Labor für Strahlenbiologie & Experimentelle Radioonkologie
Klinik für Strahlentherapie und Radioonkologie

# DNA-Reparatur als Target für die individualisierte Prostatakrebstherapie

Kai Rothkamm

Update SBRT bei Oligo-Metastasen: Indikationen beim Prostata- und Bronchialkarzinom

23. November 2018

Hubertus Wald Tumorzentrum
Universitäres Cancer Center Hamburg
Ein Kompetenznetzwerk des UKE

Universitätsklinikum Hamburg-Eppendorf

# Thanks to



**Laboratory of Radiobiology & Experimental Radiation Oncology**

AG1 Homologous Recombination & Genomic Instability
K. Borgmann, A. Parplys, F. Meyer, E. Rahlf, Y. Goy, B. Riepen, E. Matschl

**AG2 Regulation of DSB Repair in Tumors**
**W. Mansour, S. Köcher, C. Oing**, M. Elsesy, S. Fahmy, L. Nordquist, S. Meien, A. Zielinski

AG3 Signal Transduction & Molecular Targeting
M.Kriegs, T. Rieckmann, L. Bußmann, J. Bartels, K. Hoffer, F. Gatzemaier, S. Christiansen

**Collaborations:**

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S. Burdak-Rothkamm & R. Simon (Pathology)
T. Lange & U. Schumacher (Anatomy)
H. Huland, T. Schlomm, B. Beyer & P. Tennstedt (Martini-Klinik)
B. Fehse (Stem Cell Transpl.)
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R. Gatti (Pathology, LA)
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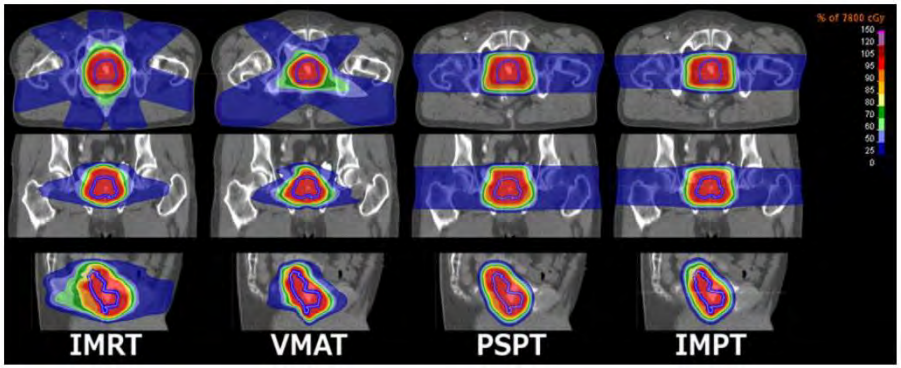
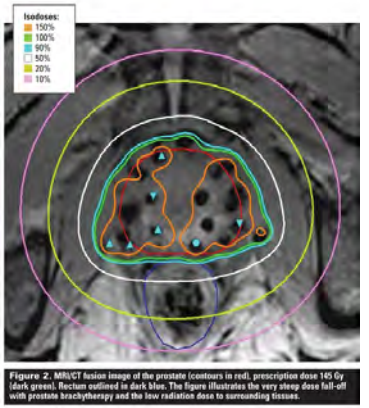
Monika Kutzner Stiftung

# Radiation treatment options for prostate cancer





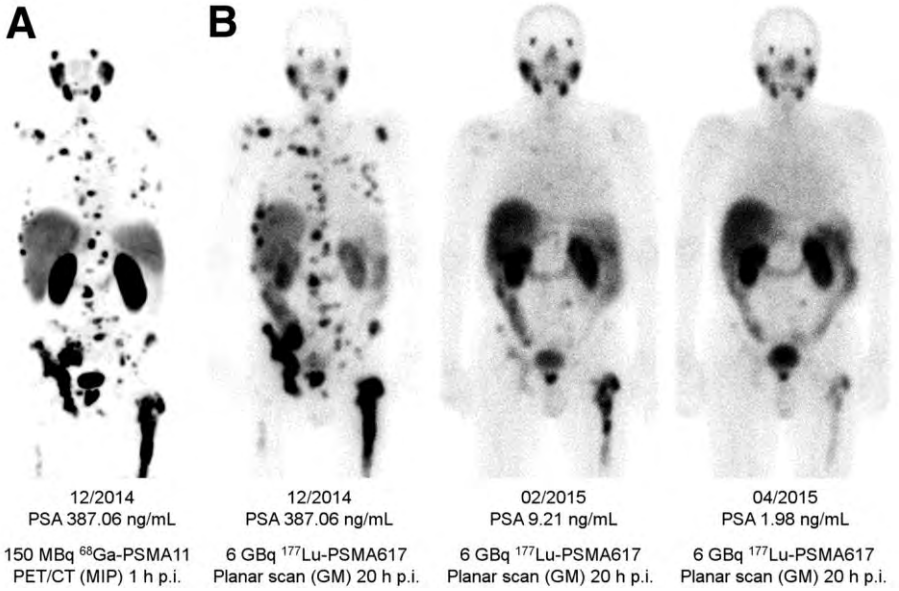

Walsh et al (2018) Cancers 10:55




Keyes et al (2010) BCMJ 52:76

- EBRT
- LDR & HDR brachytherapy

- SBRT (oligometastases)
- PSMA-targeted Lu-177 radionuclide therapy
- Ra-223 therapy (bone mets)




Kratochwil et al JNM (2016)

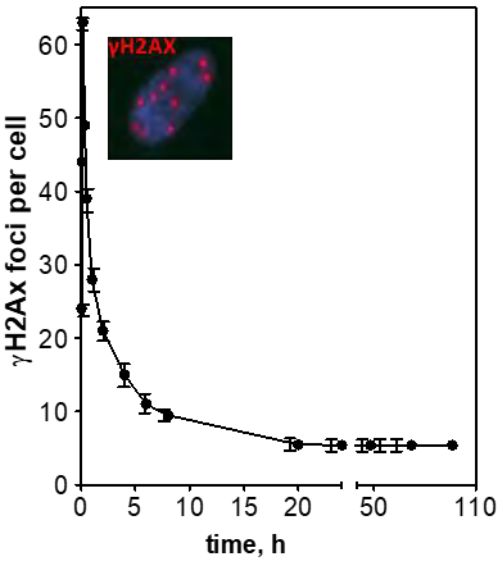
# DSB repair and cancer treatment



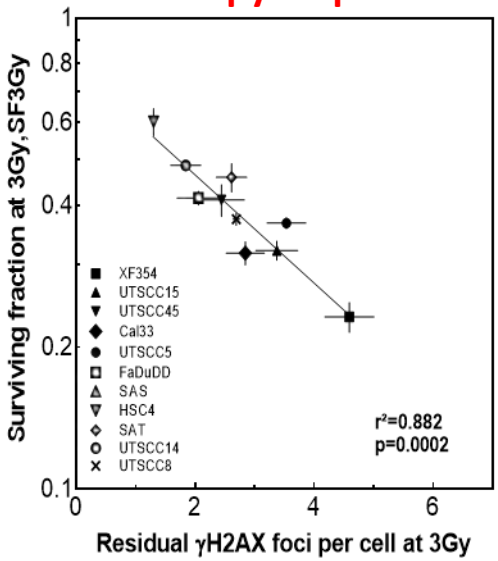
## Cancer treatment strategies:

