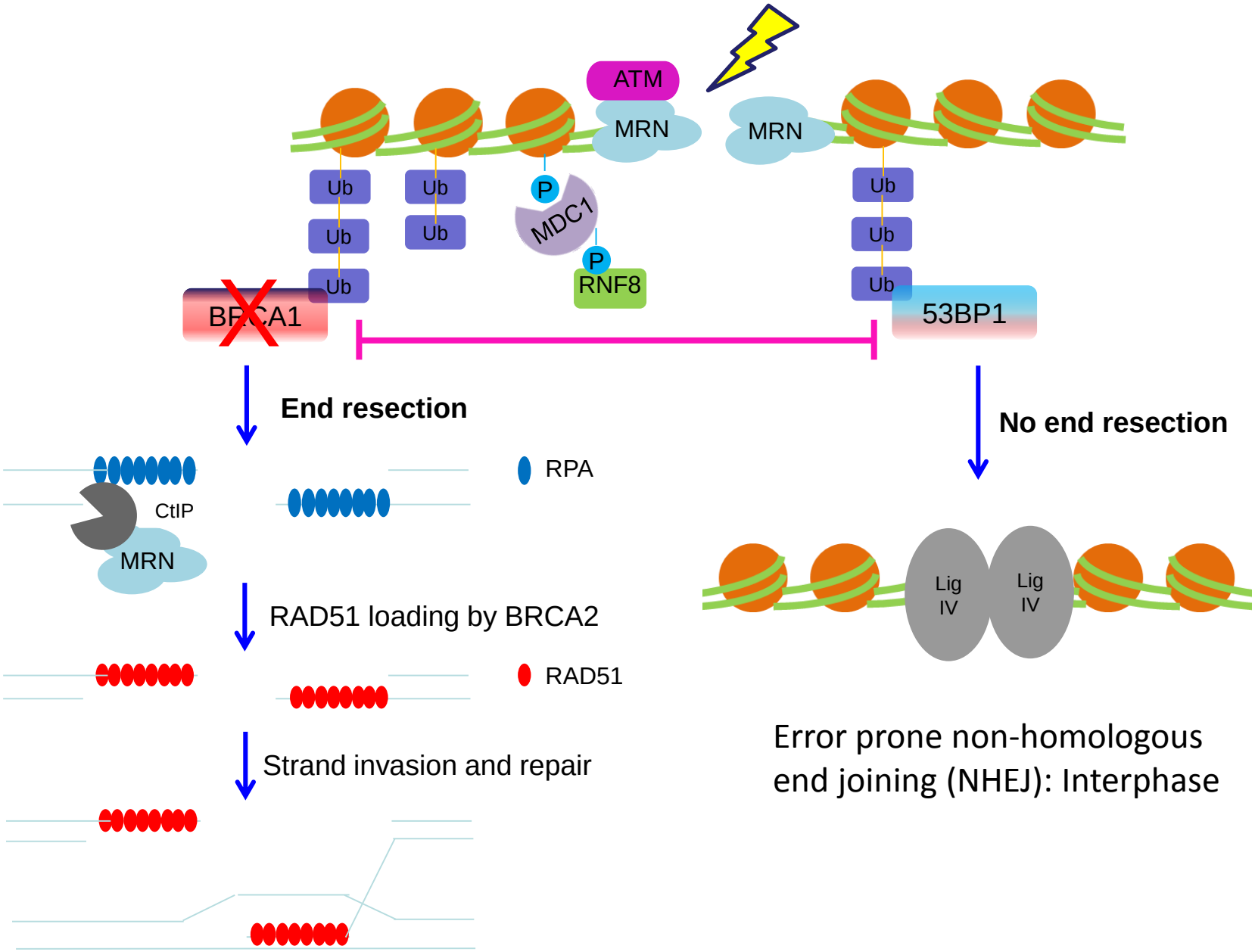

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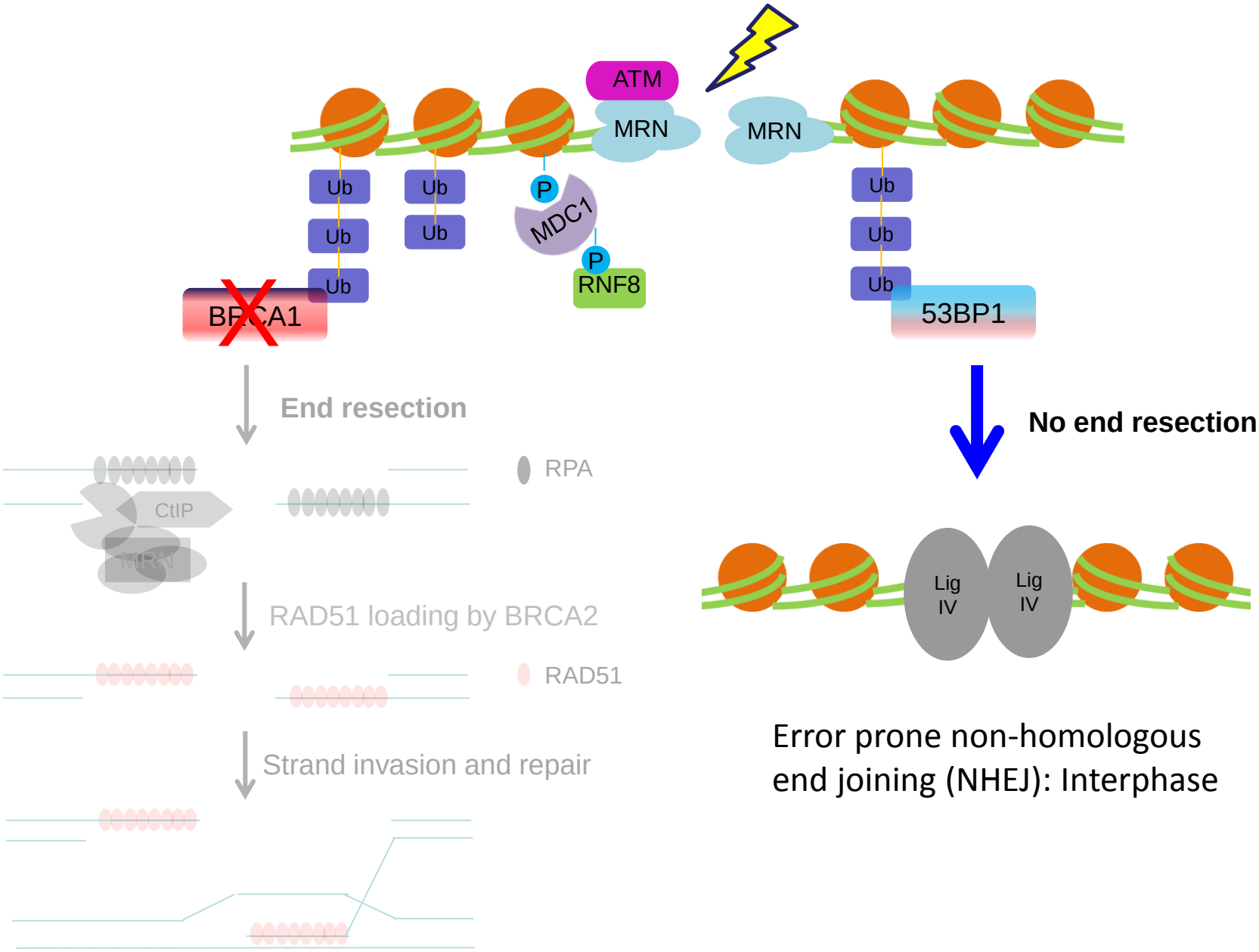
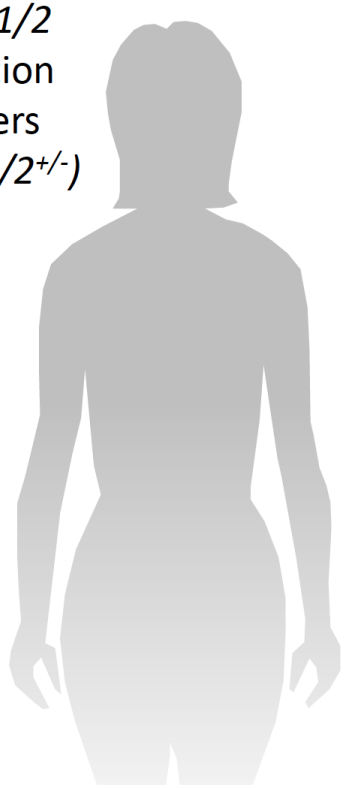


Targeting BRCA1-deficient cancers

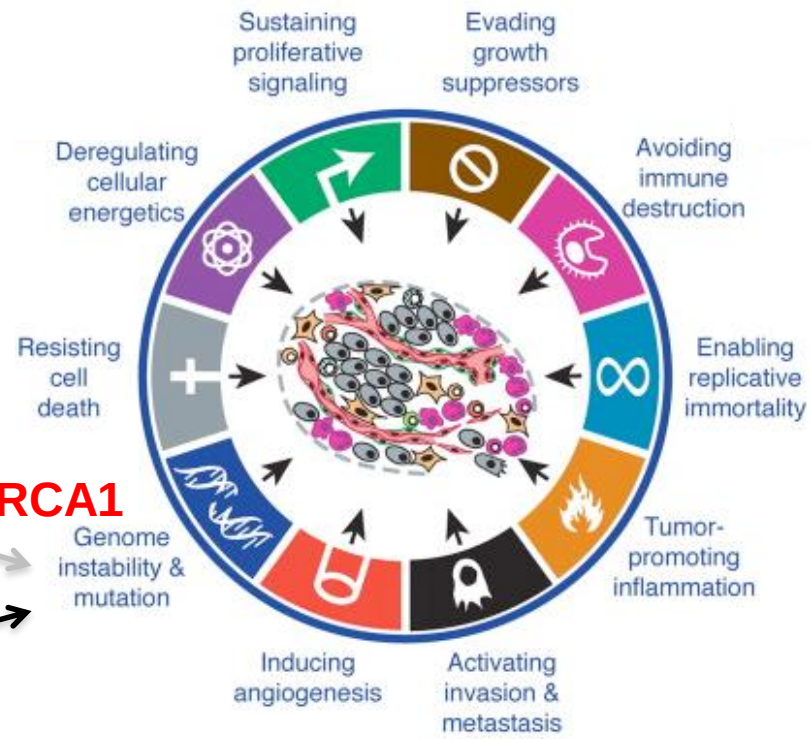
BRCA1/2 mutation carriers (*BRCA1/2*$^{+/-}$)



1. BRCA1-deficiency comprises a substantial fraction of all breast and ovarian cancers (mutation and promoter methylation)
2. BRCA1-deficient tumors have a poor prognosis
3. BRCA1-deficient cells are hypersensitive to DNA double strand breaks
4. This can be exploited by PARP inhibitors or radiotherapy

Kornelia Polyak & Judy Garber, Nature Medicine 17, 283–284 (2011)

…and we have powerful weapons to target cancer



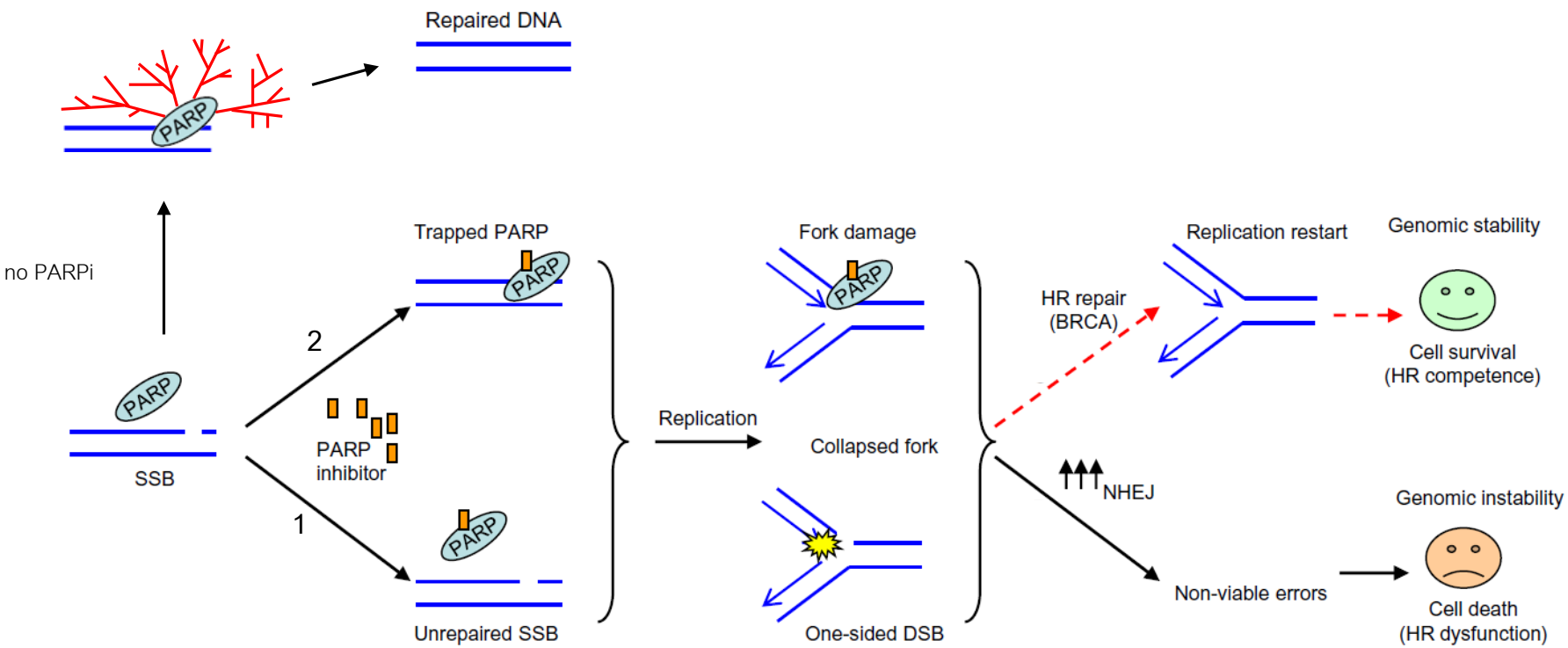
radiotherapy

BRCA1

PARP inhibitors

Hanahan & Weinberg
Cell 2011

The principle of PARP inhibition

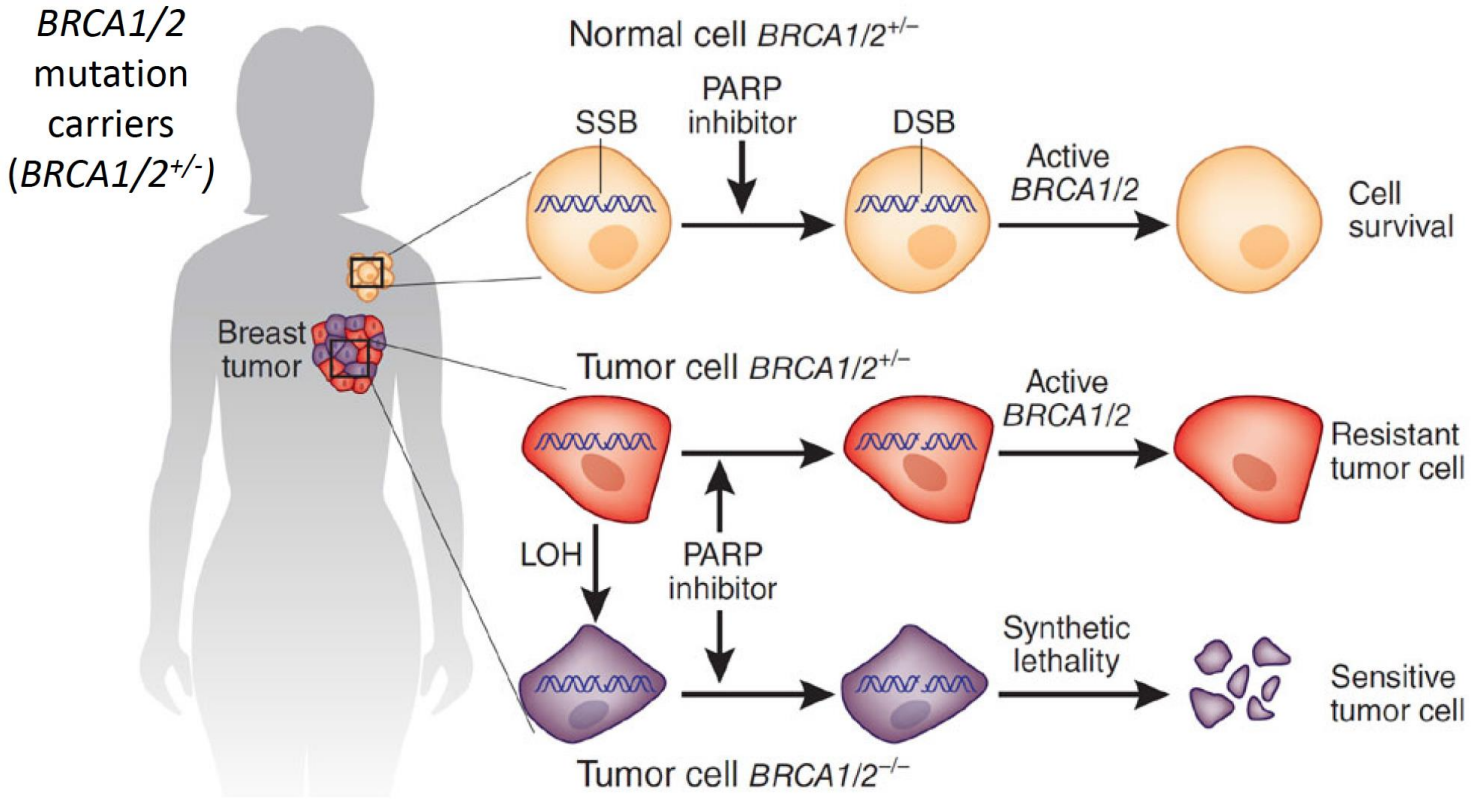


Dual mode of action of PARP inhibitors:

1. catalytic inhibition (prevents PARylation)
2. trapping activity (enzyme poisoning)

Adapted from *Lupo B., Trusolino L., BBA 2014*

BRCA1-deficient cancers are hypersensitive to PARP inhibition



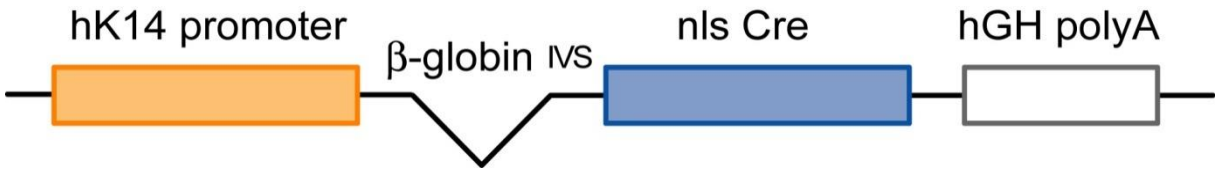
Kornelia Polyak & Judy Garber, Nature Medicine 17, 283–284 (2011)