- Surgery
- Radiotherapy
- Chemotherapy

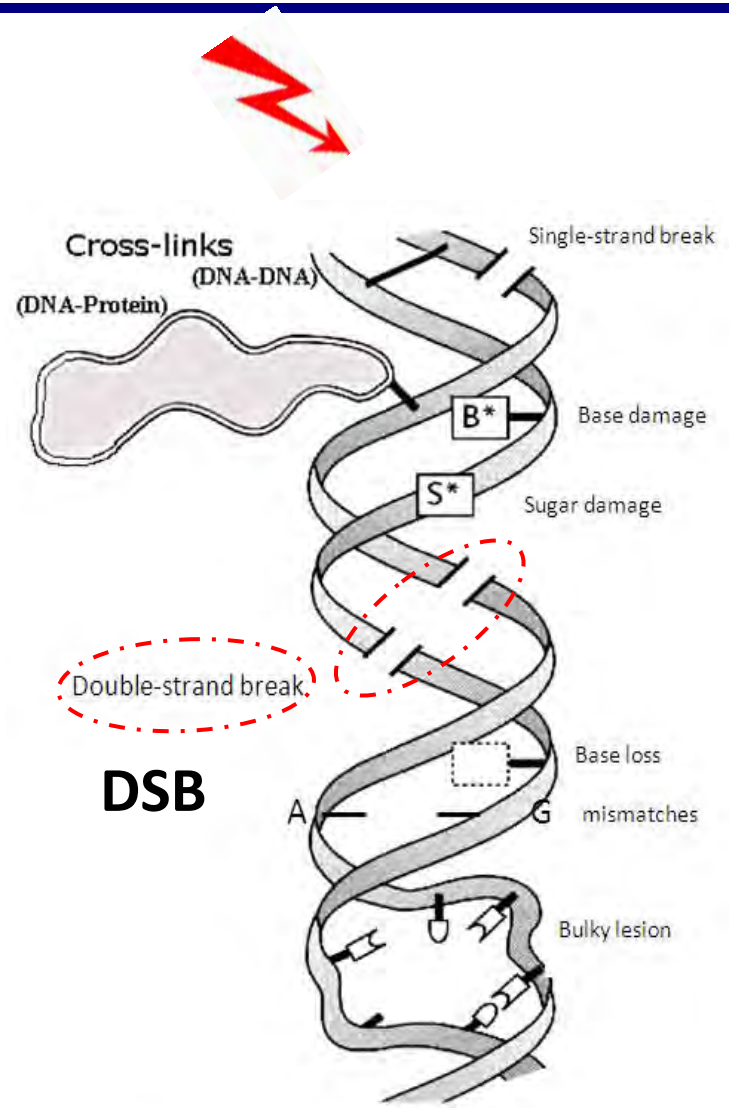
**DSB**

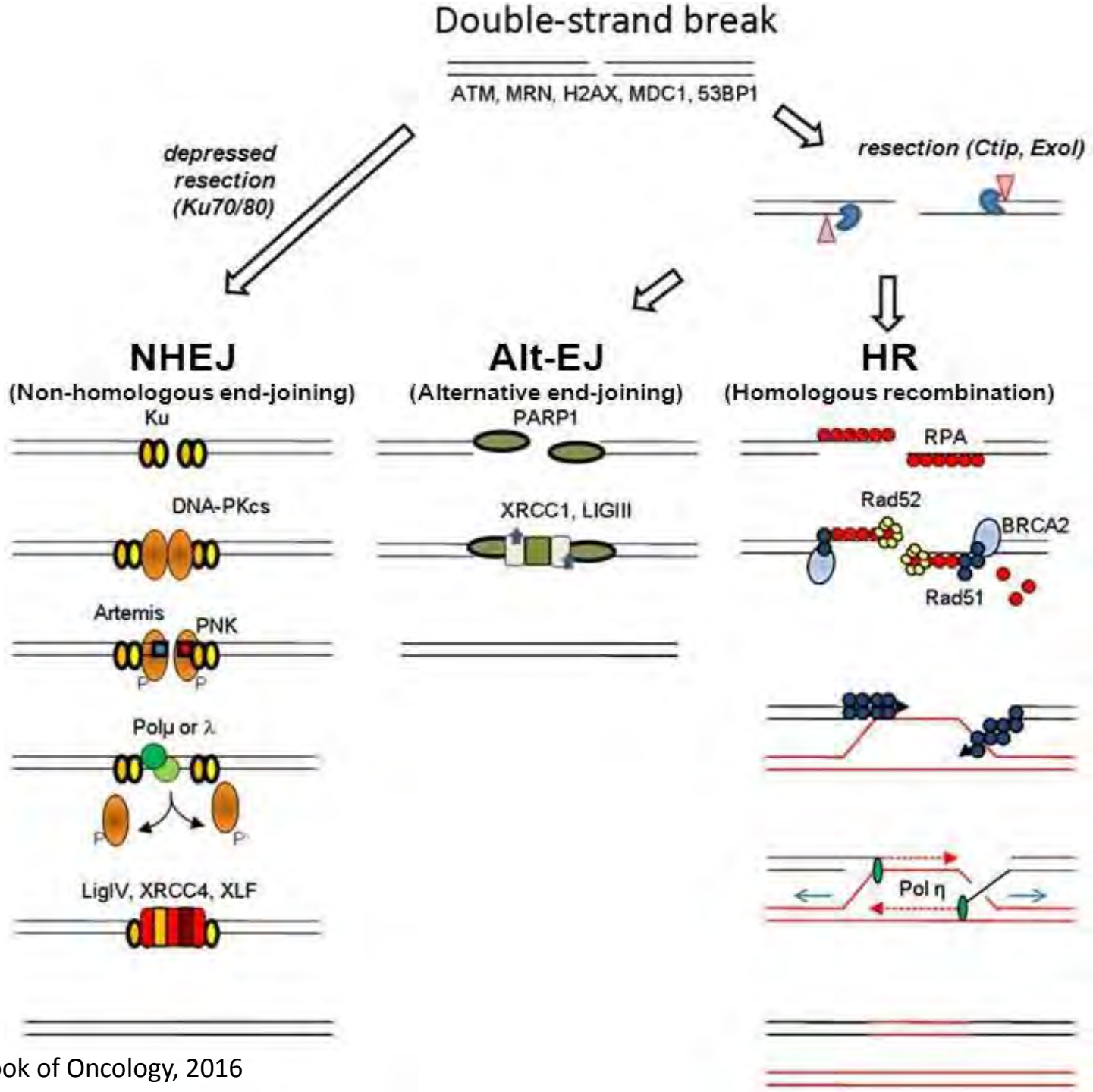



**DSB repair determines therapy response**




Unrepaired DSBs

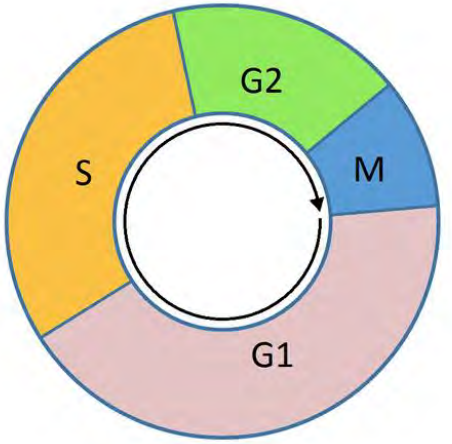





Oxford Textbook of Oncology, 2016

# Targeting DSB repair to enhance cancer treatment






**Facts:**

- *DSB repair is a potent barrier against carcinogenesis*
- *DSB repair is critical for cell reproduction*

**Hypotheses:**

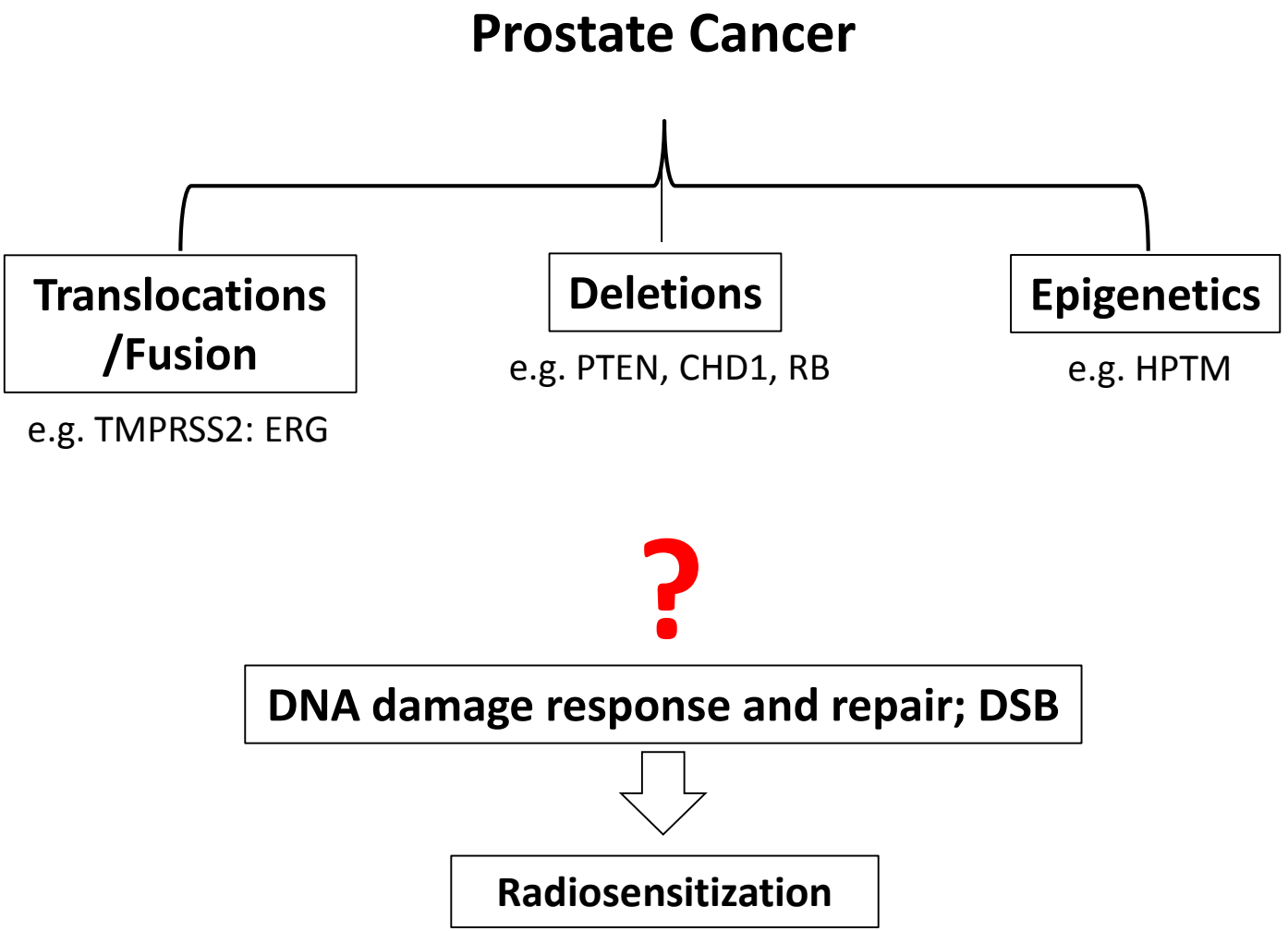
- *DSB repair is frequently deregulated in cancer to balance cell growth and the accumulation of genomic alterations*
- *This deregulation could be exploited for tumor-specific targeting*

**Tasks:**

- *Identify tumor-specific DSB repair alterations*
- *Establish tumor-specific targeting strategies to enhance radiotherapy efficacy*