Keratin 14-Cre, Brca1$^{flox/flox}$, p53$^{flox/flox}$ (KB1P) mouse model

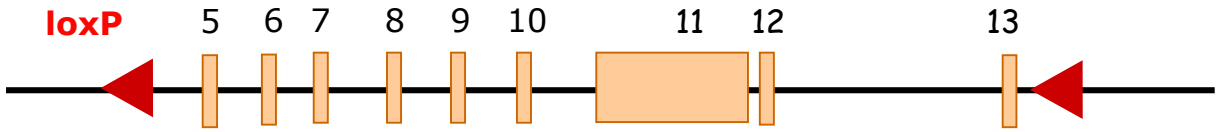
Brca1 ko embryonic lethal

→ Tissue-specific inactivation

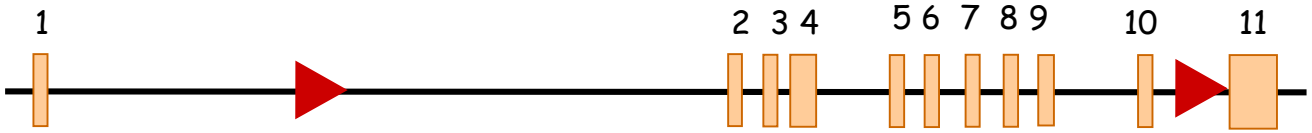
Keratin14-Cre



Brca1^{F}



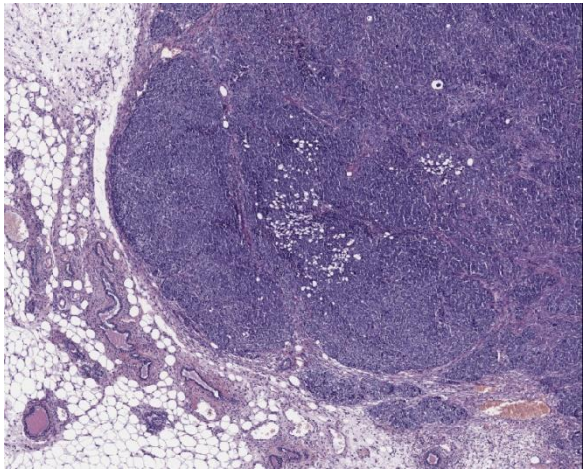
p53^{F}



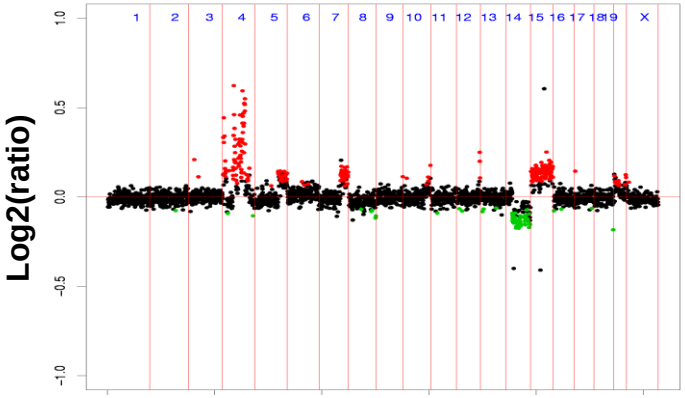
Liu et al. PNAS 2007

BRCA1 mouse mammary tumors resemble human BRCA1-associated breast cancer

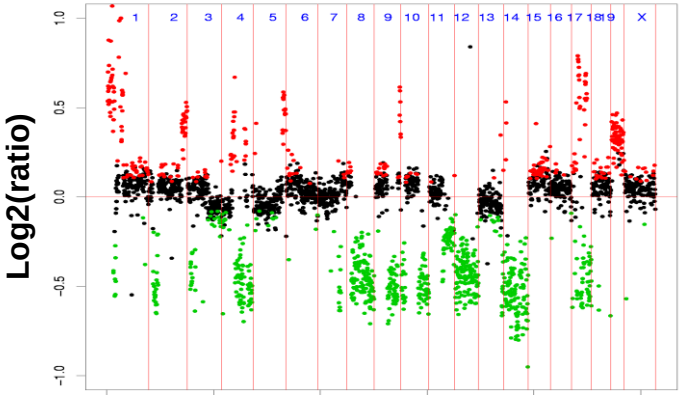
- High grade solid carcinoma (IDC)
- Undifferentiated
- Pushing margins
- ER- PR- and HER2-negative
- Basal-like
- Genomic instability



K14cre;p53$^{F/F}$

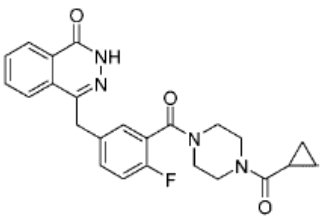


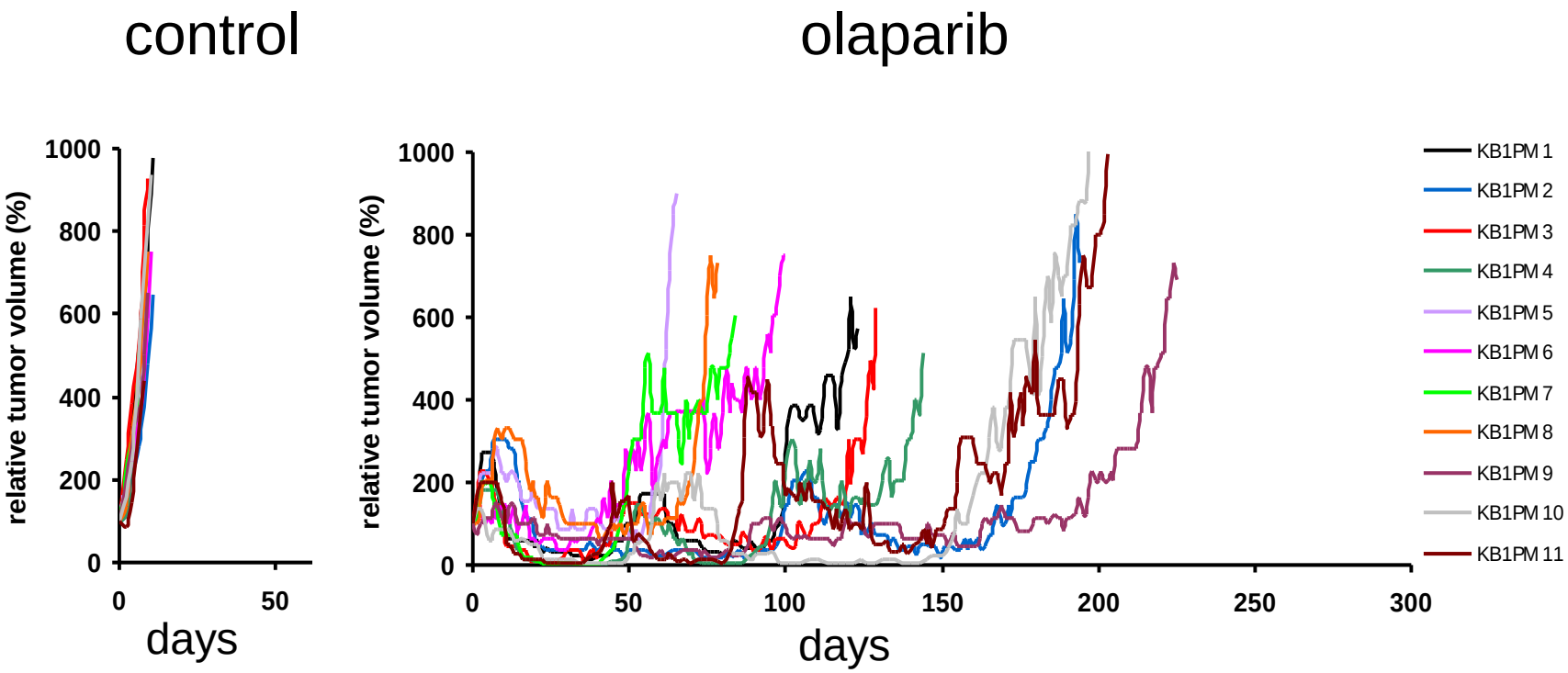
K14cre;Brca1$^{F/F}$;p53$^{F/F}$



Liu et al. PNAS 2007

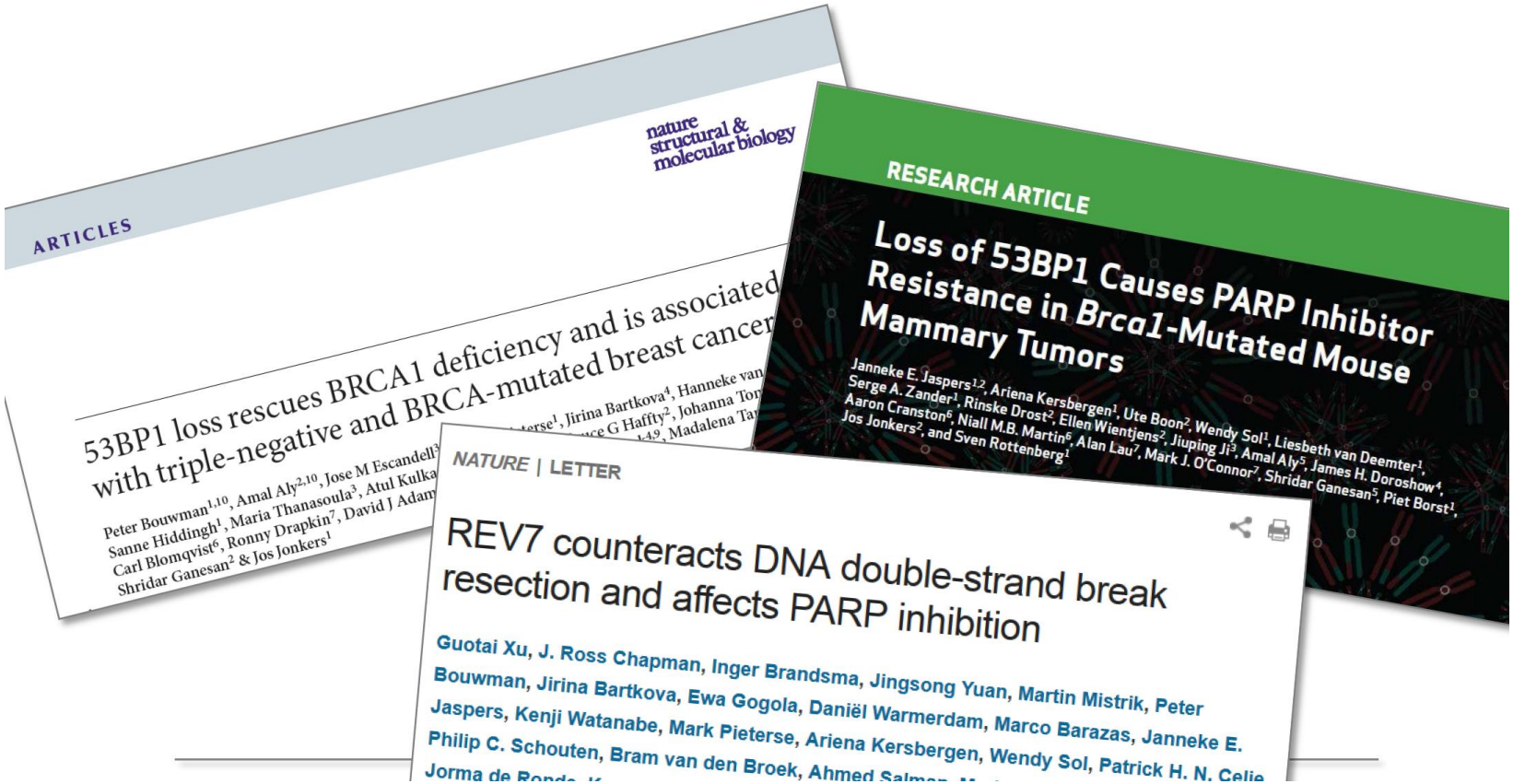
Brca1-deficient mouse mammary tumors are highly sensitive to the PARP inhibitor olaparib, but eventually acquire resistance





Jaspers et al. Cancer Disc. 2013

HR deficiency can be rescued by the loss of additional factors



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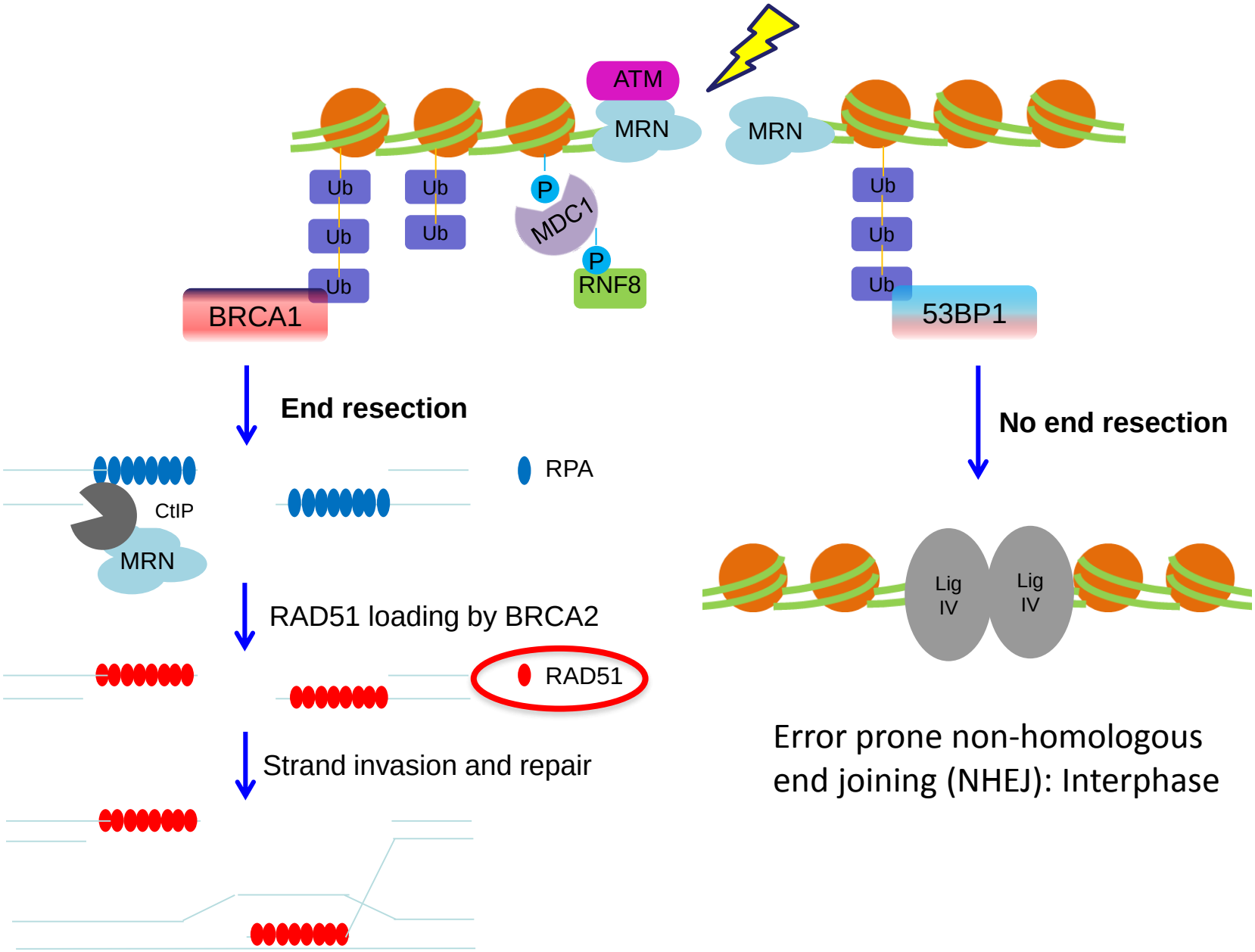

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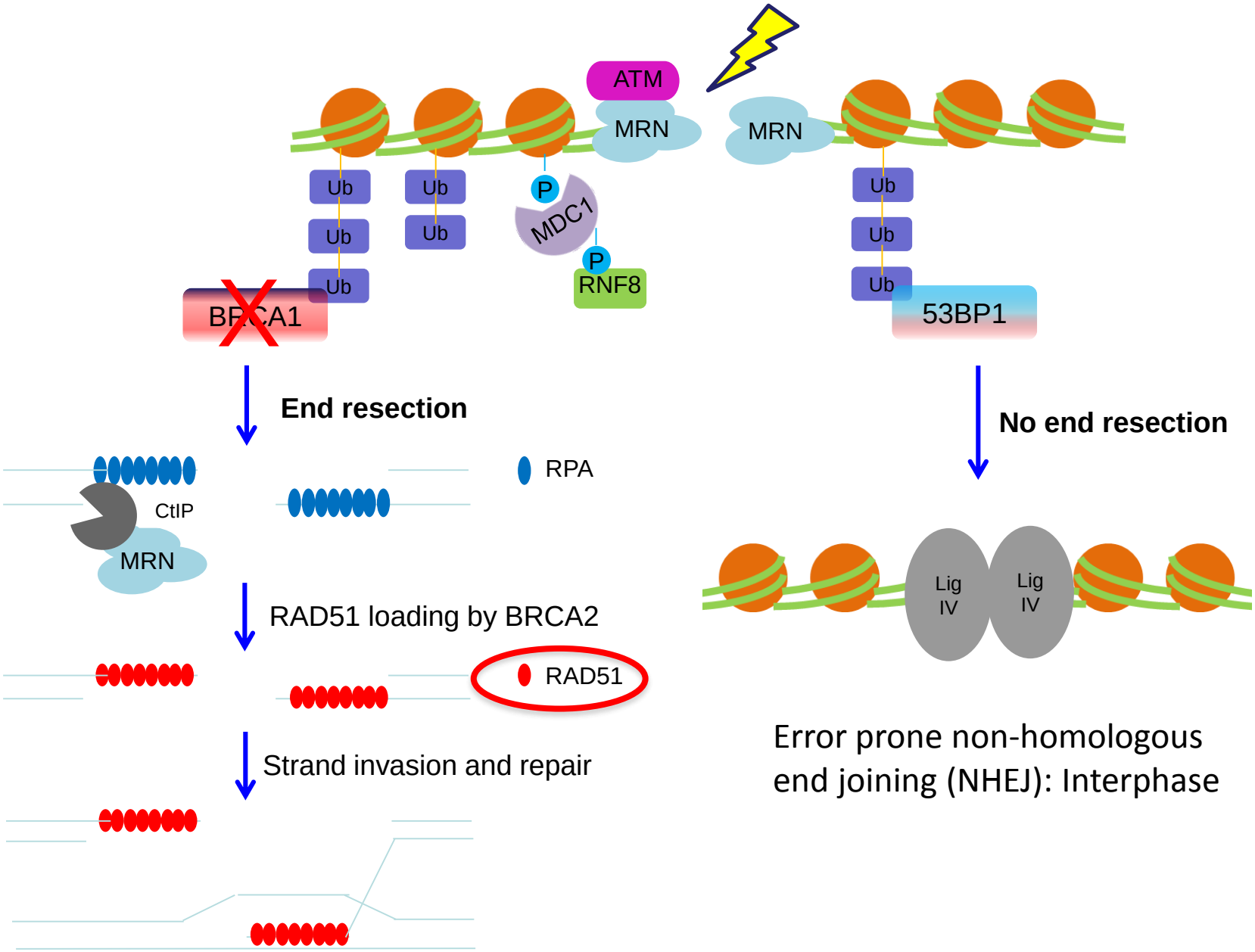

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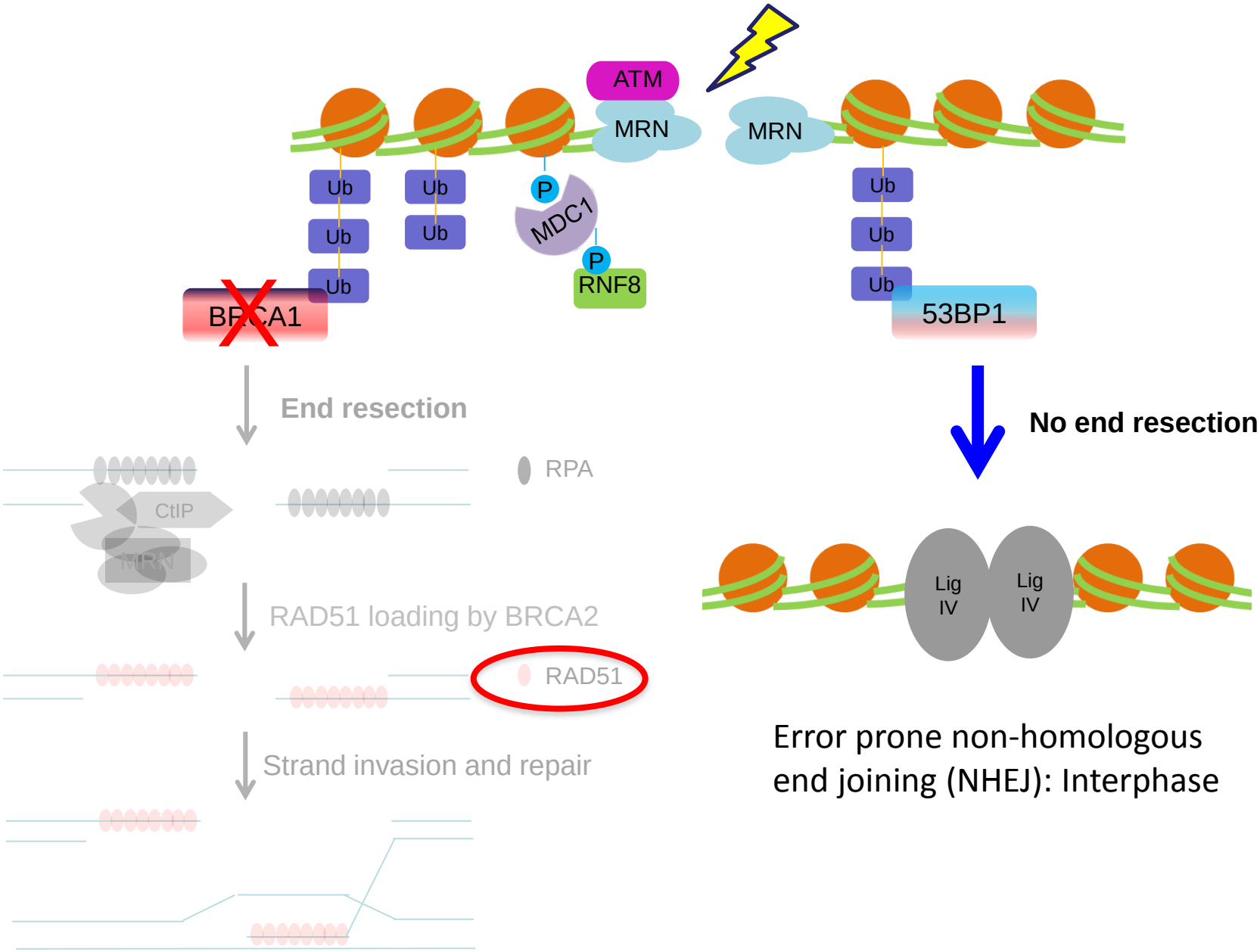
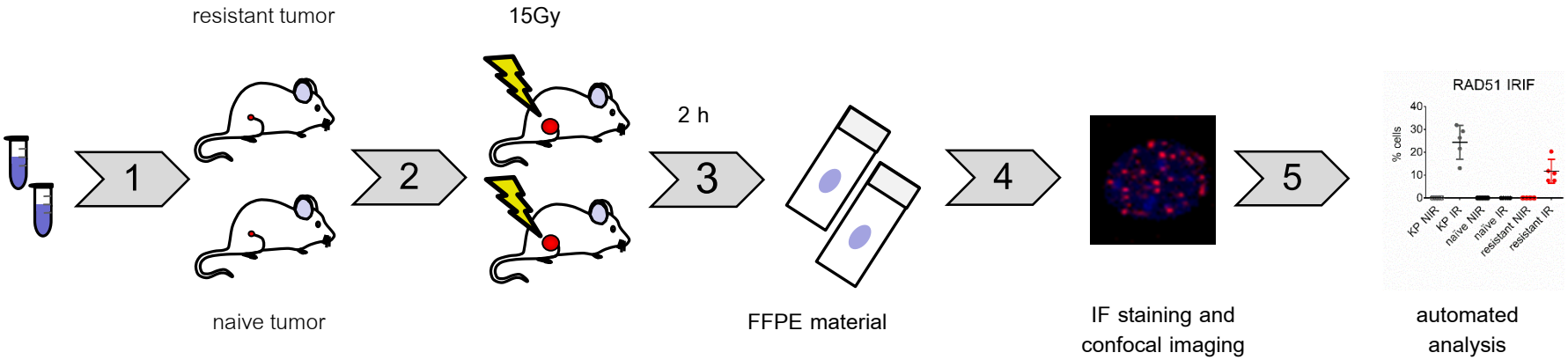