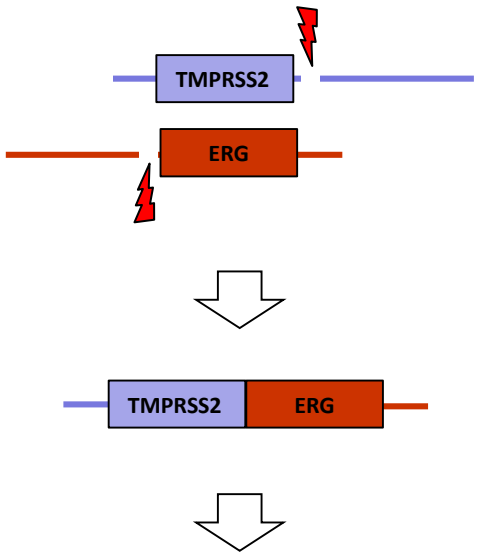
# Prostate cancer: genomic and epigenetic changes





# Prostate cancer genome: TMPRSS2:ERG fusion






**ERG overexpression**

Prostate Cancer and Prostatic Diseases (2010) 13, 228–237

1365-7852/10
www.nature.com/pcan

ERG oncoprotein expression in prostate cancer: clonal progression of ERG-positive tumor cells and potential for ERG-based stratification