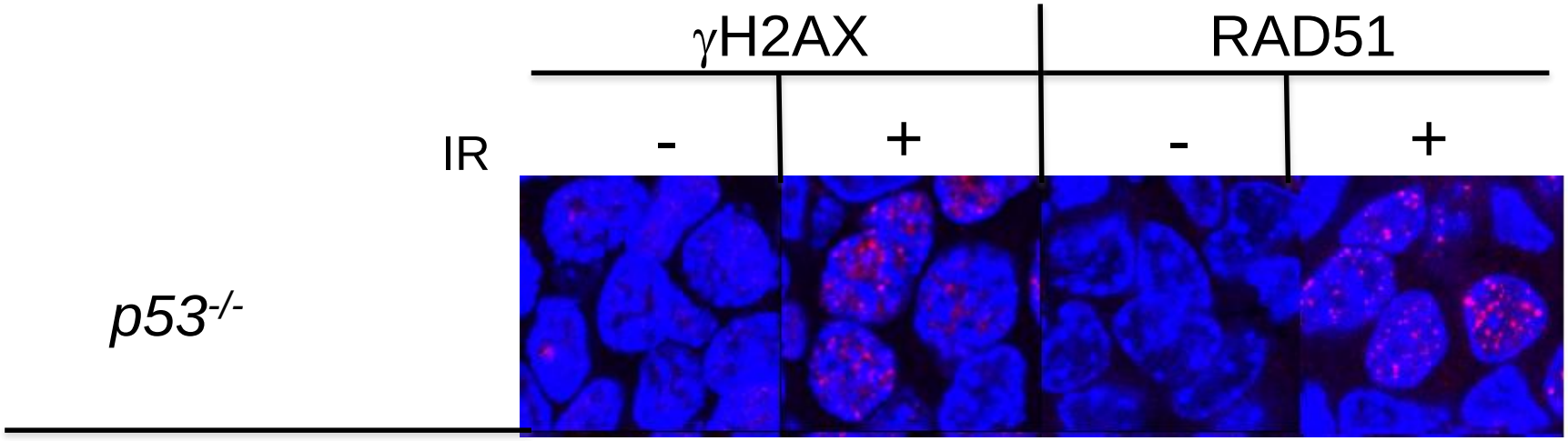
In situ RAD51 IRIF formation assay



- large panel of BRCA1- or BRCA2-deficient PARPi-resistant tumors analyzed (41 and 30 tumors respectively)

Ewa Gogola

Several olaparib-resistant *Brca1*$^{-/-}$;*p53*$^{-/-}$ tumors restore the formation of RAD51 foci



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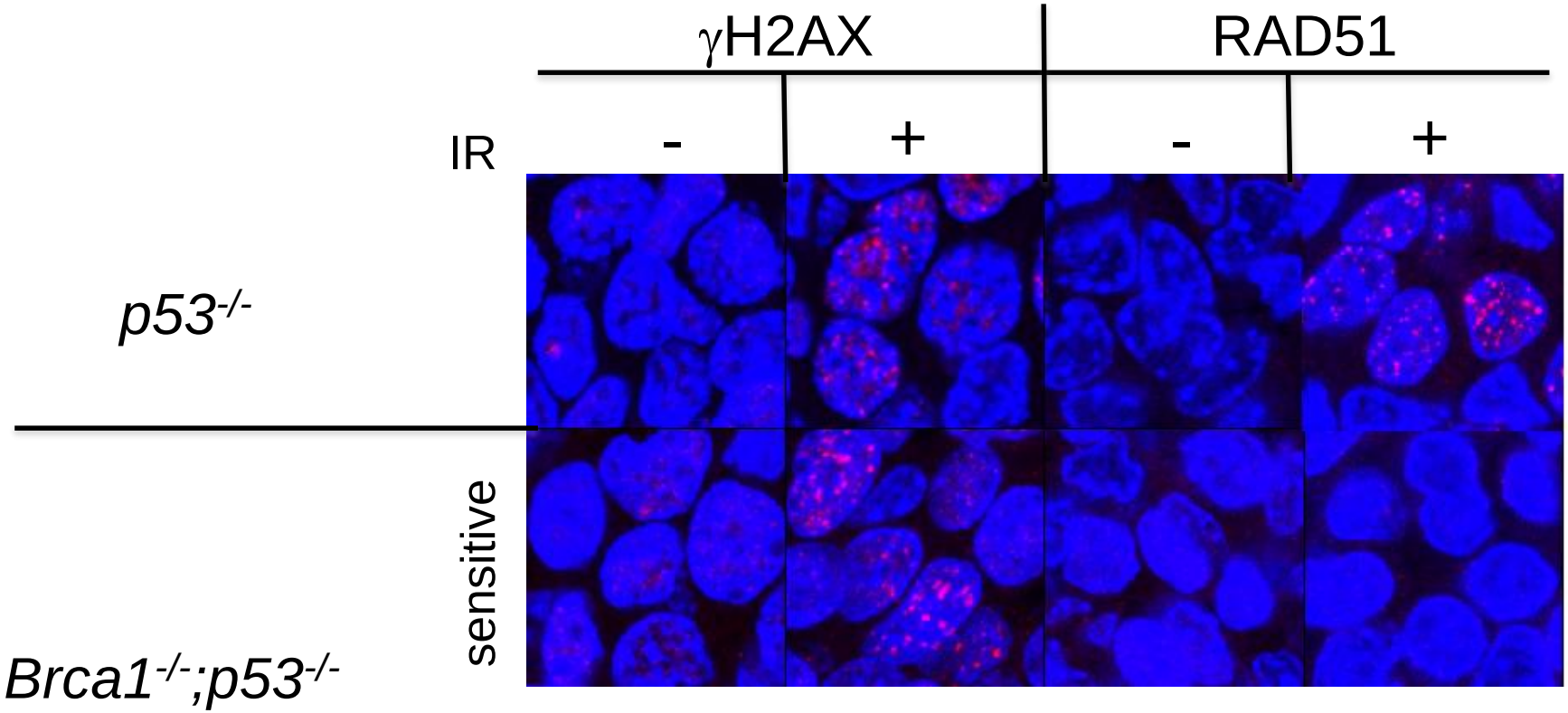

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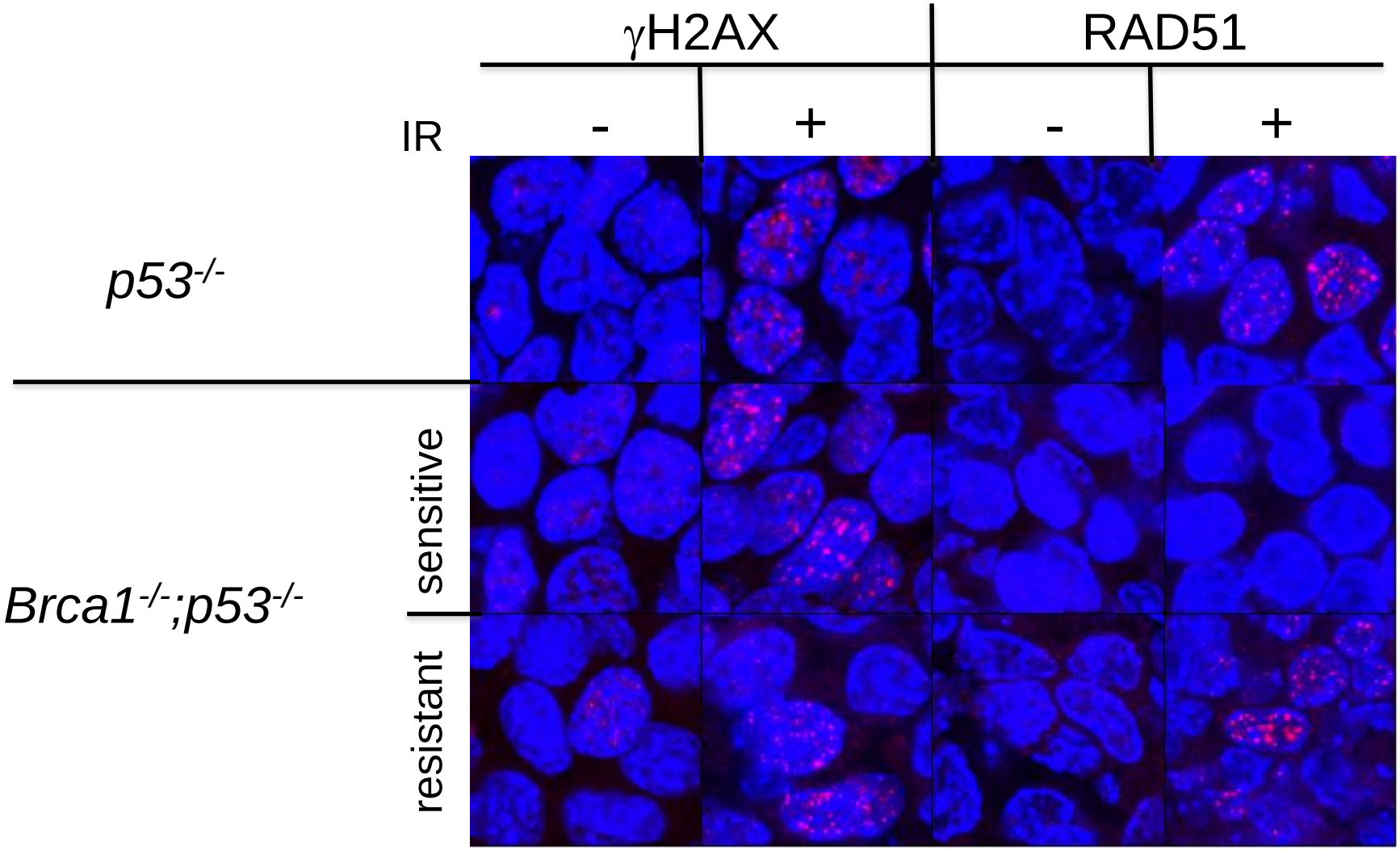

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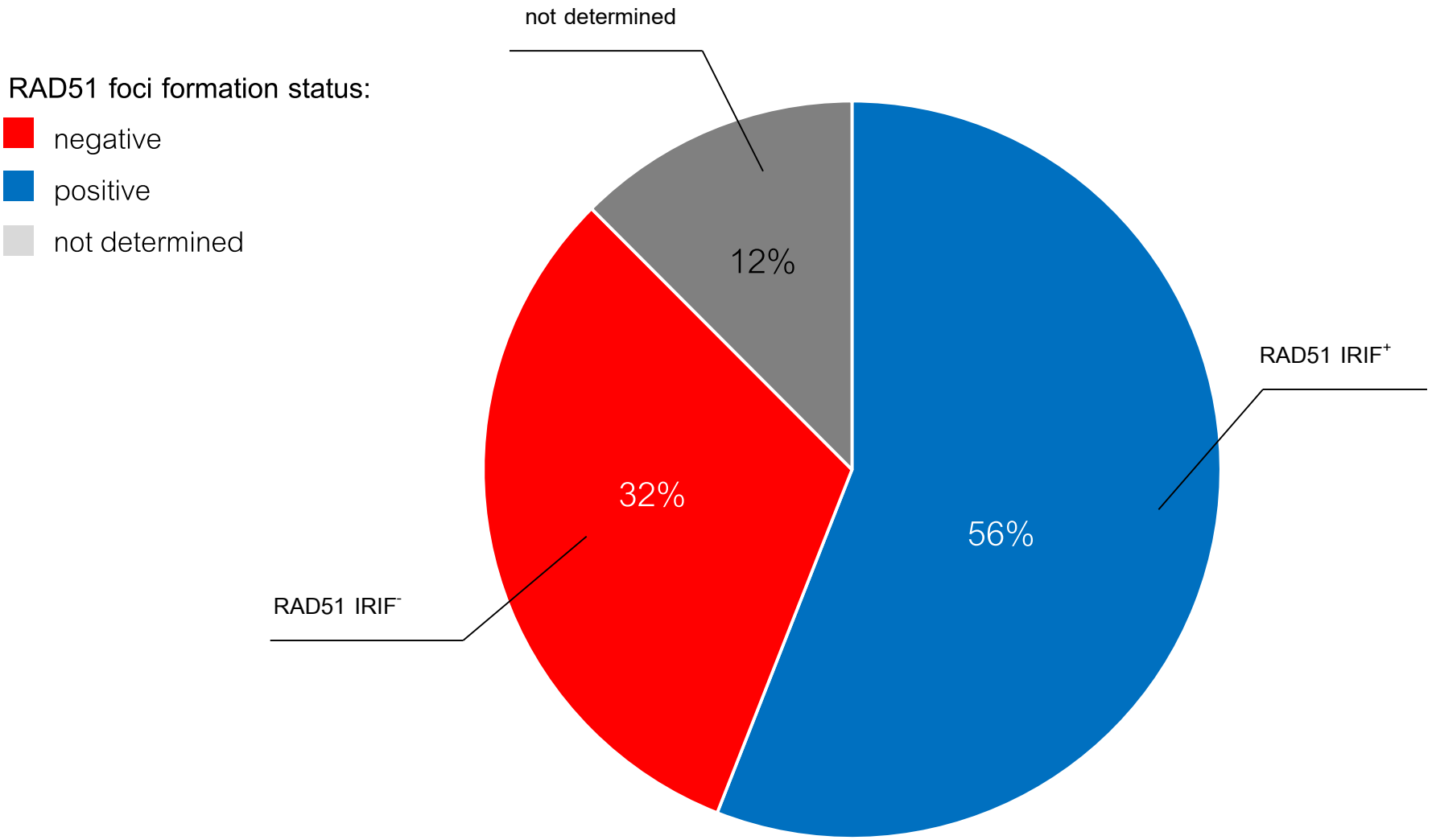
RAD51 IRIF formation in BRCA1-deficient PARPi-resistant tumors



53BP1 loss rescues BRCA1 deficiency and is associated with triple-negative and BRCA-mutated breast cancers

Peter Bouwman[1,10], Amal Aly[2,10], Jose M Escandell[3,10], Mark Pieterse[1], Jirina Bartkova[4], Hanneke van der Gulden[1], Sanne Hiddingh[1], Maria Thanasoula[3], Atul Kulkarni[2], Qifeng Yang[2], Bruce G Haffty[2], Johanna Tommiska[5], Carl Blomqvist[6], Ronny Drapkin[7], David J Adams[8], Heli Nevanlinna[5], Jiri Bartek[4,9], Madalena Tarsounas[3], Shridar Ganesan[2] & Jos Jonkers[1]

Nat Struct Mol Biol. 2010

53BP1 Inhibits Homologous Recombination in *Brca1*-Deficient Cells by Blocking Resection of DNA Breaks

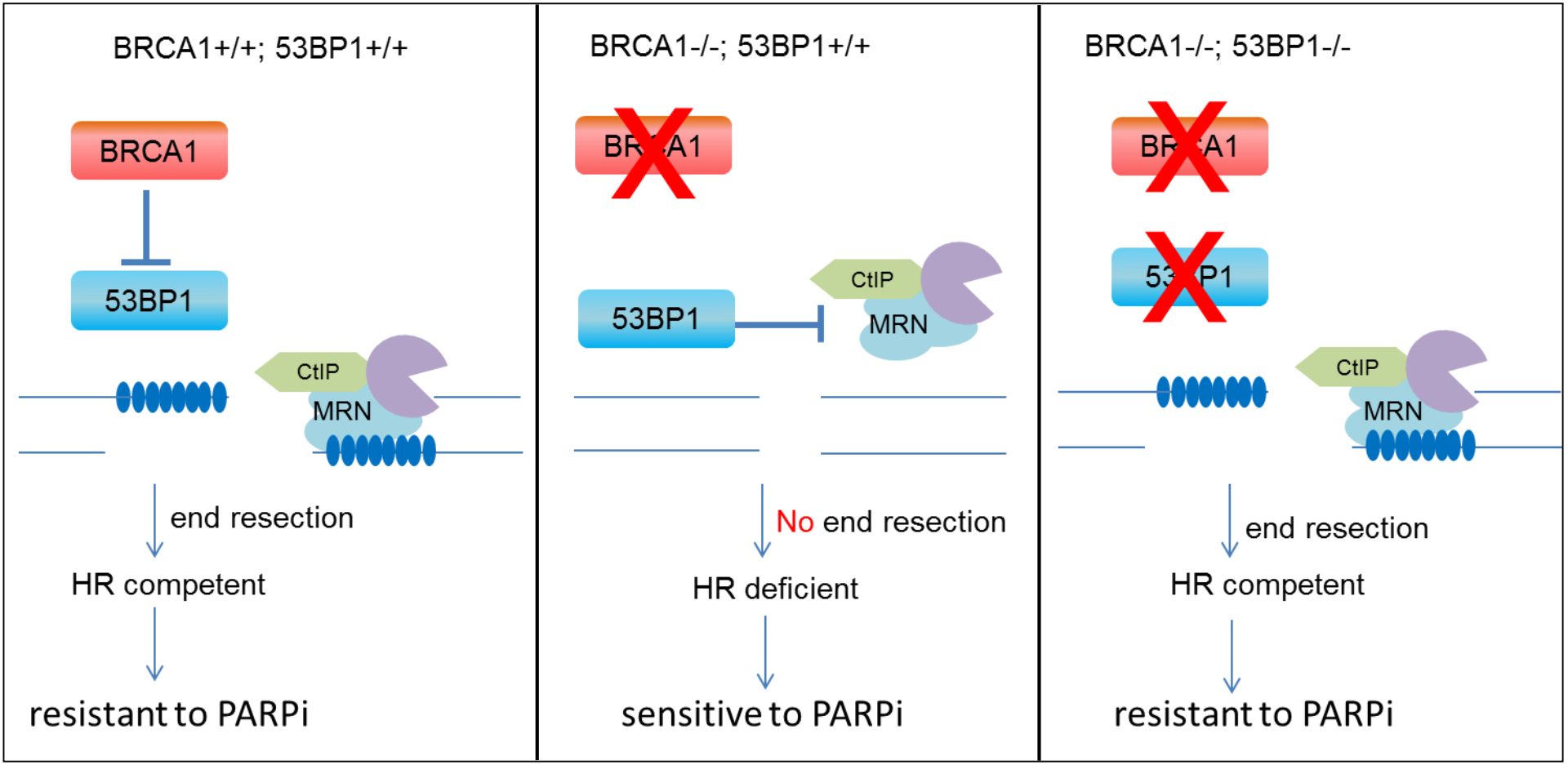
Samuel F. Bunting,[1] Elsa Callén,[1,8] Nancy Wong,[1,8] Hua-Tang Chen,[1] Federica Polato,[1] Amanda Gunn,[4] Anne Bothmer,[5] Niklas Feldhahn,[5] Oscar Fernandez-Capetillo,[7] Liu Cao,[2] Xiaoling Xu,[3] Chu-Xia Deng,[3] Toren Finkel,[2] Michel Nussenzweig,[5,6] Jeremy M. Stark,[4] and André Nussenzweig[1,*]
[1]Experimental Immunology Branch, National Cancer Institute
[2]Translational Medicine Branch, National Heart, Lung, and Blood Institute
[3]Genetics of Development and Disease Branch, National Institute for Diabetes and Digestive and Kidney Diseases
National Institutes of Health, Bethesda, MD 20892, USA
[4]Department of Cancer Biology and Irell and Manella Graduate School of Biological Sciences, Beckman Research Institute of the City of Hope, Duarte, CA 91010, USA
[5]Laboratory of Molecular Immunology
[6]Howard Hughes Medical Institute
Rockefeller University, New York, NY 10065, USA
[7]Genomic Instability Group, Spanish National Cancer Research Centre (CNIO), 28029 Madrid, Spain
[8]These authors contributed equally to this work
*Correspondence: andre_nussenzweig@nih.gov
DOI 10.1016/j.cell.2010.03.012