**~ 50% of PCa**

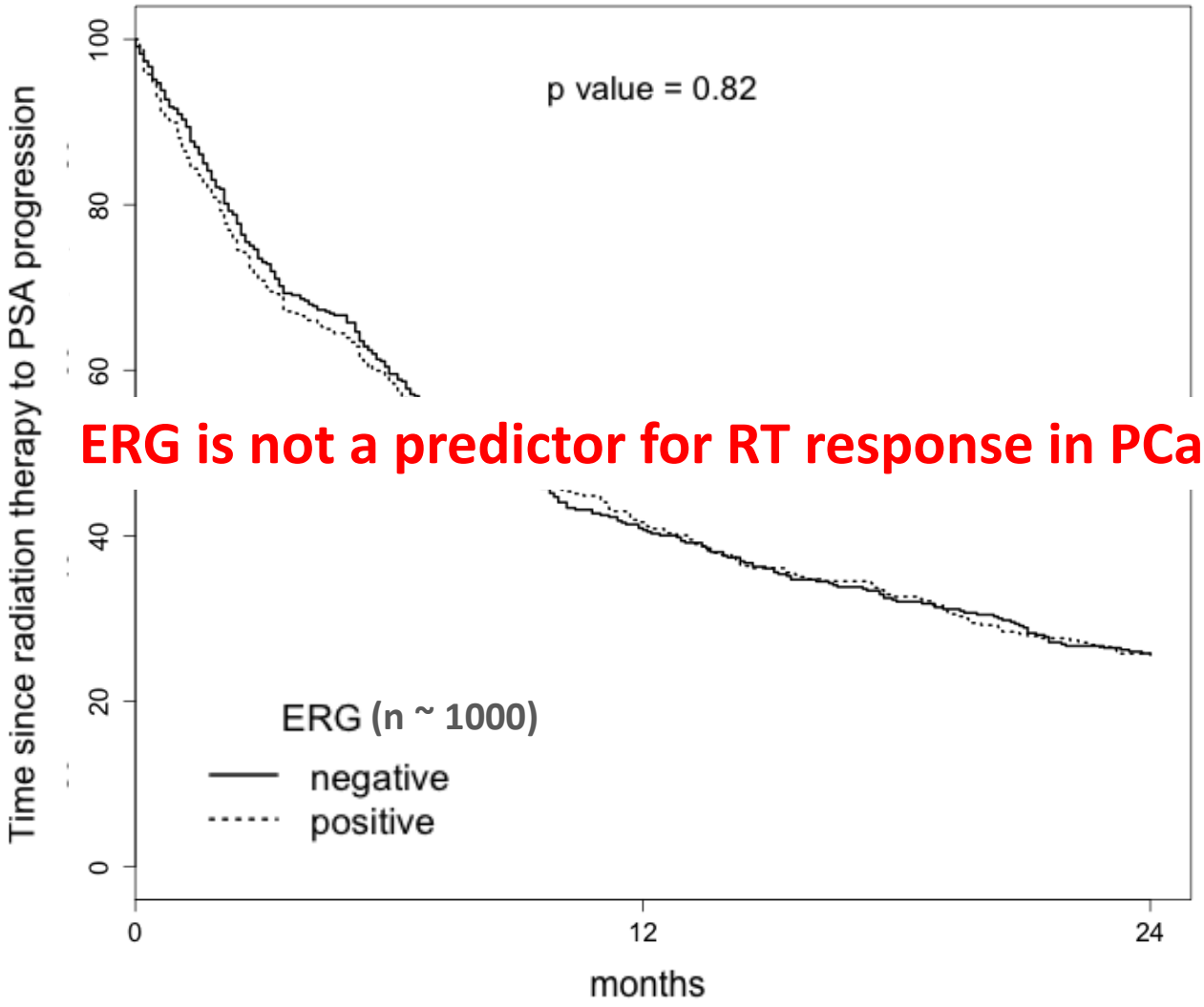
THE JOURNAL OF UROLOGY®


**Molecular Diagnosis of Prostate Cancer: *PCA3* and *TMPRSS2:ERG* Gene Fusion**

Maciej Salagierski and Jack A. Schalken*

# ERG does not affect the response to RT in PCa patients

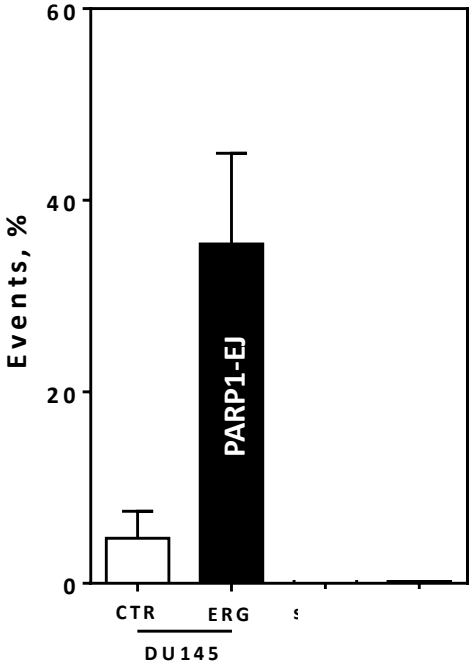




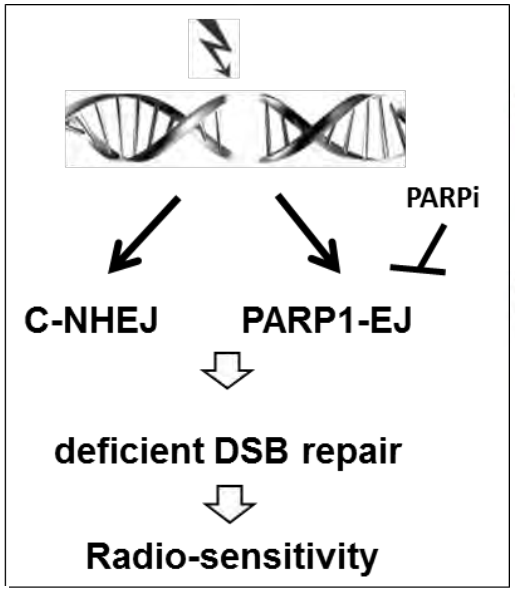

**ERG is not a predictor for RT response in PCa**

# DSB repair in ERG positive PCa: *in vitro* analysis



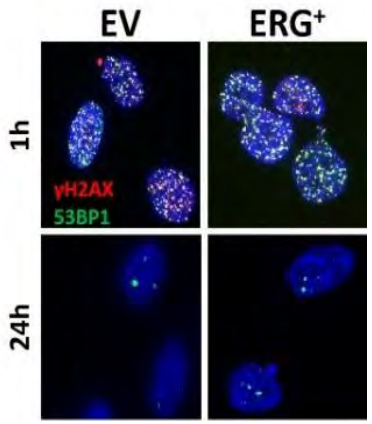



ERG⁺ cells ???

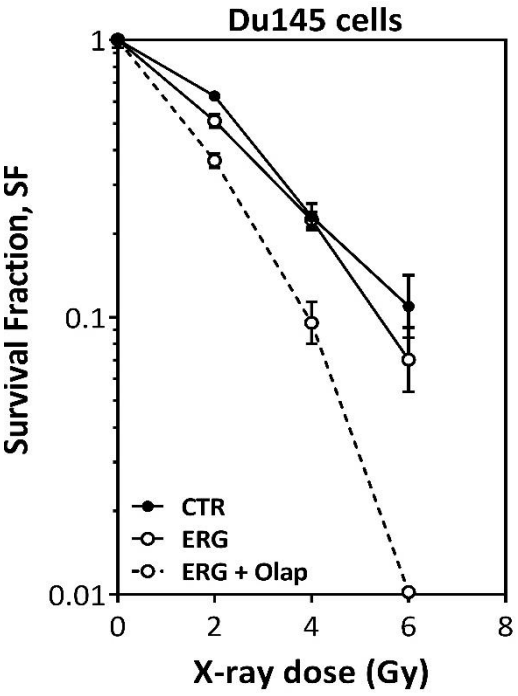



*Kötter et al., Mol Oncology 2014*

More residual DSBs after PARPi




Radiosensitized by PARPi



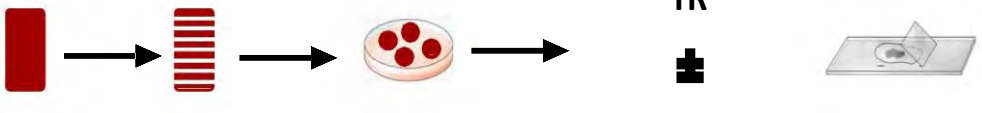

**ERG overexpression leads to repair switch to PARP1-EJ**

*In collaboration with Martini-Klinik*

*S. Köcher*

## Functional *ex-vivo* assay

PCa patients



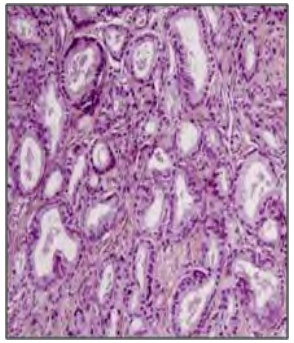
**Tumor biopsy** — Surgeon, IHC/ Pathology ✓

**300µm slices**

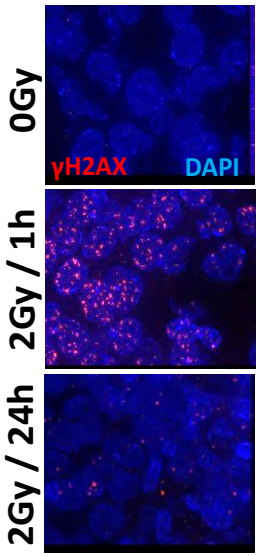
***ex-vivo* culture** — Conditions: **Oxygenation**, **Proliferation** ✓

**IR** + **Treatment**

**3-7µm slices**



**Pathology** — IHC/ Pathology ✓




**IF analysis**

DSB repair

Köcher et al, Int J Cancer, in press

# *Ex vivo* analysis to confirm ERG-mediated PARP1-EJ switch

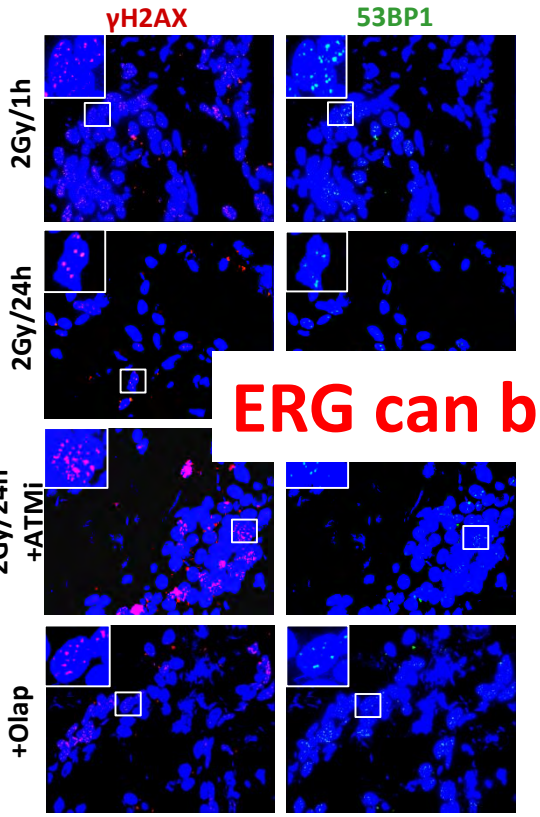
(50 samples)