Cell 2010

53BP1 loss restores HR and causes PARPi resistance of BRCA1-deficient cells

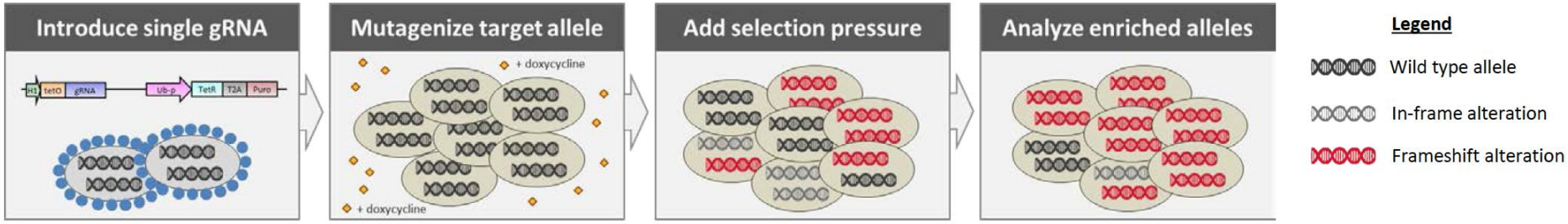
Loss of 53BP1 relieves enzymes to resect DSB ends and promotes HR, resulting in PARPi resistance of BRCA1-deficient cells

Bouwman et al. 2010; Bunting et al. 2010



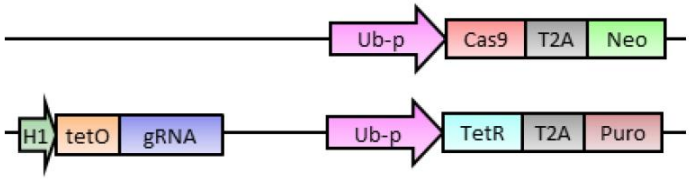
Competition assay to confirm selection of knock outs

Competition assay in polyclonal cell lines



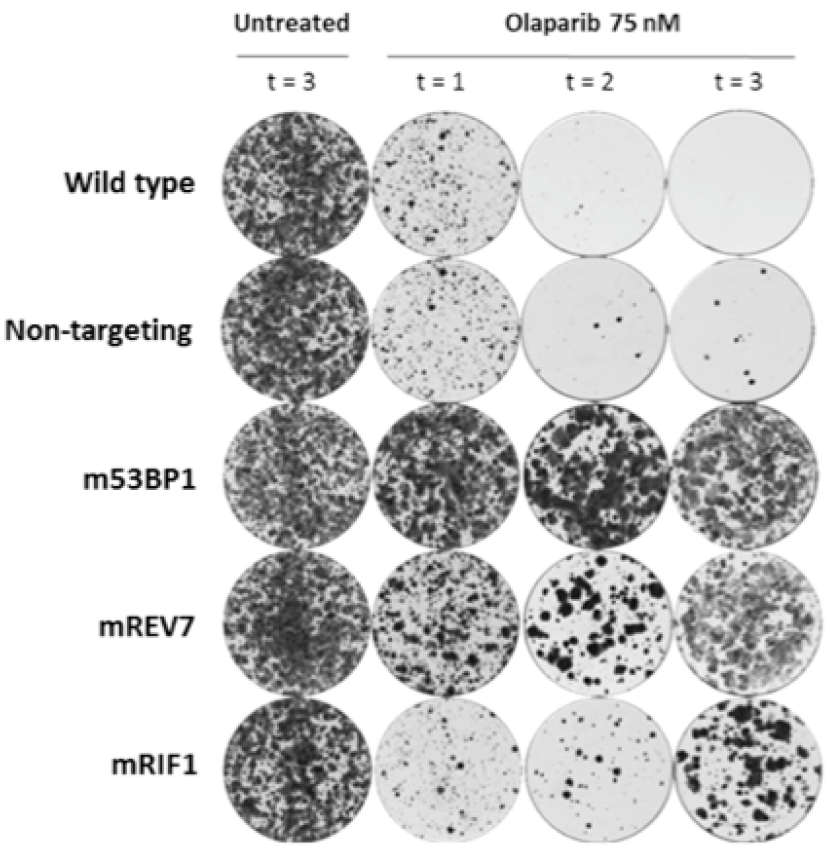
Virus-mediated plasmid delivery

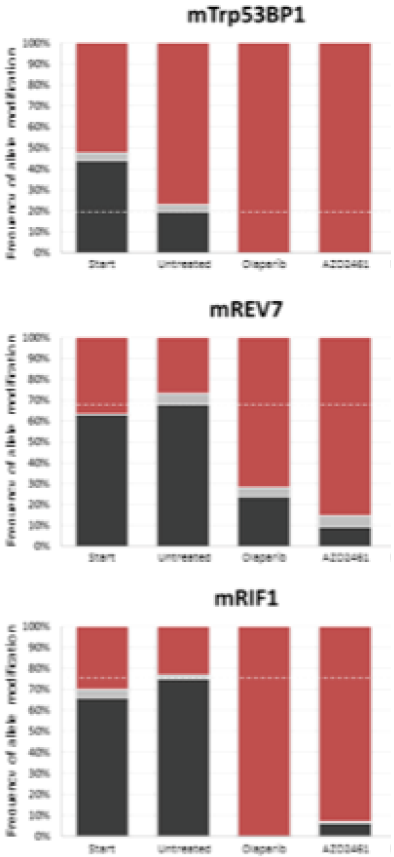
pGSC_Cas9_Neo + iKRUNC (Bastiaan Evers)



Experimental details

1. Single cells were plated to allow clonogenic outgrowth
2. Cells were harvested and re-plated every 10 days for 3 times
3. The 'evolution' of the polyclonal population was monitored by TIDE





Left to right:

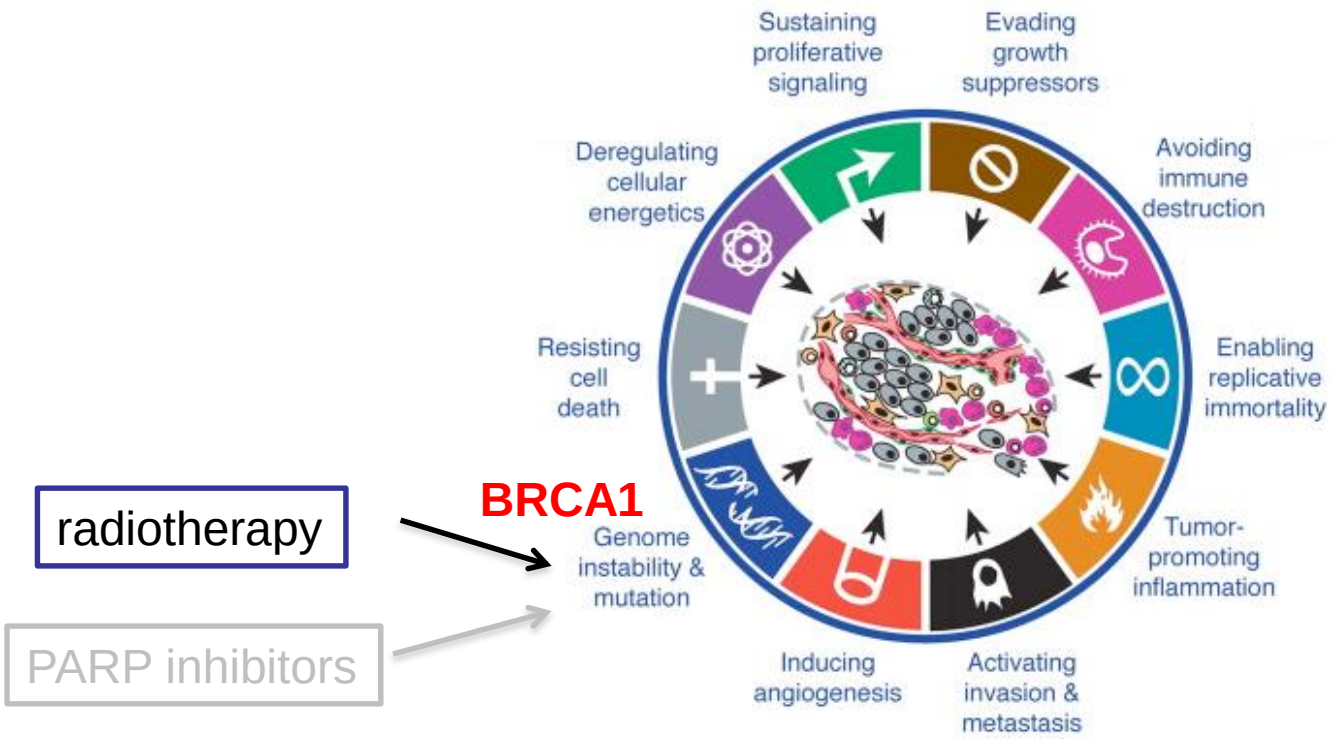
- Start
- Untreated
- Olaparib (75 nM)
- AZD2461 (250 nM)

%Frameshift alleles

%In-frame alleles

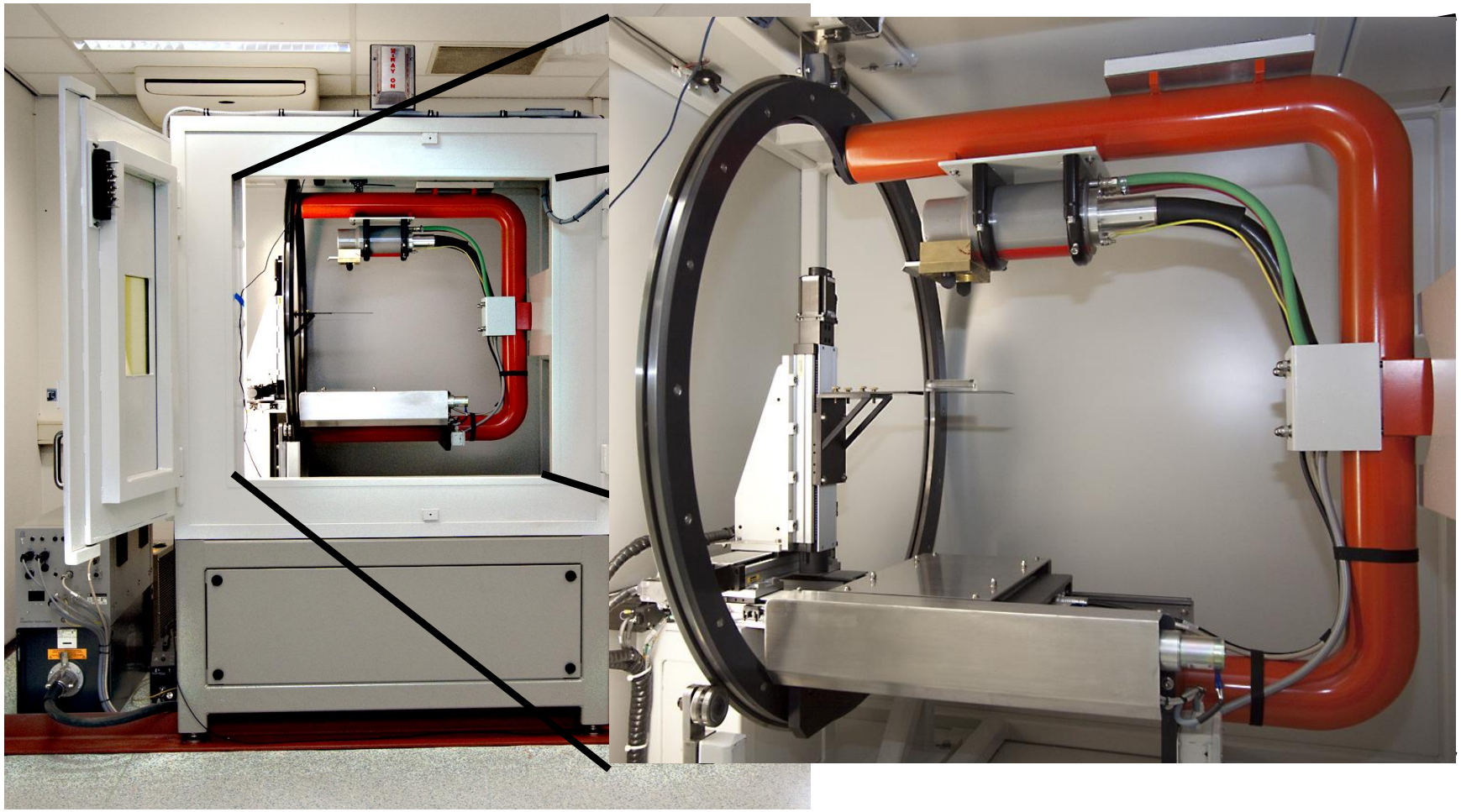
%Wild type alleles

…and we have powerful weapons to target cancer



Hanahan & Weinberg
Cell 2011

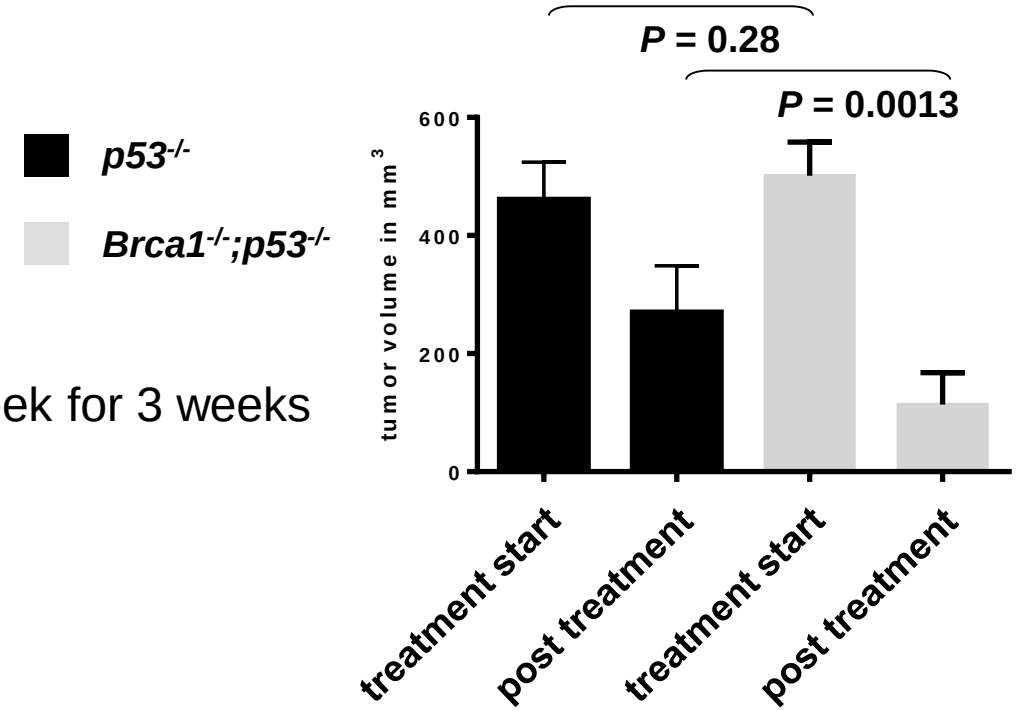
Xrad 225Cx



Developed by Princess Margaret Hospital (PMH, Toronto) in collaboration with Precision X-ray Inc (PXI, USA)

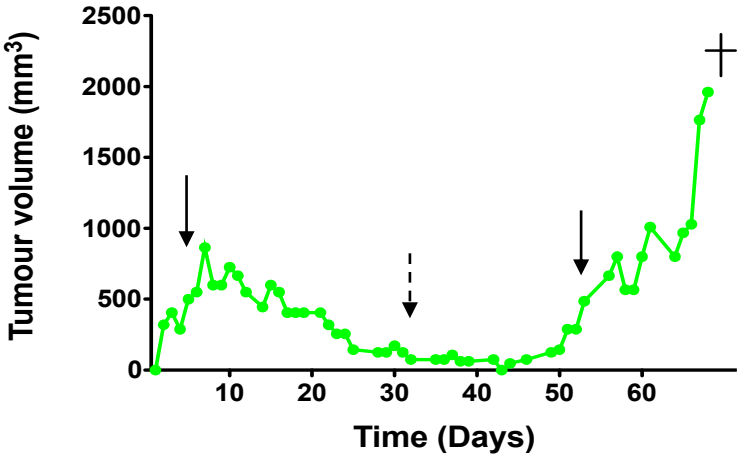
Pre-clinical μIGRT using defined GEMMs for breast cancer

- *K14cre;p53$^{F/F}$*
- *K14cre;Brca1$^{F/F}$;p53$^{F/F}$*



4Gy/fr, 3fr/week for 3 weeks

Some BRCA1-deficient mammary tumors acquire radioresistance



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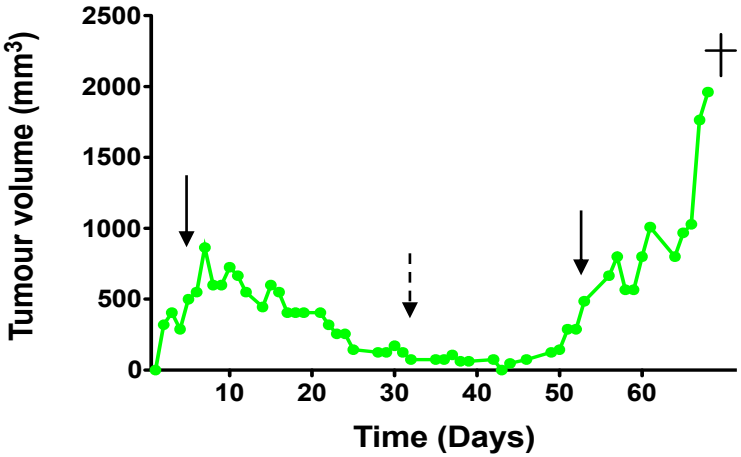
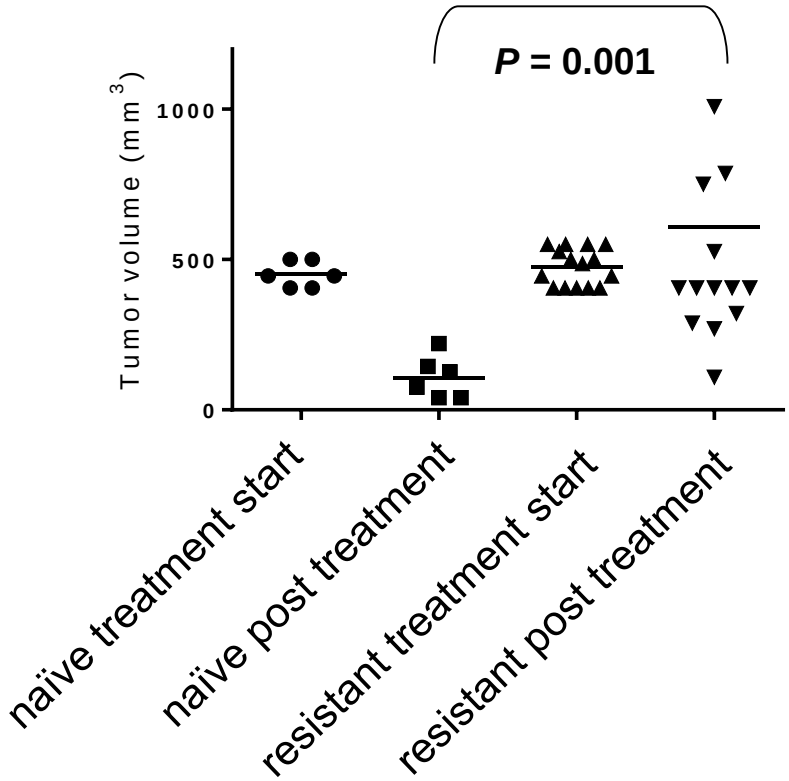




4Gy/fr, 3fr/week for 3 weeks

A paradox: none of the radioresistant tumors shows restoration of HR

- No restoration of RAD51 foci formation in any of the radioresistant tumors
- No loss of 53BP1, REV7, or RIF1

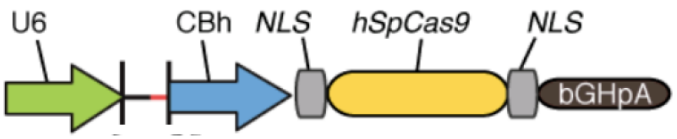
Hypothesis:

Restoration of homologous recombination through promotion of end-resection sensitizes BRCA1-deficient cells to ionizing radiation

Clonogenic assay in polyclonal cell lines

Transfection-mediated plasmid delivery

pX330_hPGKpuroR (Zhang' lab, modified by Tim Harmsen)