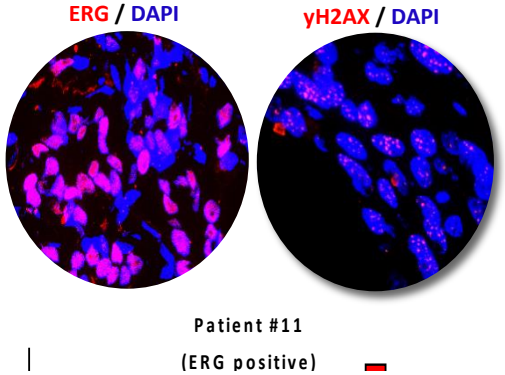


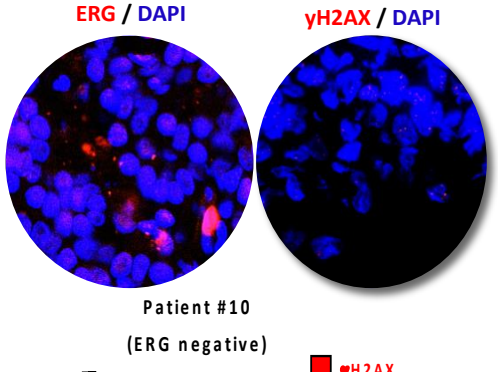
## *In cooperation with Martini-Klinik*

**Fresh tumor biopsies**
(~100 samples from ~ 50 PC patients)

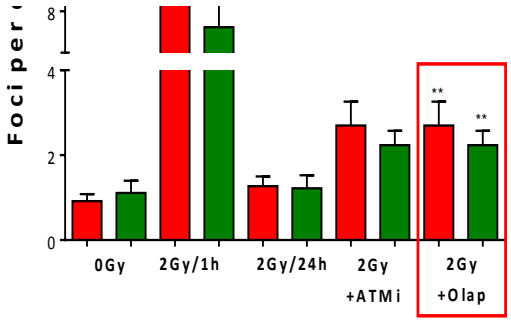
*Tissue + IR (2Gy) ± ATMi/ PARPi -------- γH2AX foci (1h & 24h)*



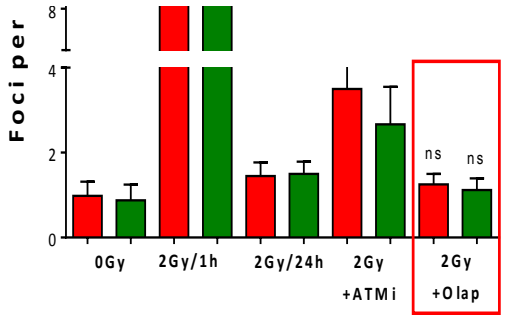




**ERG can be used to modulate RT response in PCa**




Radiosensitization by ATMi

Radiosensitization by PARPi




Radiosensitization by ATMi

NO radiosensitization by PARPi

Cancer Letters 423 (2018) 60–70

Contents lists available at ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet

Original Articles

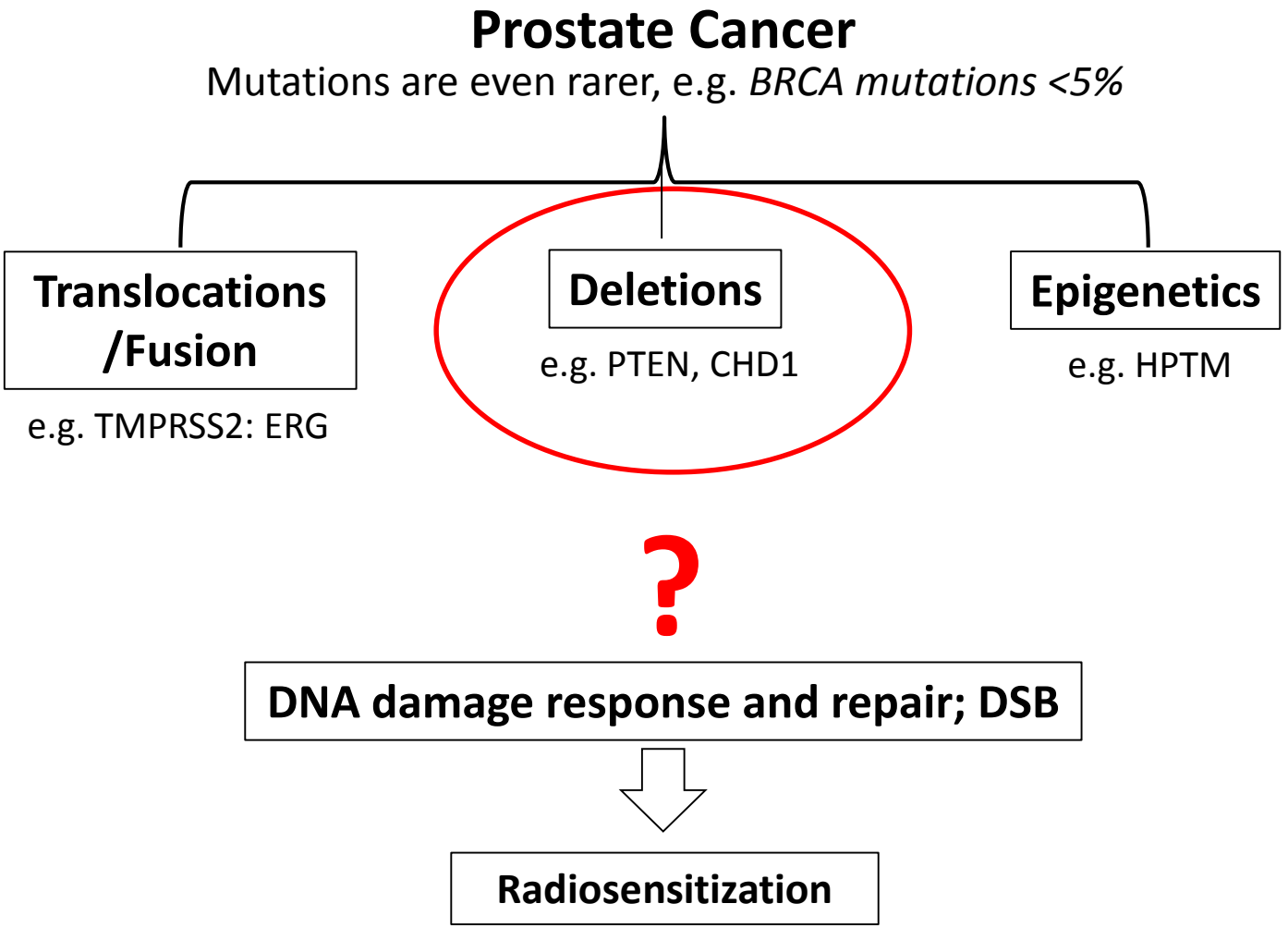
# BCL2-overexpressing prostate cancer cells rely on PARP1-dependent end-joining and are sensitive to combined PARP inhibitor and radiation therapy