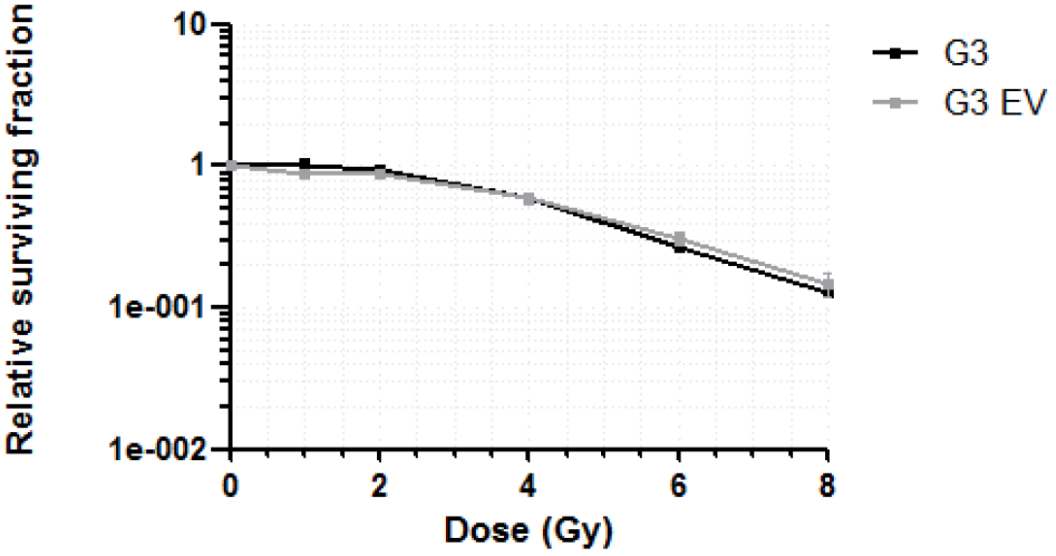
Experimental details

1. Single cells were plated to allow clonogenic outgrowth (day 0)
2. Cells were irradiated on day 1 and stained on day 10
3. Colonies were counted manually and averaged between replicate wells

$$PE = \frac{\#counted\ colonies}{\#plated\ cells} \times 100\% \qquad SF = \frac{PE\ after\ treatment}{PE\ without\ treatment} \times 100\%$$

Promotion of end-resection sensitizes BRCA1$^{-/-}$ cells to IR

BRCA1$^{-/-}$;p53$^{-/-}$ log-dose response



1. Restoration of HR through reconstitution of BRCA1-protein expression reduces sensitivity to radiotherapy
2. 53BP1, REV7 and RIF1 knockout sensitize KB1P cells to radiotherapy from doses of >2 Gy

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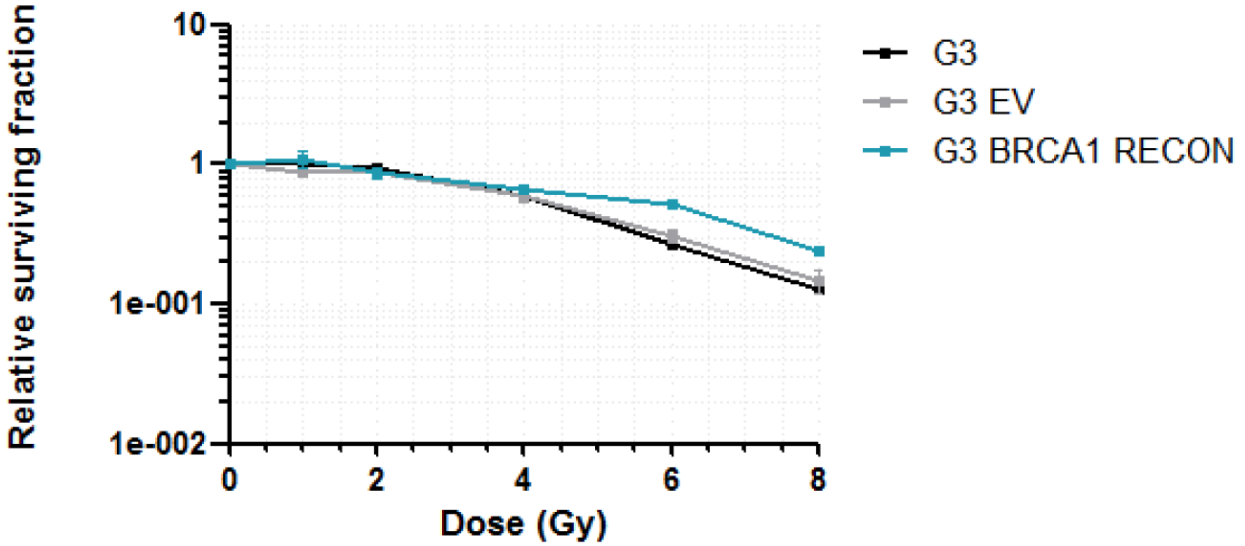

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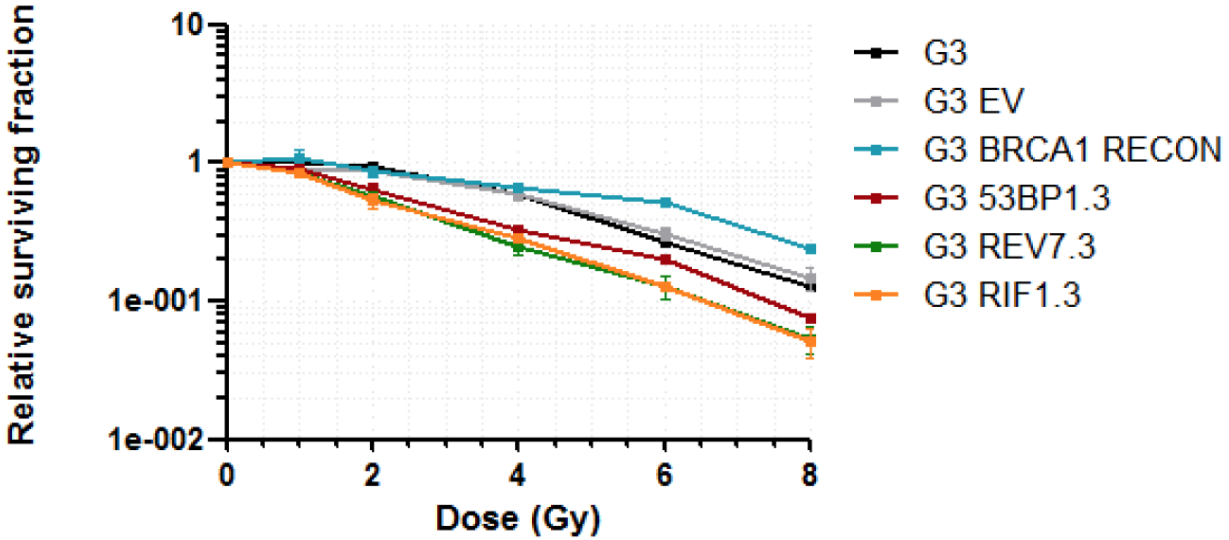

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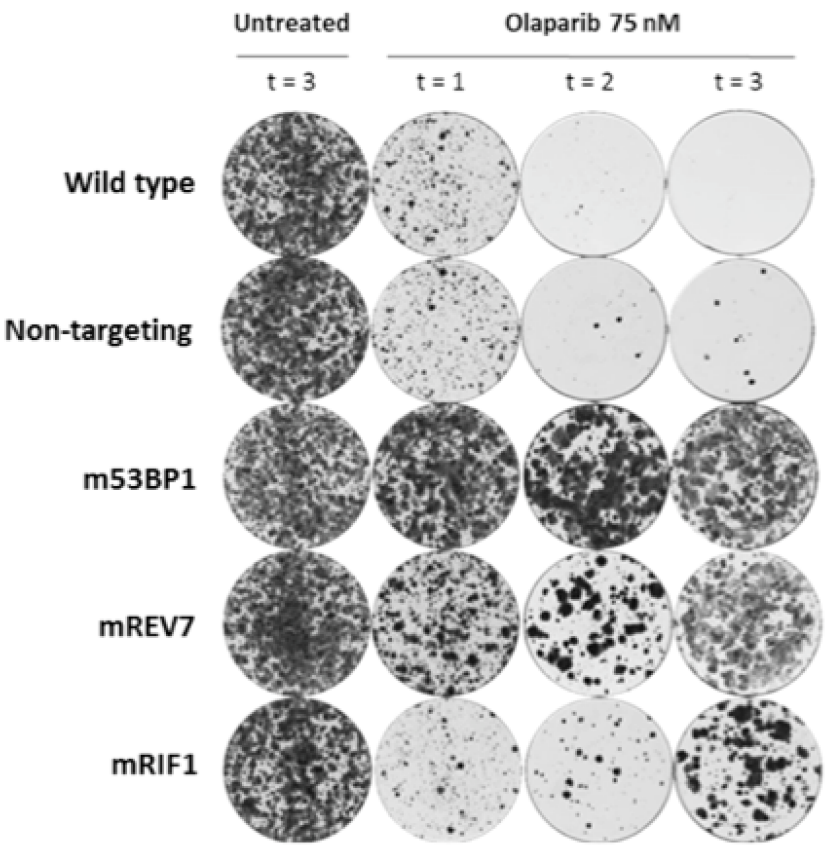
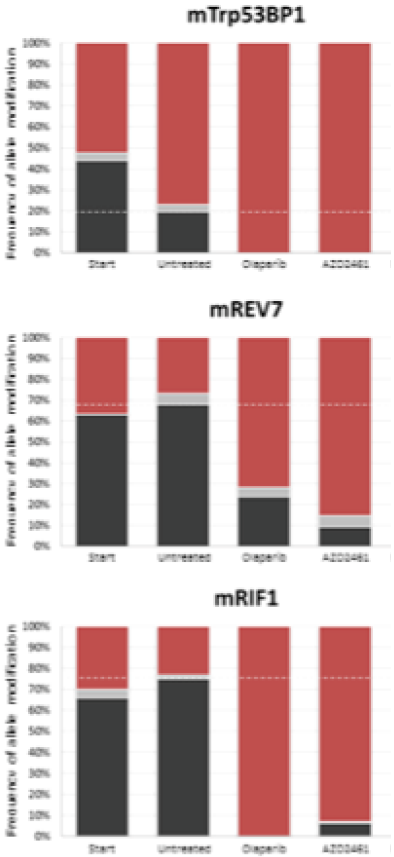
Promotion of end-resection sensitizes BRCA1$^{-/-}$ cells to IR





Left to right:

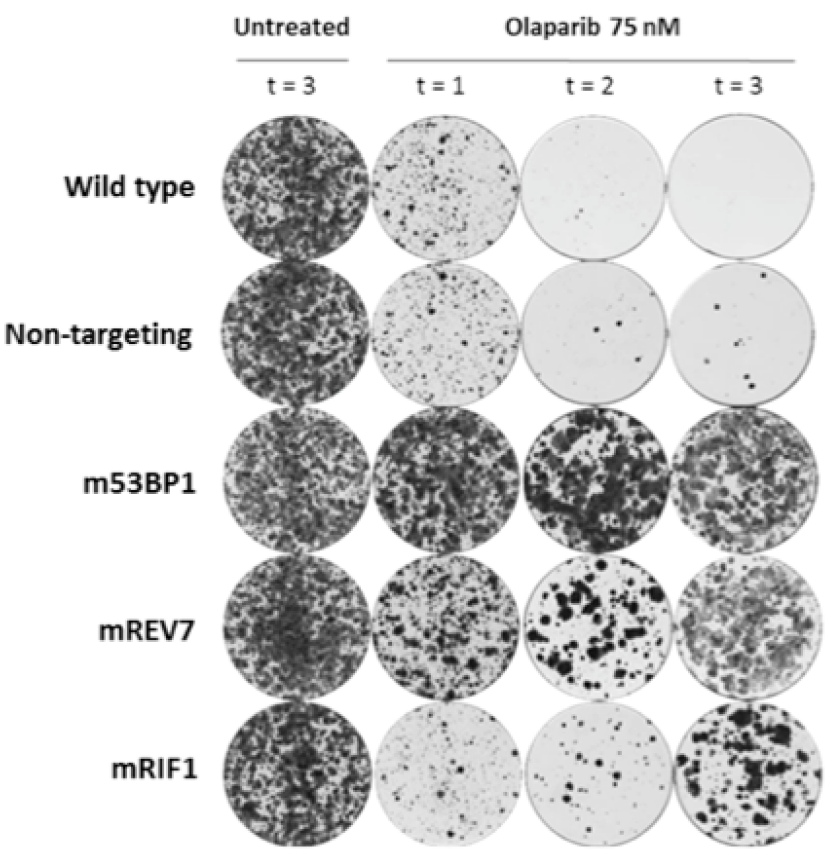
- Start
- Untreated
- Olaparib (75 nM)
- AZD2461 (250 nM)

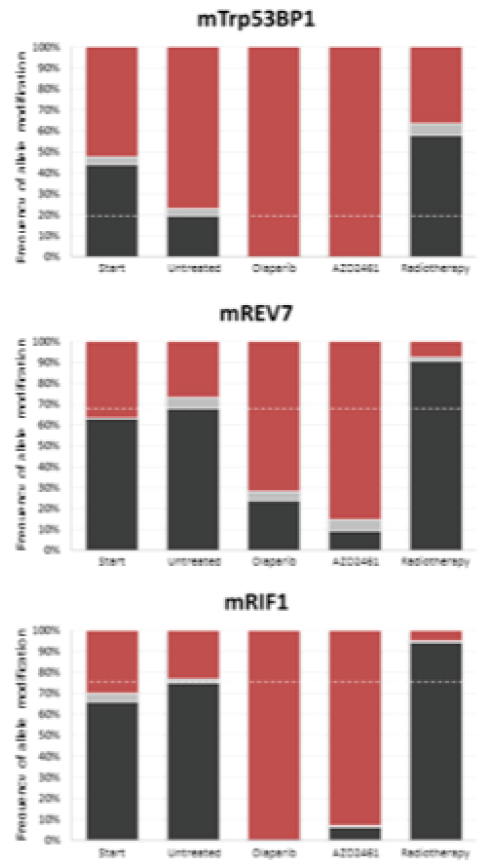
%Frameshift alleles

%In-frame alleles

%Wild type alleles

Promotion of end-resection sensitizes BRCA1$^{-/-}$ cells to IR





Left to right:

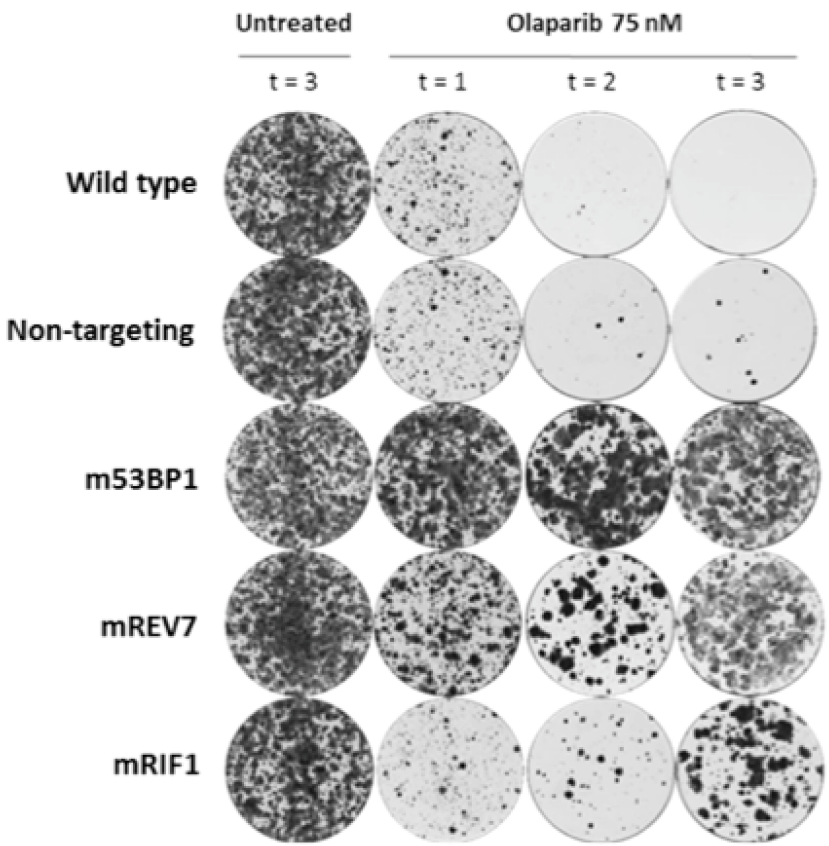
- Start
- Untreated
- Olaparib (75 nM)
- AZD2461 (250 nM)
- Radiotherapy (3x 4Gy)

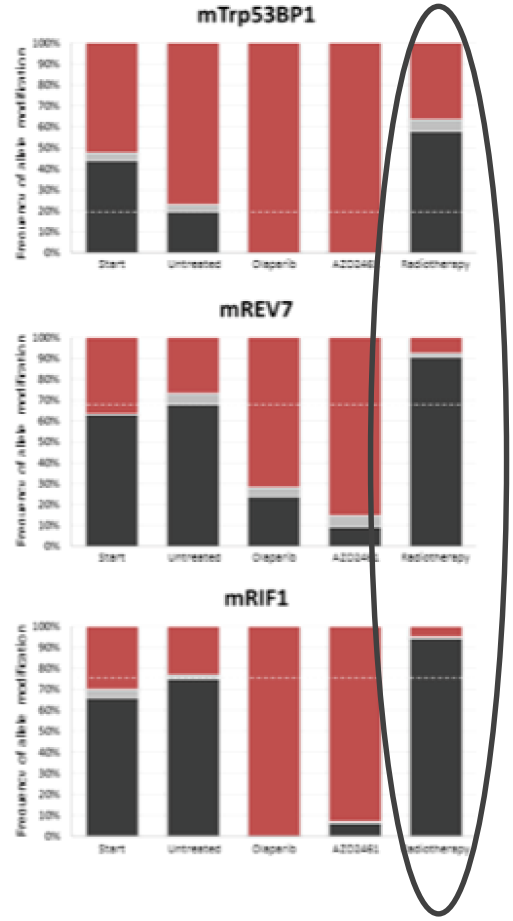
%Frameshift alleles

%In-frame alleles

%Wild type alleles

Promotion of end-resection sensitizes BRCA1$^{-/-}$ cells to IR





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- Radiotherapy (3x 4Gy)

%Frameshift alleles

%In-frame alleles

%Wild type alleles

Promotion of end-resection sensitizes BRCA1$^{-/-}$ cells to IR

How the response to PARP inhibition and the response to radiotherapy might be reconciled

PARP inhibition: conversion of SSBs into DSBs requires passing of the replication fork, template for HR in close proximity

Radiotherapy: acute damage independent of cell cycle status; cells might be more reliant on NHEJ, explaining the radio sensitization effect.

Acknowledgements

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Rottenberg group

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Collaborators NKI

Gerben Borst

Piet Borst

Alessia Gasparini

Jos Jonkers

Jan-Jakob Sonke

Marcel Verheij

DUTCH CANCER SOCIETY

NWO
Netherlands Organisation for Scientific Research

FNSNF
FONDS NATIONAL SUISSE
SCHWEIZERISCHER NATIONALFONDS
FONDO NAZIONALE SVIZZERO
SWISS NATIONAL SCIENCE FOUNDATION

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Molecular Oncology

Piet Borst

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Wendy Sol

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Gerben Borst

Alessia Gasparini

Jan-Jakob Sonke

Marcel Verheij

Molecular Pathology

Jos Jonkers

DUTCH CANCER SOCIETY

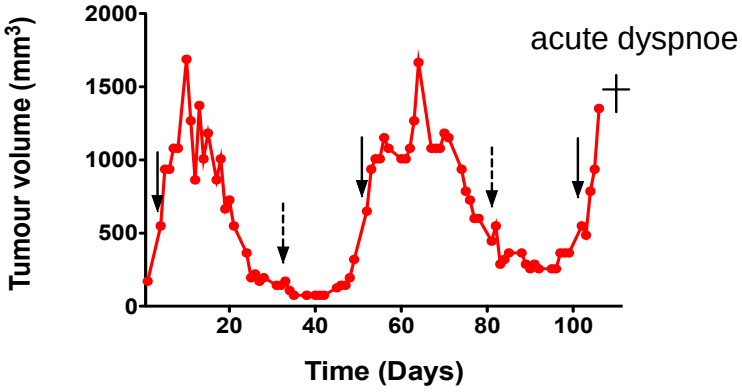
NWO
Netherlands Organisation for Scientific Research

Summary

K14cre;Brca1$^{F/F}$*;p53*$^{F/F}$ breast cancer model is useful to study 3 mechanisms of escape from radiotherapy:

- selection of cells with an increased potential to form metastasis
- lack of tumor eradication (residual disease)
- development of radioresistance

Some BRCA1-deficient mammary tumors develop metastasis



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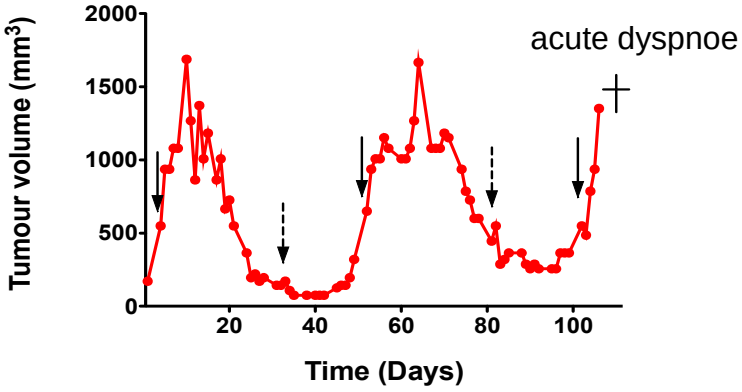
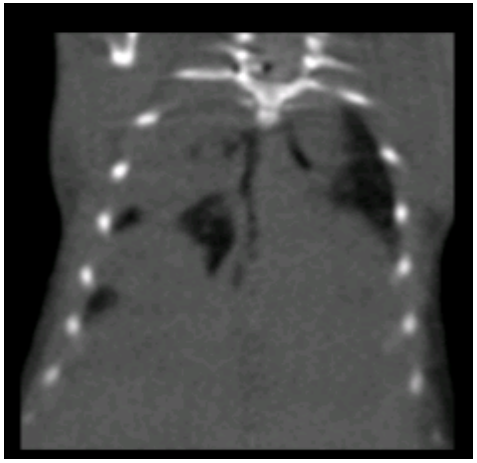
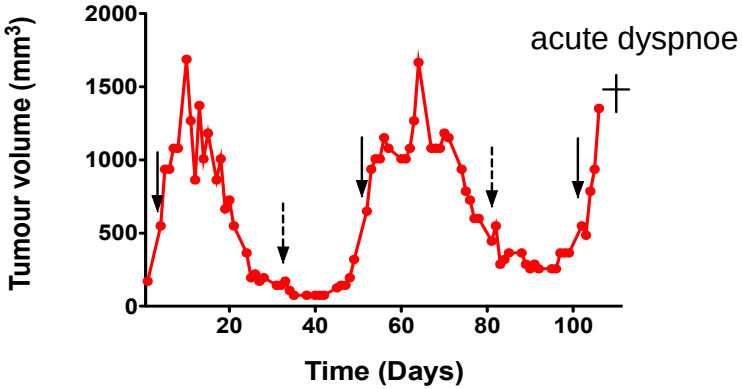
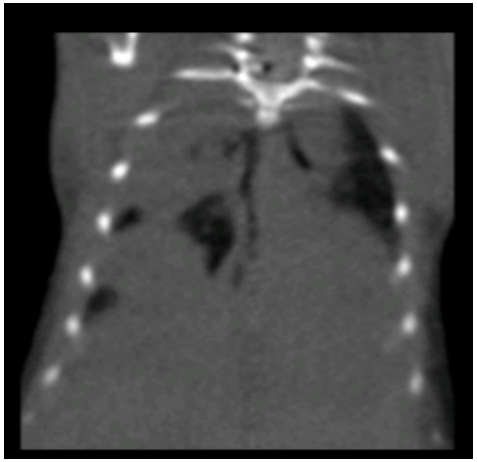
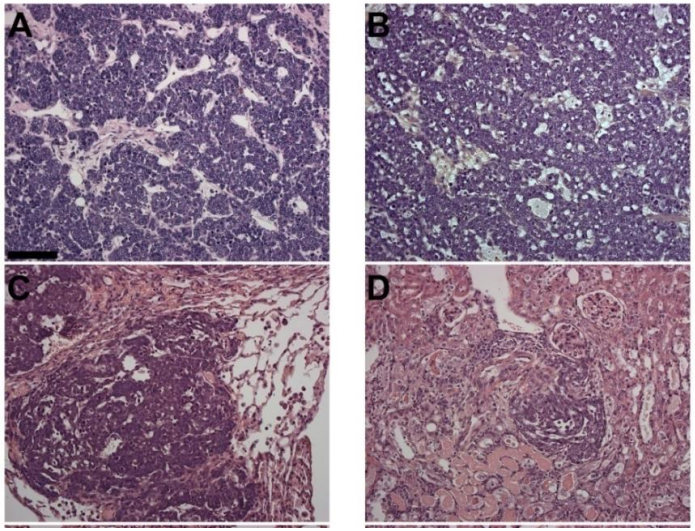




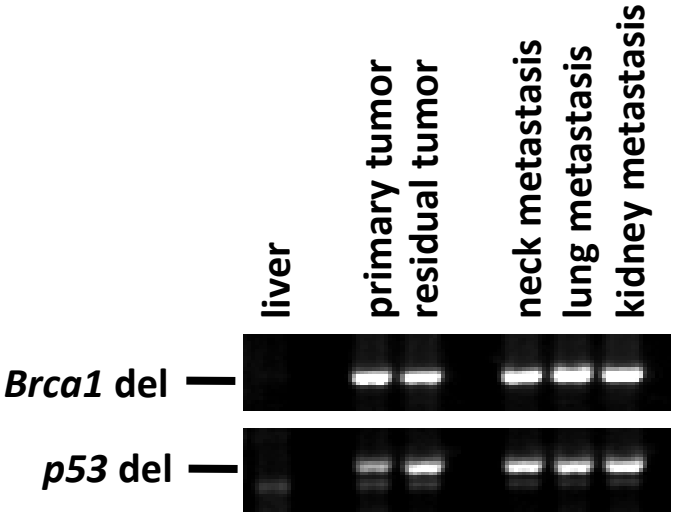
Some BRCA1-deficient mammary tumors develop metastasis

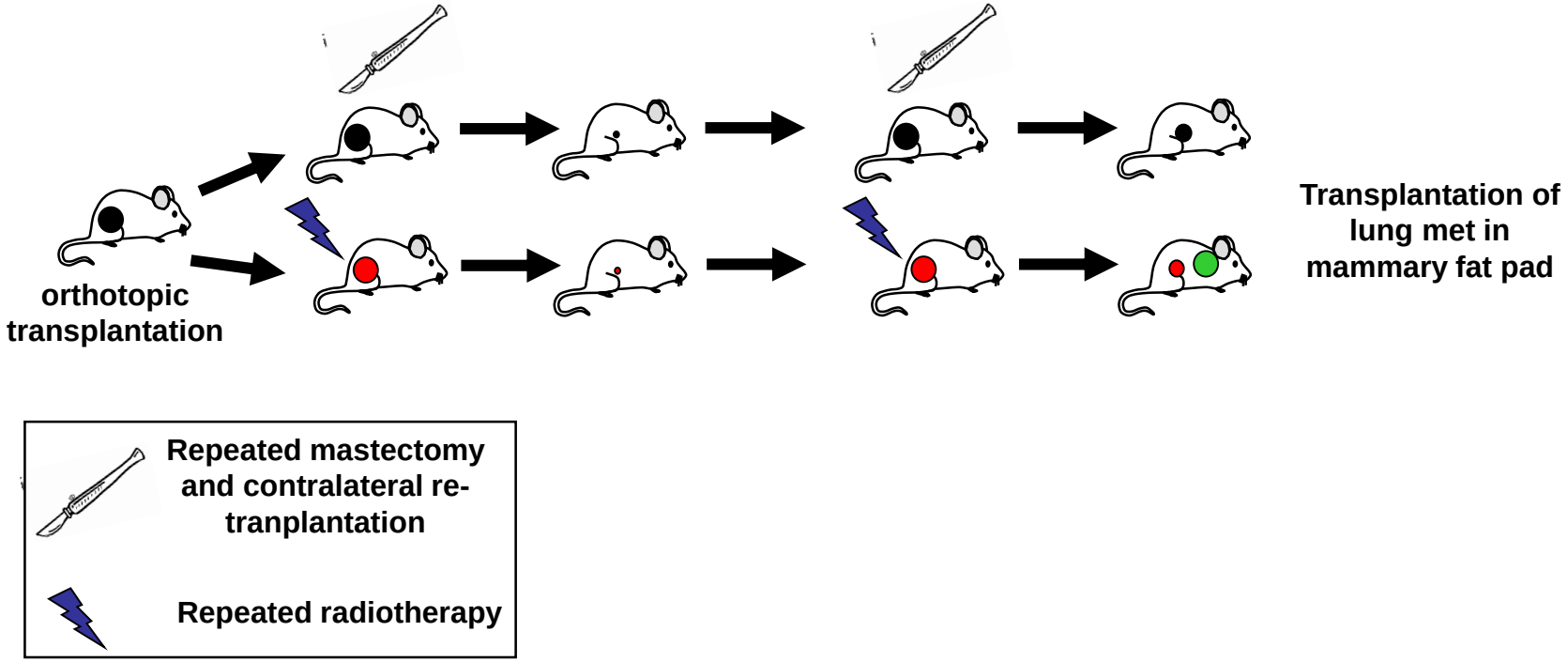


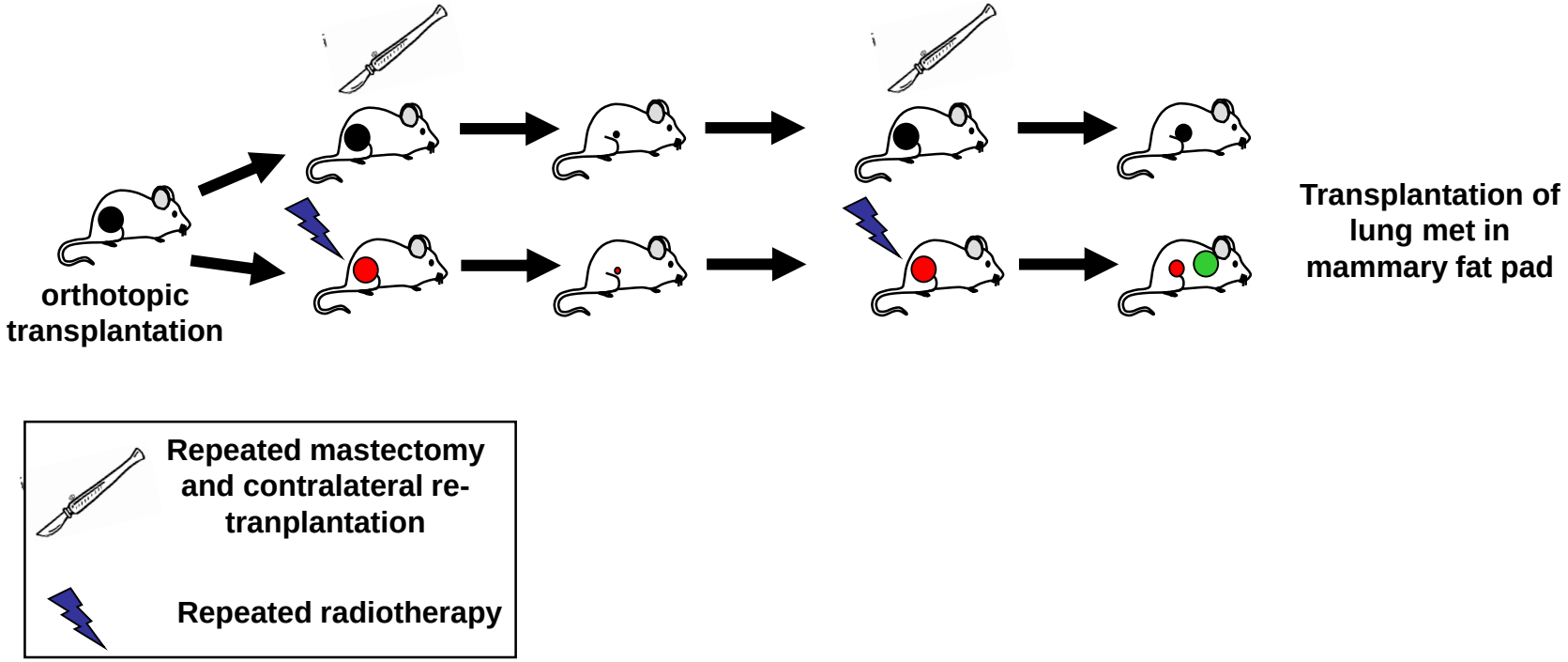




primary tumor (A), metastasis to the neck (B), lung (C), kidney (D)




orthotopic transplantation
Transplantation of lung met in mammary fat pad
Repeated mastectomy and contralateral re-tranplantation
Repeated radiotherapy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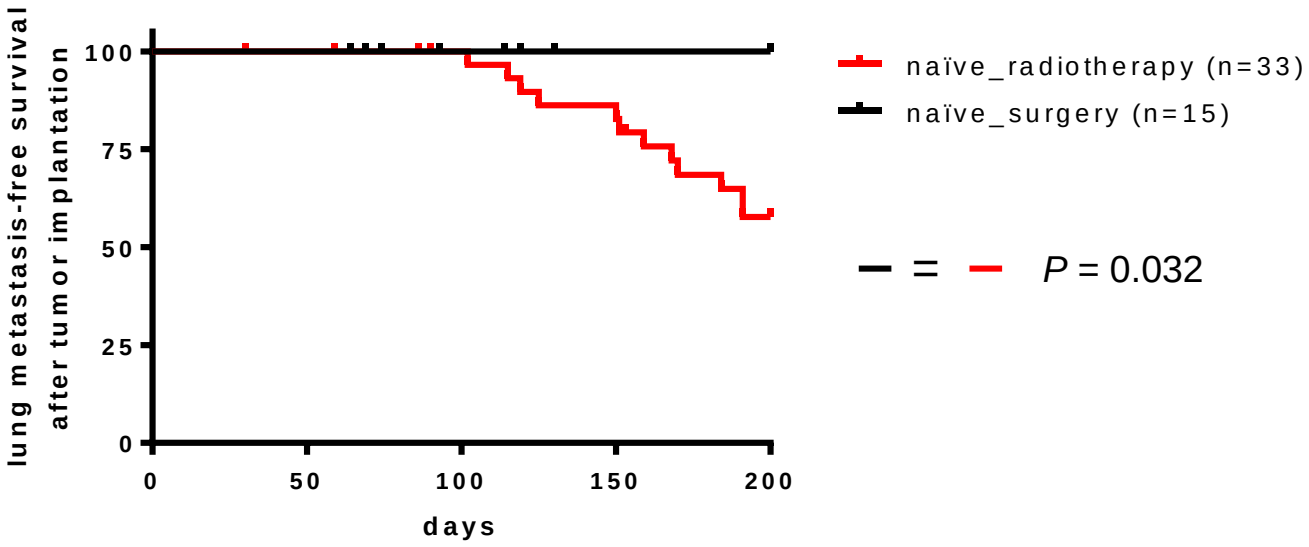
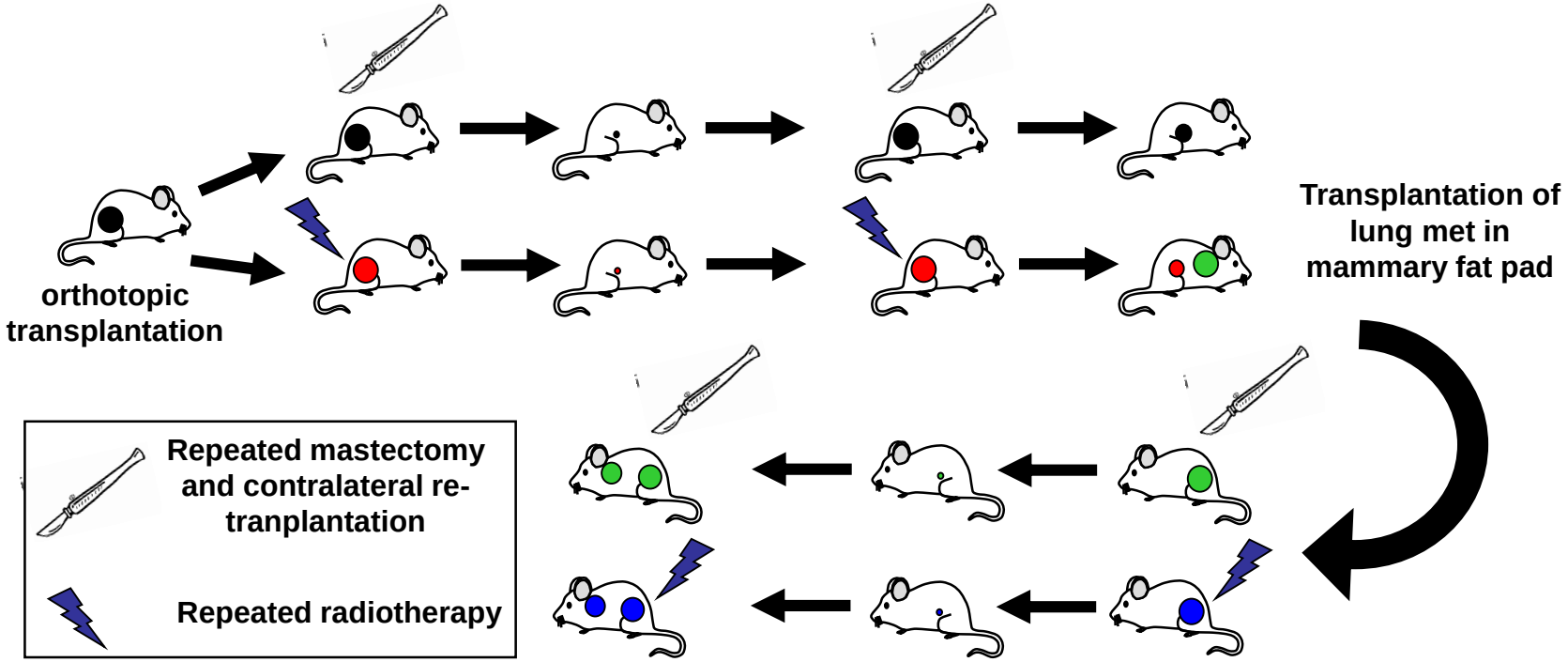
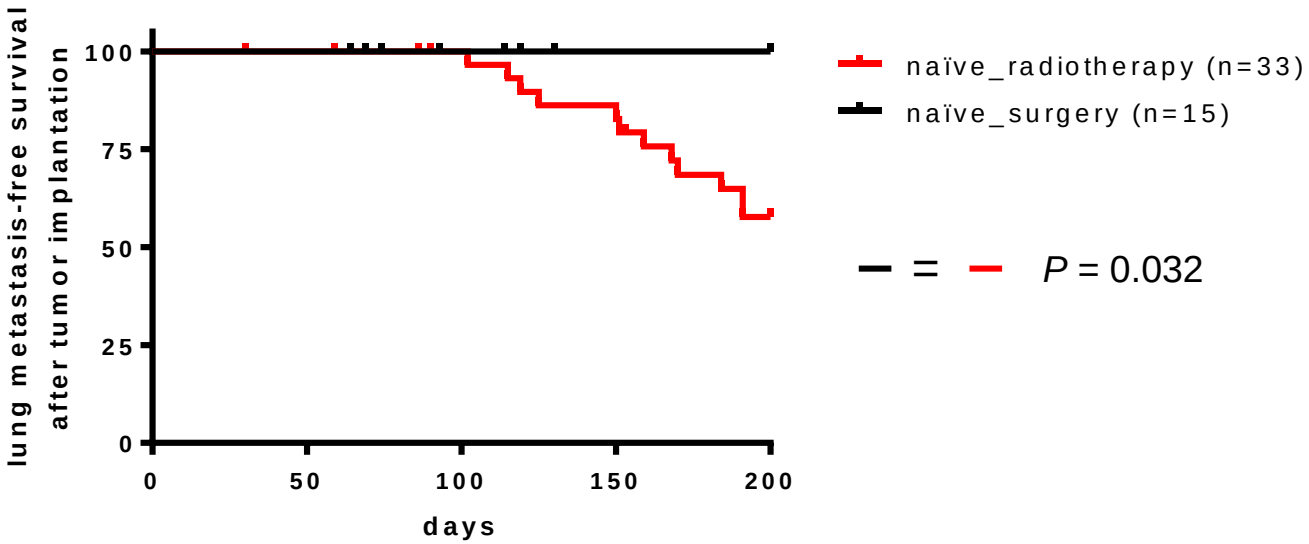

lung metastasis-free survival after tumor implantation
100
75
50
25
0
0
50
100
150
200
days
naïve_radiotherapy (n=33)
naïve_surgery (n=15)
= P = 0.032