Christoph Oing [a, b, 1], Pierre Tennstedt [c, 1], Ronald Simon [d, 1], Jennifer Volquardsen [a], Kerstin Borgmann [a], Carsten Bokemeyer [b], Cordula Petersen [e], Ekkehard Dikomey [a], Kai Rothkamm [a], Wael Y. Mansour [a, f, *]

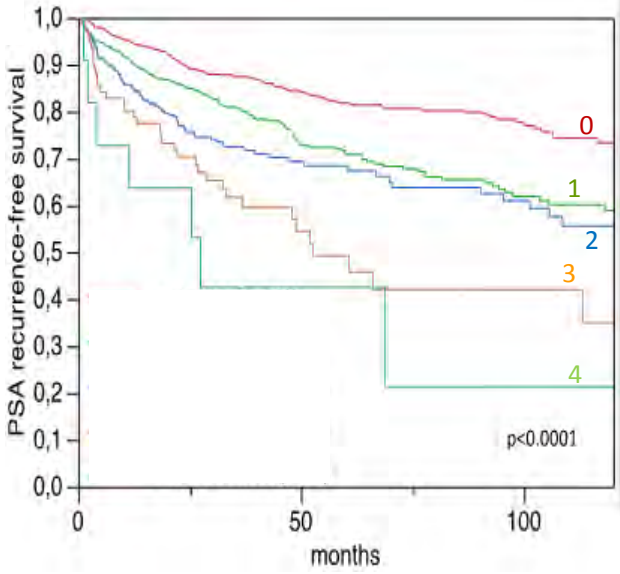
[a] *Laboratory of Radiobiology and Experimental Radiation Oncology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany*
[b] *Department of Oncology, Hematology and Bone Marrow Transplantation with Section of Pneumology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany*
[c] *Martini-Clinic, University Medical Center Hamburg Eppendorf, Hamburg, Germany*
[d] *Department of Pathology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany*
[e] *Department of Radiotherapy and Radiooncology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany*
[f] *Department of Tumor Biology, National Cancer Center, Cairo University, Cairo, Egypt*

# Prostate cancer Epi/genome

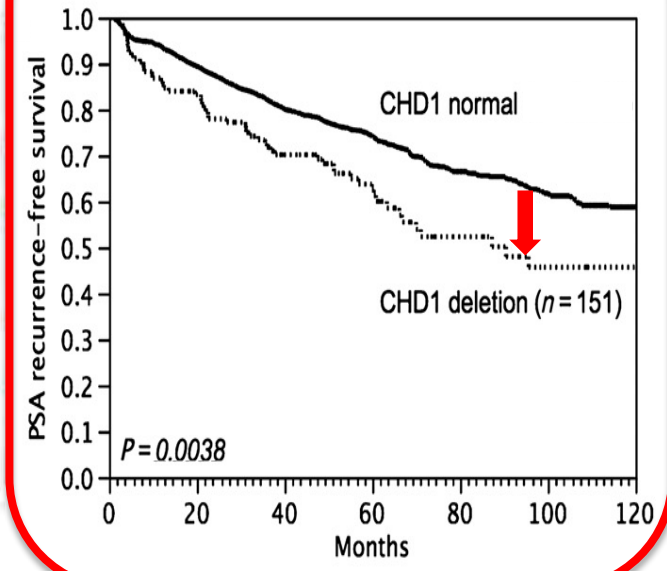




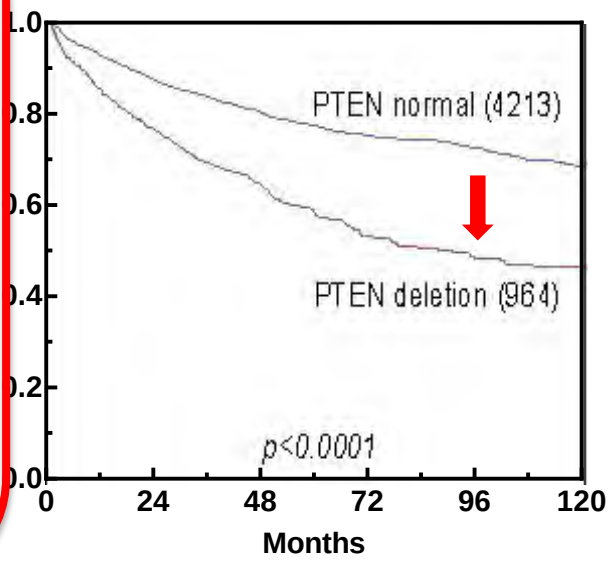
# Deletions affect survival of PCa patients




## CHD1 deletion




## PTEN deletion

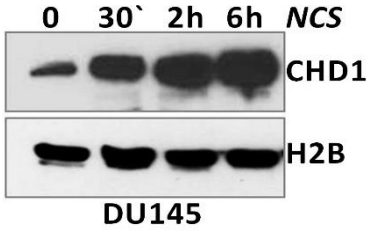



*Burkhardt et al. Ca Res, 2013*

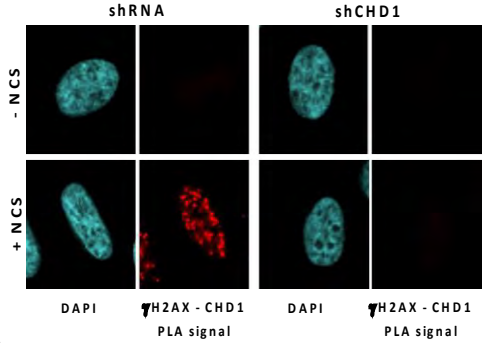
# CHD1 is required for DSB repair via homologous recombination