orthotopic transplantation
Transplantation of lung met in mammary fat pad
Repeated mastectomy and contralateral re-tranplantation
Repeated radiotherapy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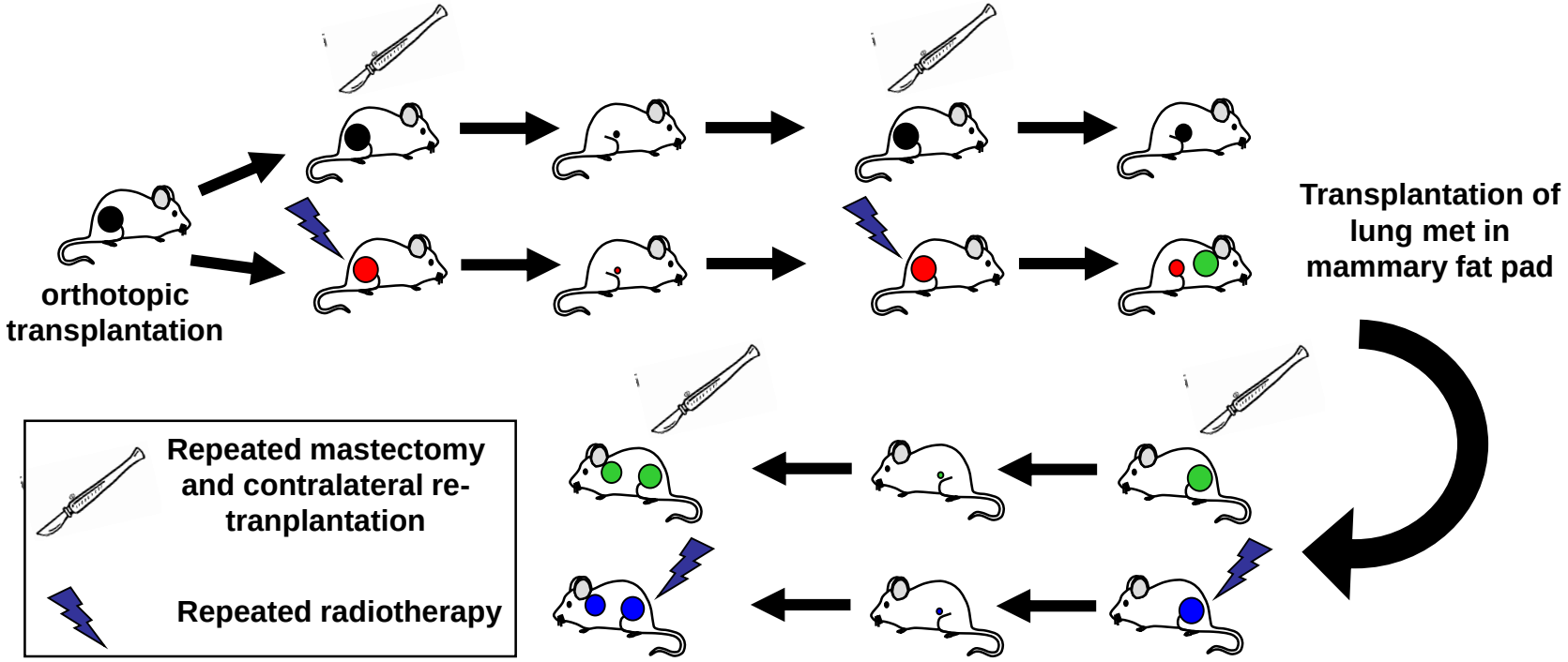
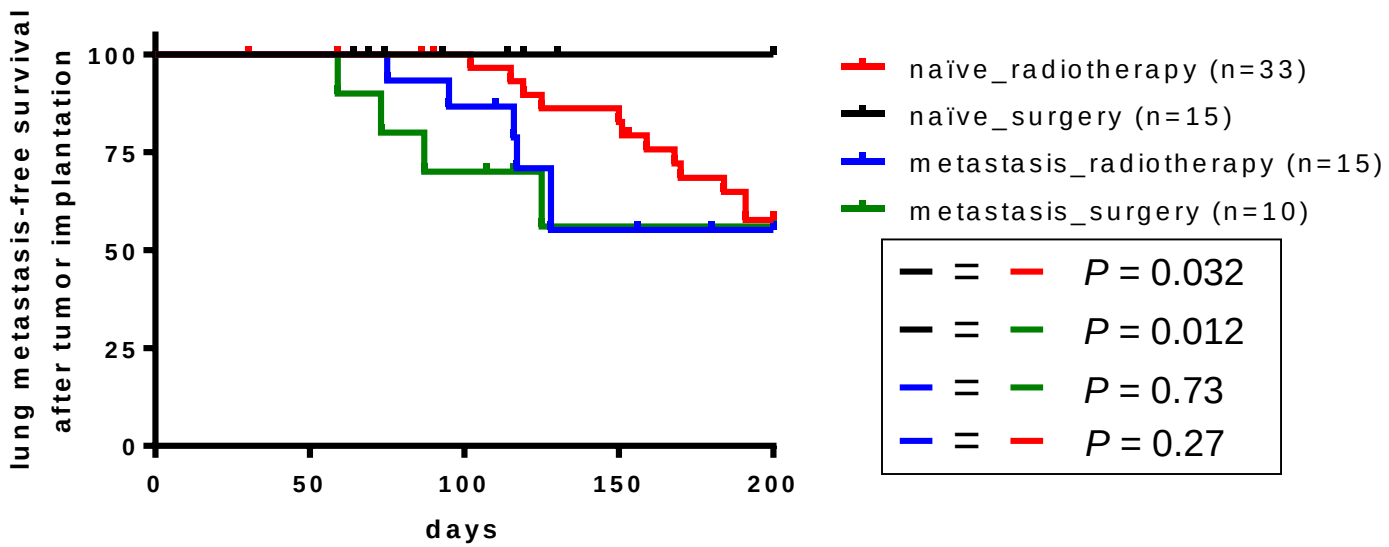
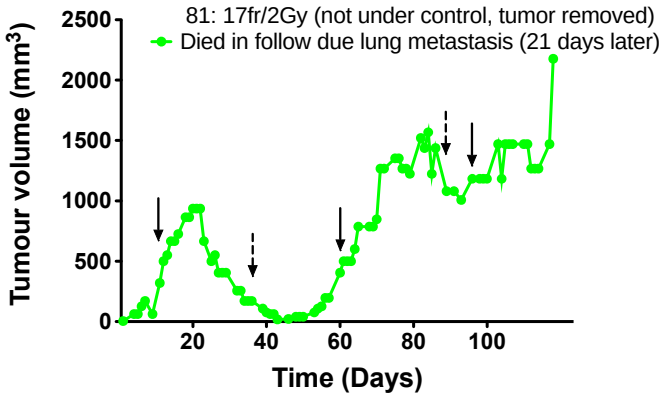
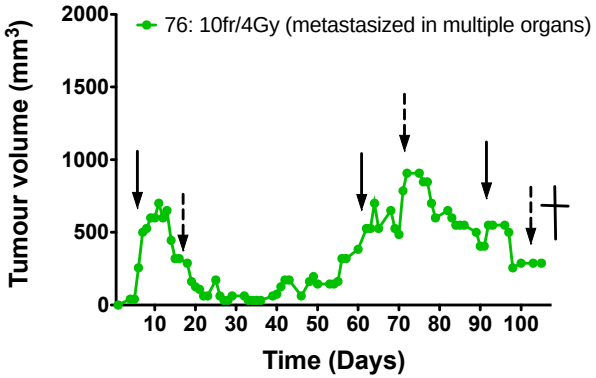
naïve_surgery (n=15)
= P = 0.032

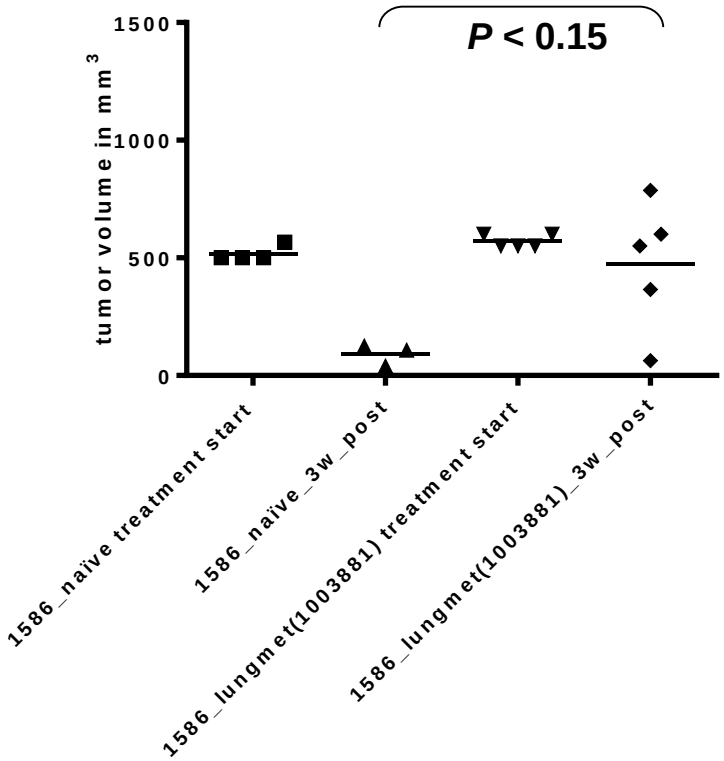

orthotopic transplantation
Transplantation of lung met in mammary fat pad
Repeated mastectomy and contralateral re-tranplantation
Repeated radiotherapy

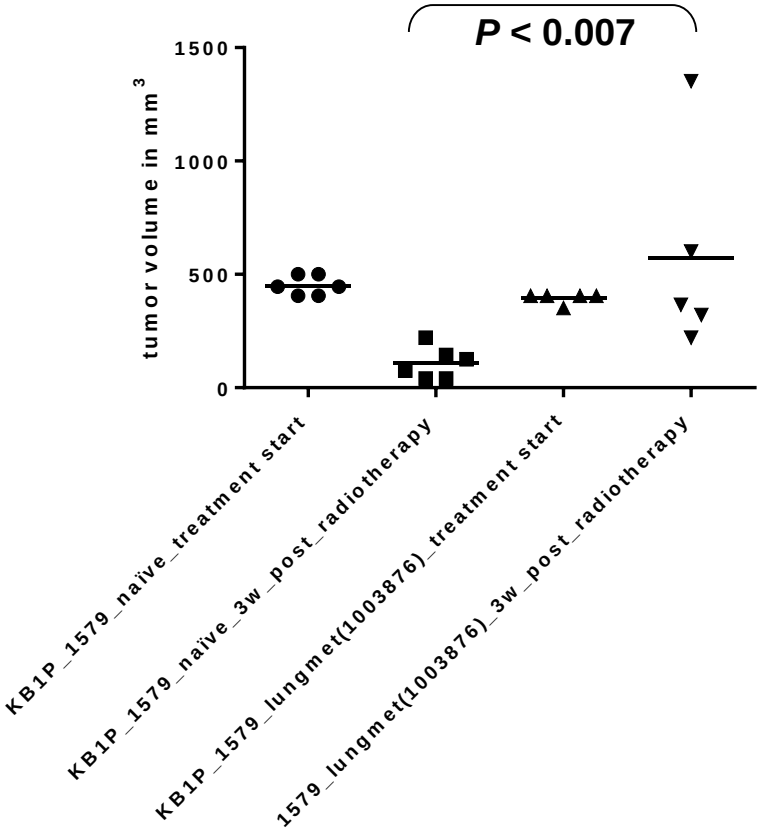

lung metastasis-free survival after tumor implantation
100
75
50
25
0
0
50
100
150
200
days
naïve_radiotherapy (n=33)
naïve_surgery (n=15)
metastasis_radiotherapy (n=15)
metastasis_surgery (n=10)
= P = 0.032
= P = 0.012
= P = 0.73
= P = 0.27

Testing stable radio-resistance of lung metastases



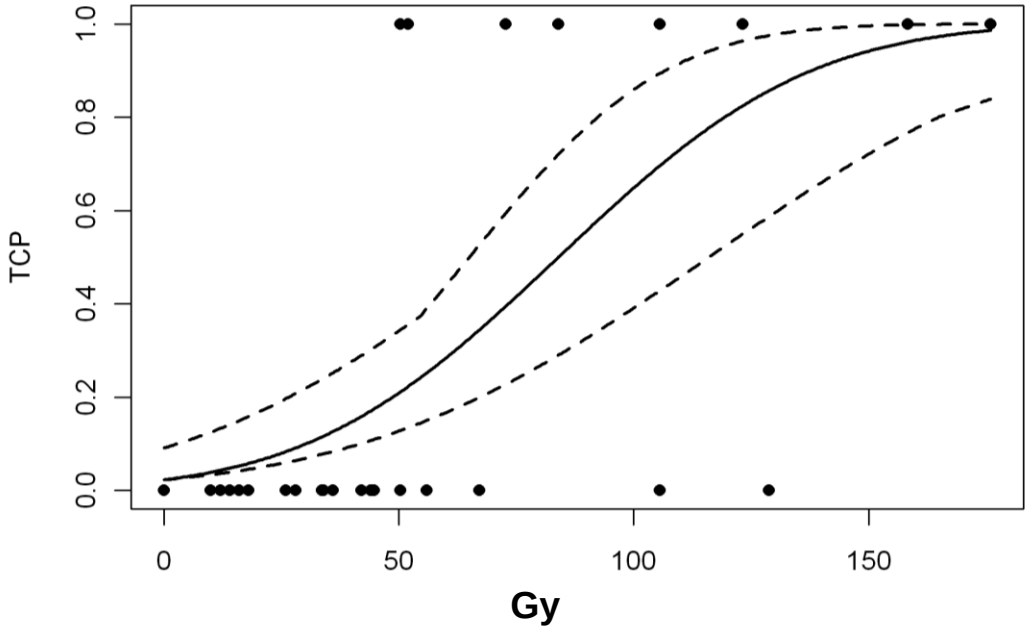






Tumor control propability (TCP)

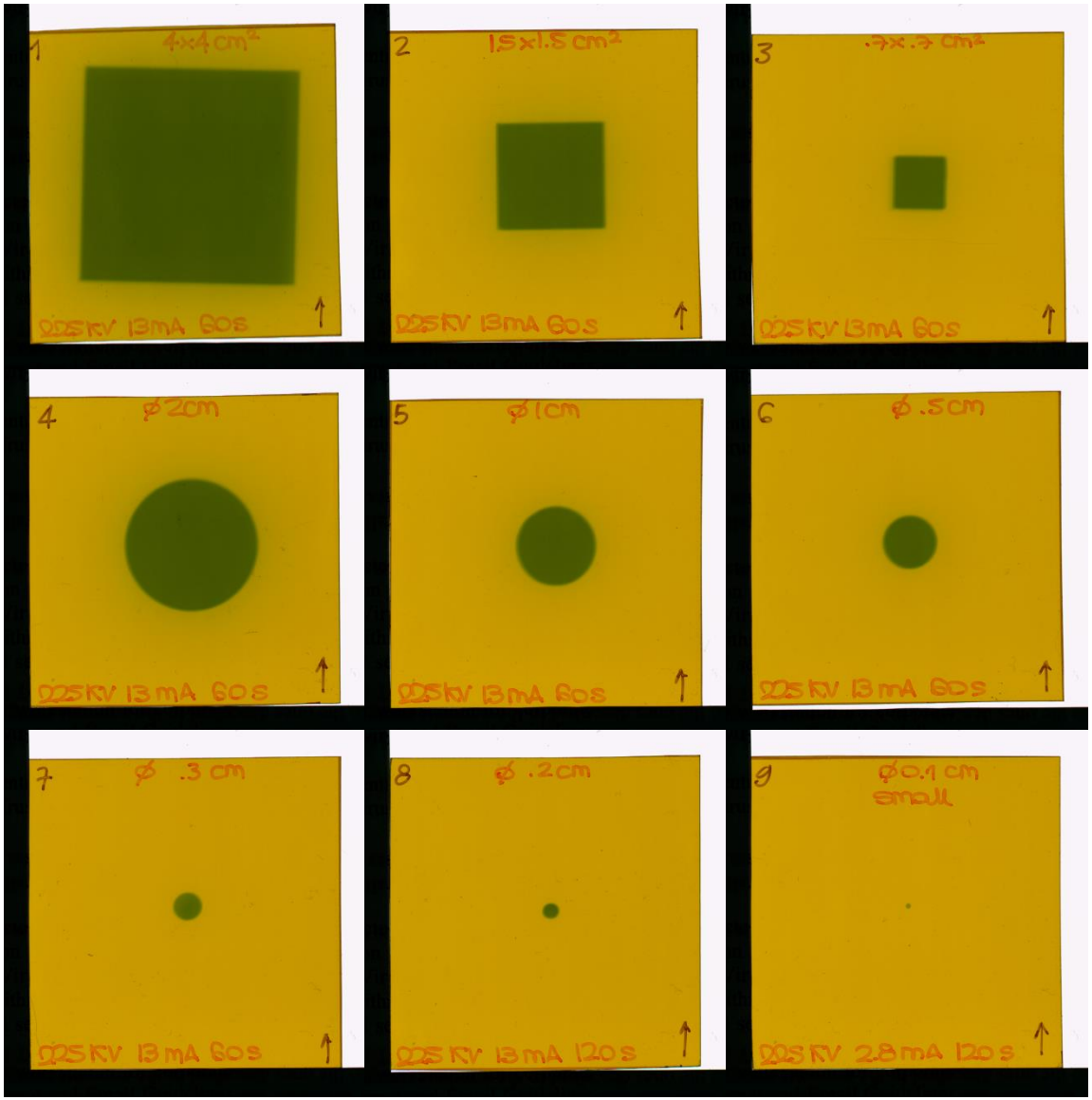
1. High doses are needed to achieve tumor control (D50=84Gy, follow up 6 months)
2. Still only few mice cured (follow up 6months)
3. Tumor grows during treatment if irradiated with 2Gy every other day



XRAD225 in NKI

Collimators set…..

Beam Characteristics



EBT2 films

Scanned pre and post irradiation

Film @ isocenter

(1 cm in solid water)

Accuracy

85 μm/pixel when scanned at 300 dpi

Why study radiotherapy responses in genetically engineered mouse cancer models?

- Lack of local radiotherapy control is a handicap in the clinic
- Clinical decision making for local tumor control: surgery versus radiotherapy
- Xenograft models are often hypersensitive to RT
- Is tumor eradication achieved with clinically relevant doses?
- Induction of DNA damage: the question whether a DNA-damaging drug reaches all tumor cells is not an issue
- Use as *in situ* assay for DNA damage response (*e.g.* RAD51 foci)

BRCA1- or BRCA2-deficient cells are sensitive to poly(ADP-ribose) polymerase (PARP) inhibition

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]

[1]*Cancer Research UK Gene Function and Regulation Group and* [2]*The Breakthrough Breast Cancer Research Centre Institute of Cancer Research, Fulham Road, London SW3 6JB, UK*
[3]*Guy's Hospital, St Thomas' Street, London SE1 9RT, UK*
[4]*KuDOS Pharmaceuticals Ltd, Cambridge Science Park, Cambridge CB4 0WG, UK*
[5]*Wellcome Trust and Cancer Research UK, Gurdon Institute of Cancer and Developmental Biology, and Department of Zoology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QN, UK*

* These authors contributed equally to this work
† Present address: Division of Experimental Oncology1, CRO-IRCCS, Aviano 33081 PN, Italy

Nature 434:917-21, 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]

[1]*The Institute for Cancer Studies, University of Sheffield, Medical School, Beech Hill Road, Sheffield S10 2RX, UK*
[2]*Department of Genetics, Microbiology and Toxicology, Arrhenius Laboratory, Stockholm University, S-106 91 Stockholm, Sweden*
[3]*Northern Institute for Cancer Research, University of Newcastle upon Tyne, Medical School, Newcastle upon Tyne, NE2 4HH, UK*

Nature 434:913-7, 2005

PARPi Lynparza (olaparib) approved for the clinical use

AstraZeneca

Lynparza™ approved in the European Union as first-in-class treatment for advanced BRCA-mutated ovarian cancer

Thursday, 18 December 2014

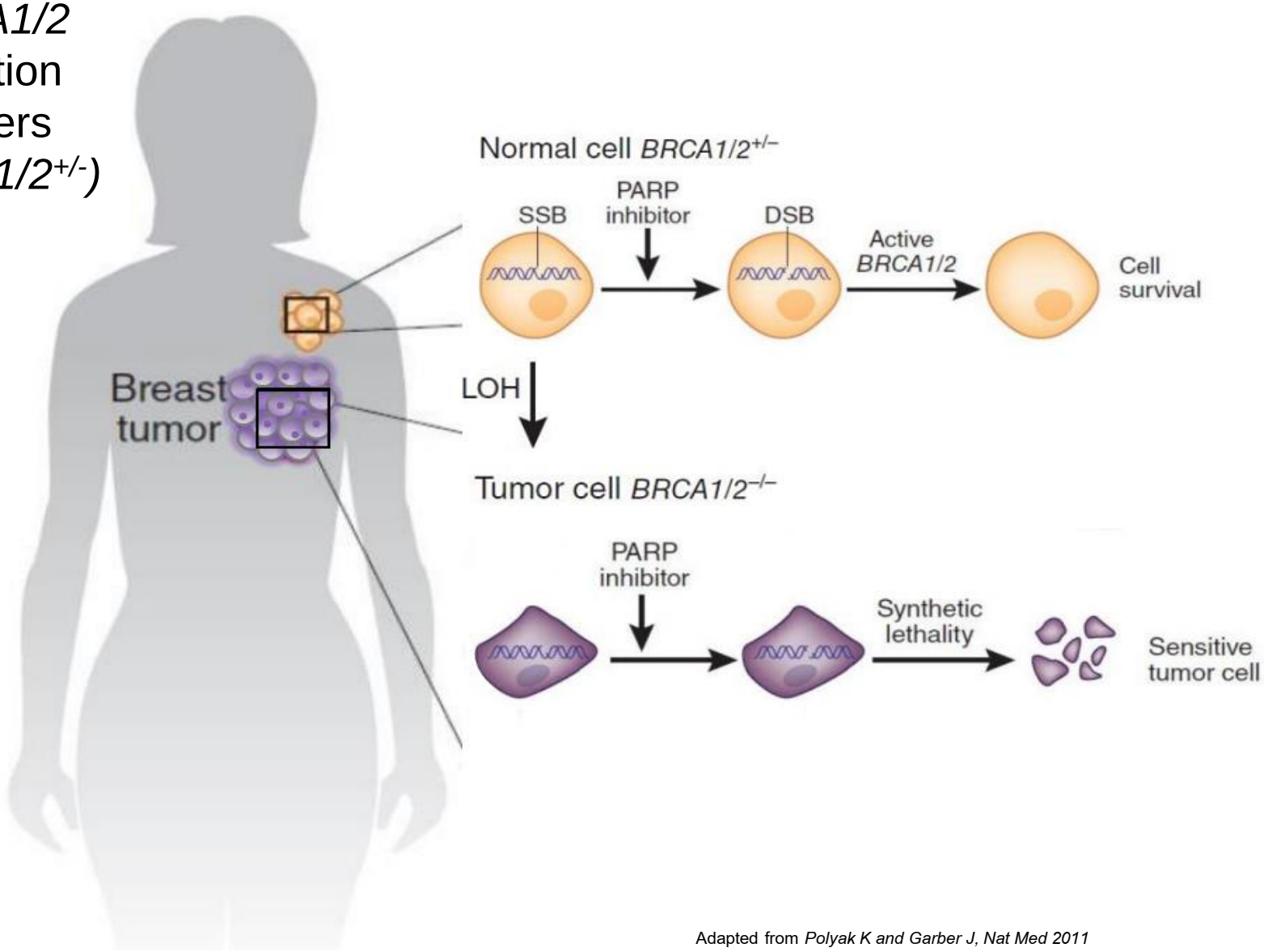
LYNPARZA™ approved by the US food and drug administration for the treatment of advanced ovarian cancer in patients with germline BRCA-mutations

Friday, 19 December 2014

www. astrazeneca.com

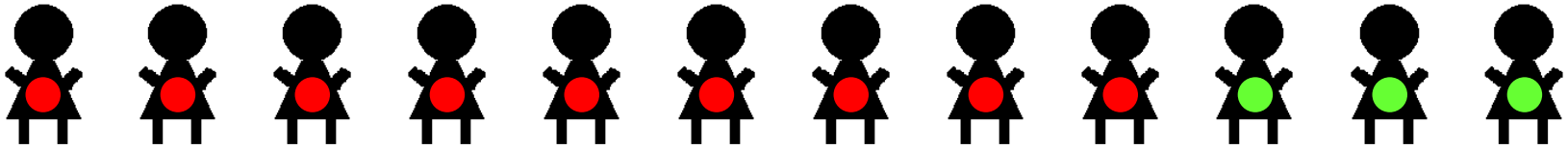
The principle of PARP inhibition

BRCA1/2 mutation carriers (*BRCA1/2*$^{+/-}$)



Adapted from *Polyak K and Garber J, Nat Med 2011*

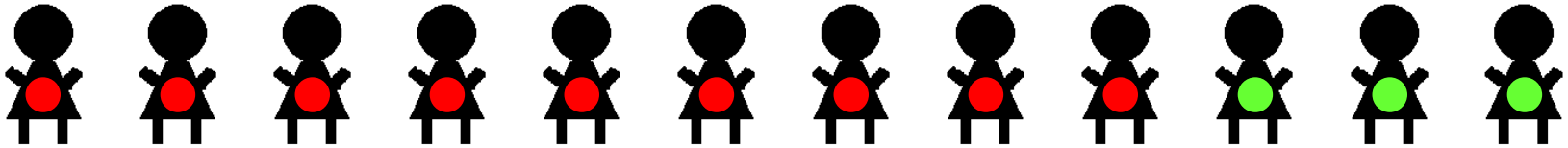
PARP inhibition in BRCA1/2-associated cancers



: BRCA1/2 deficient → therapy sensitive

: BRCA1/2 proficient → therapy resistant

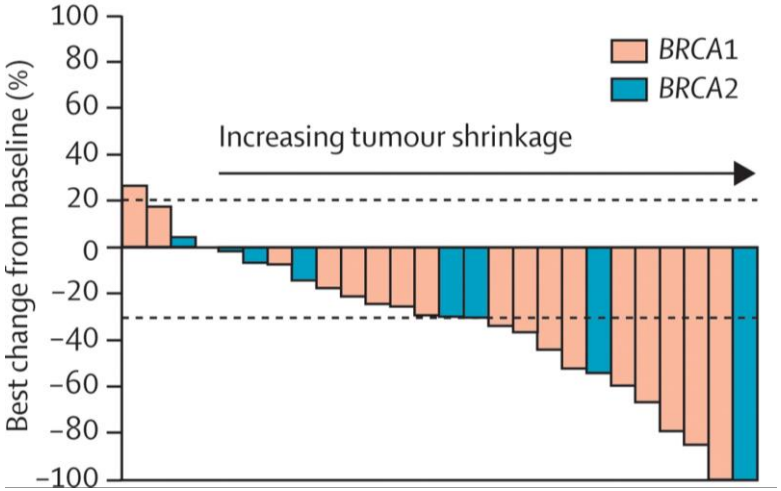
PARP inhibition in BRCA1/2-associated cancers



: BRCA1/2 deficient → therapy sensitive

: BRCA1/2 proficient → therapy resistant

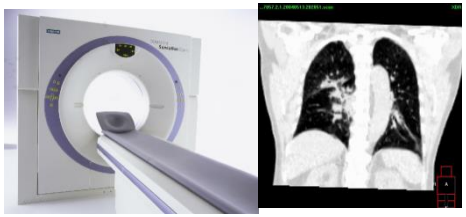
olaparib 400 mg twice daily



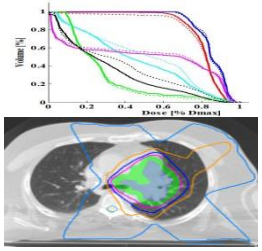
Tutt et al. (2010) Lancet 376, 235-44.

The modern radiotherapy process

Pre-treatment Imaging



Treatment Planning



In Room Imaging

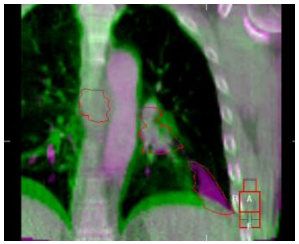
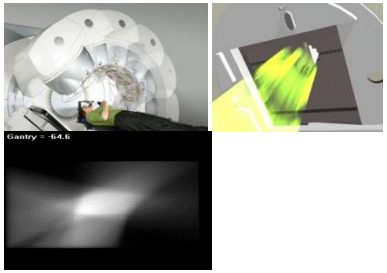


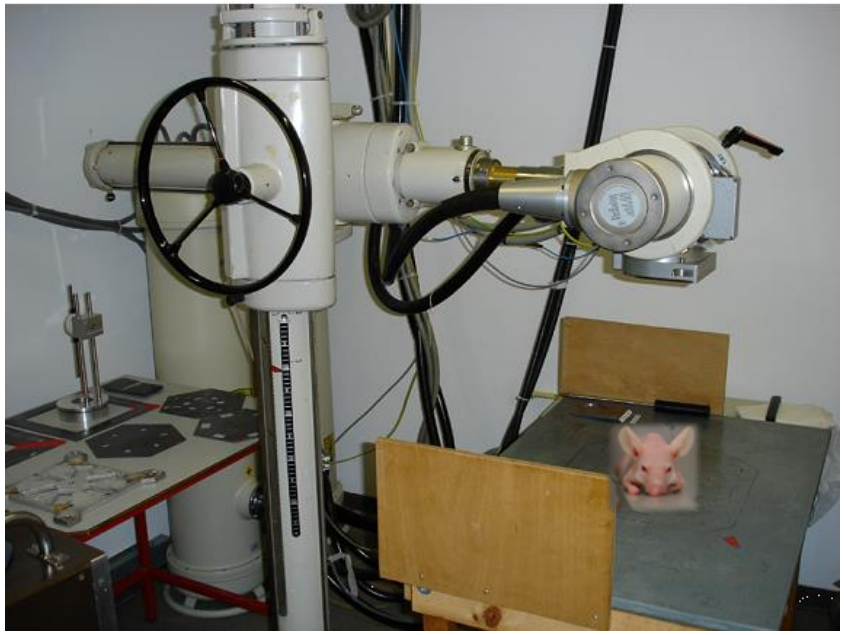
Image Registration
& Correction

Treatment Delivery

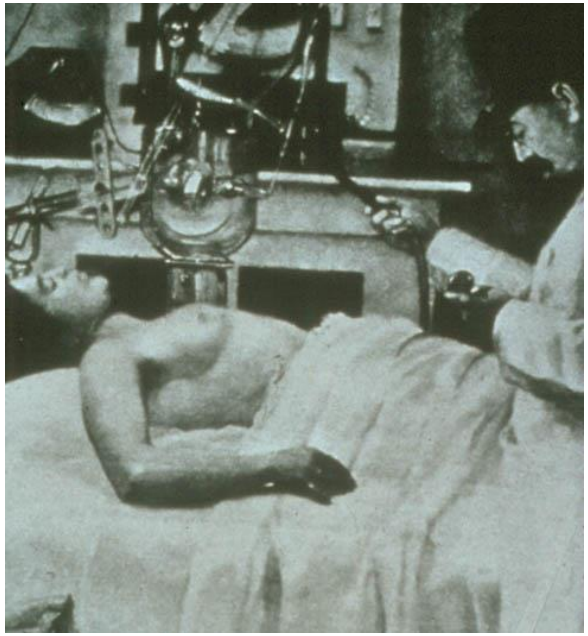


Dosimetry

Preclinical RT Technology



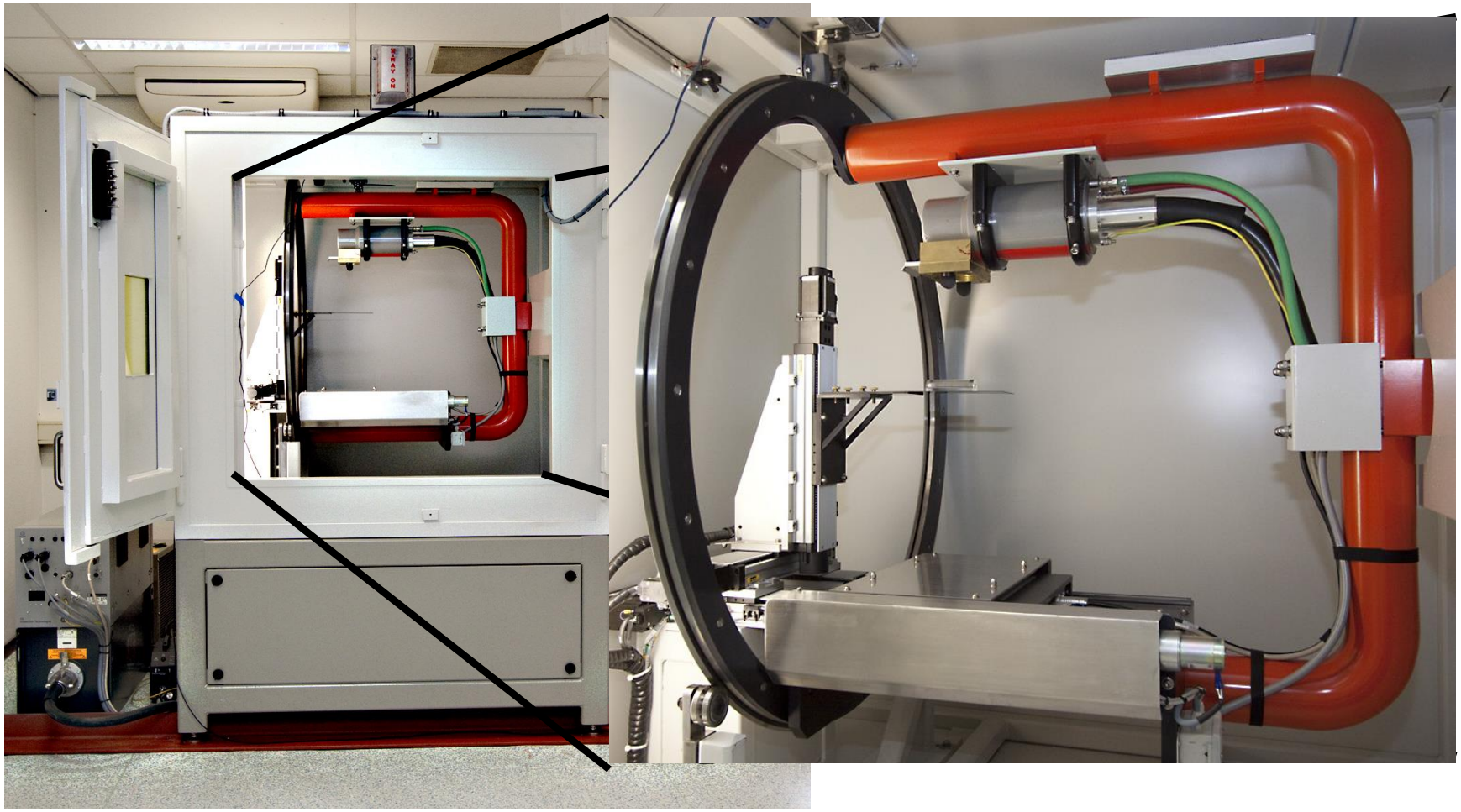
Pantak HF320 irradiation machine



Clinical RT in 1900

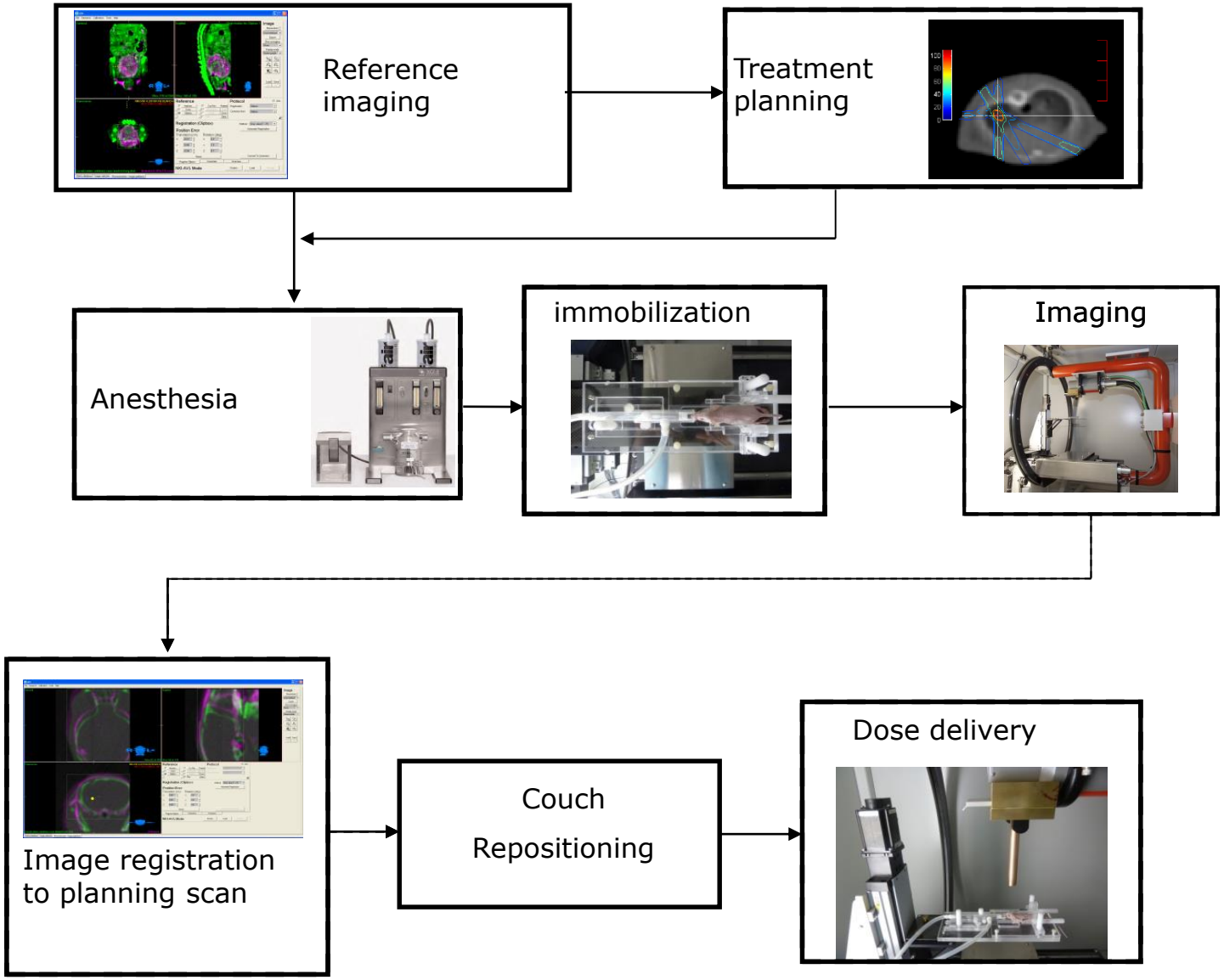
1. Large field sizes
2. High dose to normal tissues
3. No image guidance
4. No treatment planning
5. No dose verification

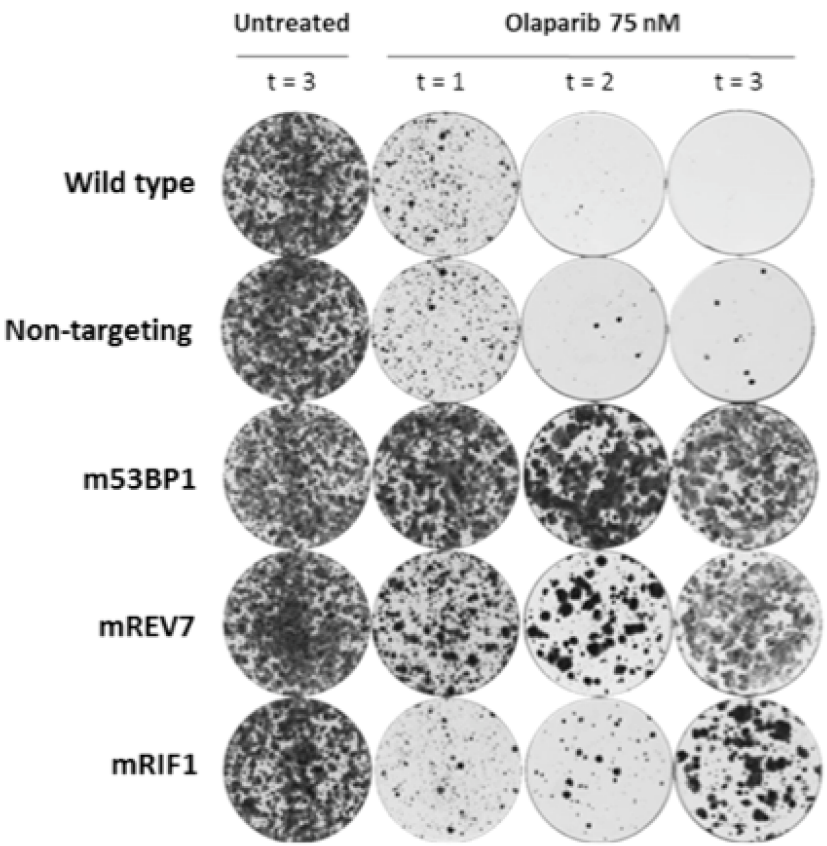
Xrad 225Cx

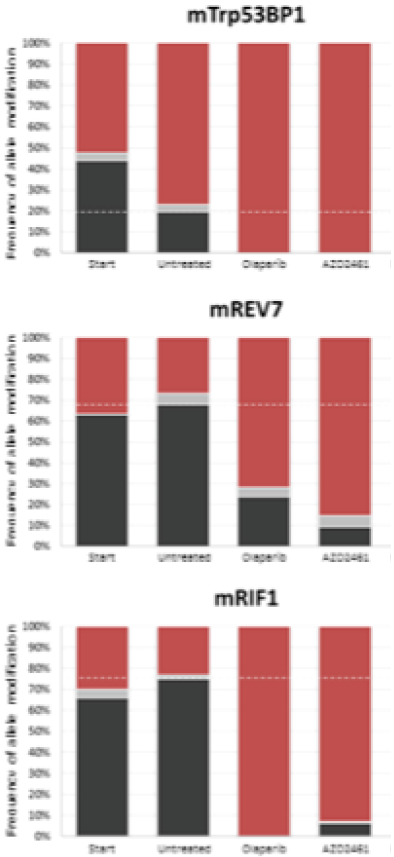


Developed by Princess Margaret Hospital (PMH, Toronto) in collaboration with Precision X-ray Inc (PXI, USA)

Pre-clinical μIGRT







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%Frameshift alleles

%In-frame alleles

%Wild type alleles