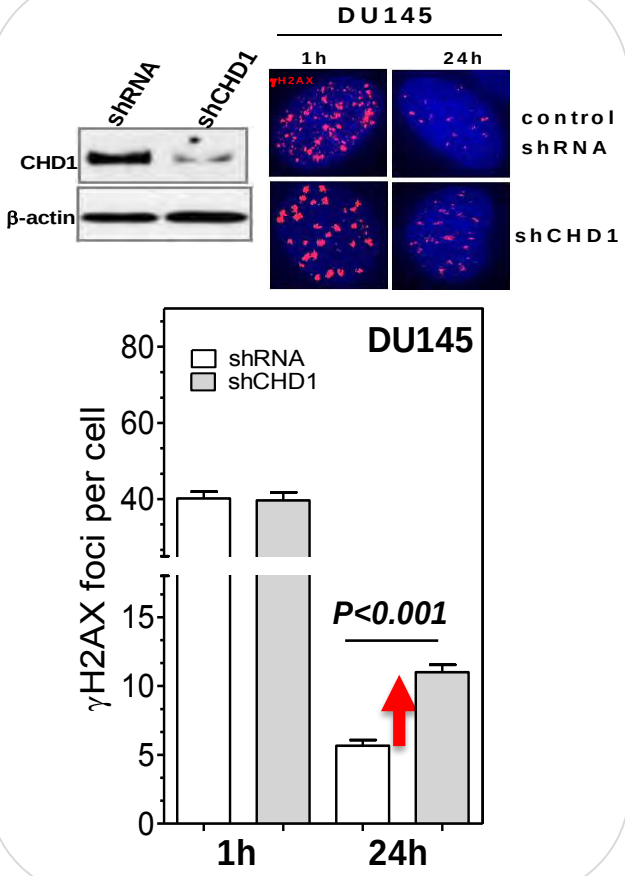
*(in collaboration with Prof. Dr. S. Johnsen, Göttingen)*

## Chromatin fraction

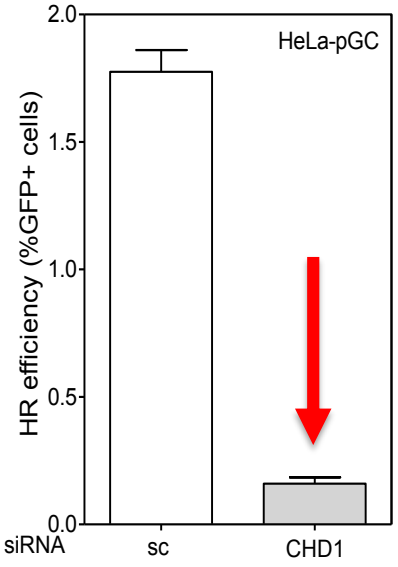



## Proximity Ligation Assay (PLA)



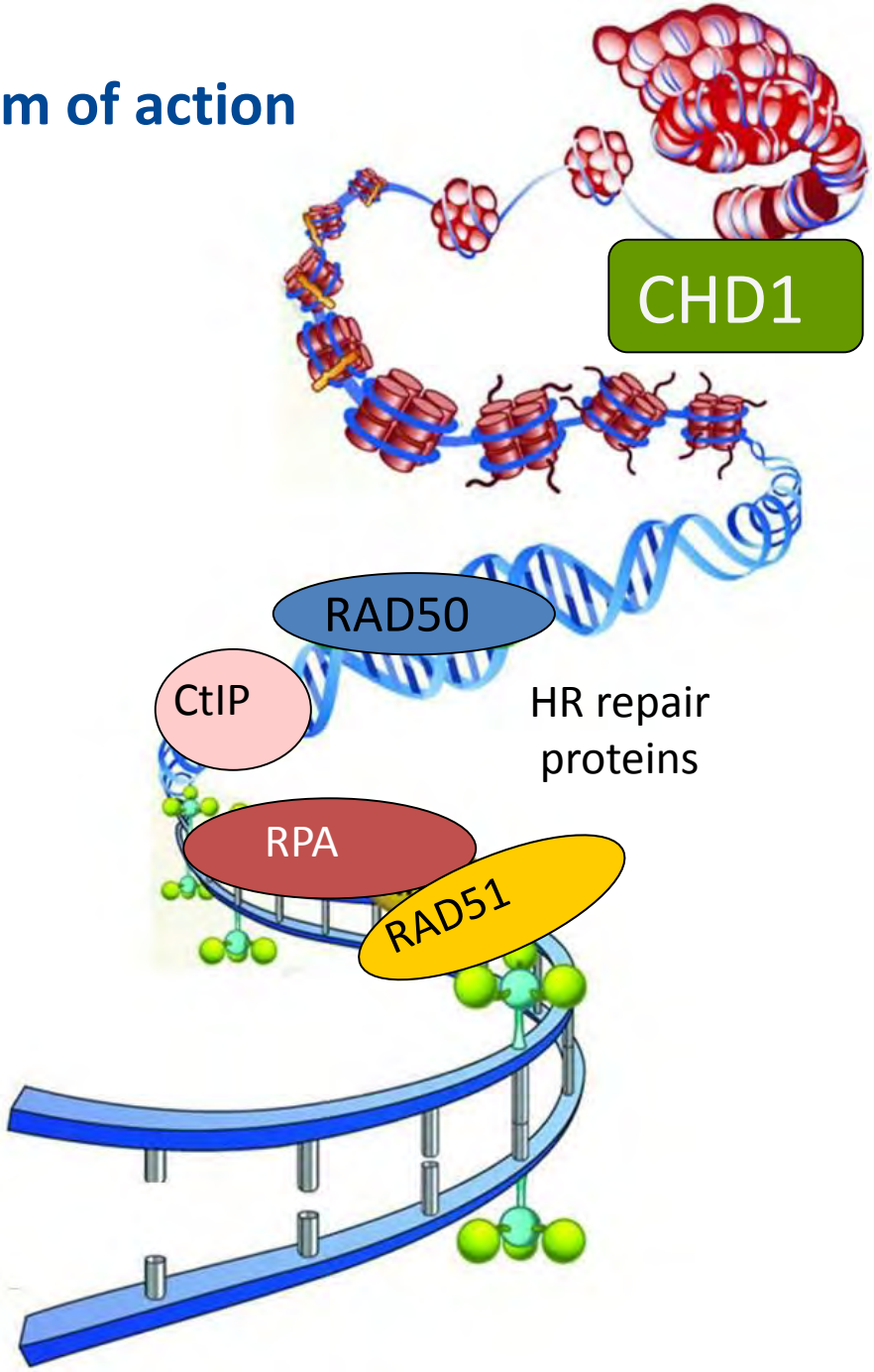




## Homologous Recombination


*Kari & Mansour et al., EMBO Rep, 2016*

# CHD1 mechanism of action

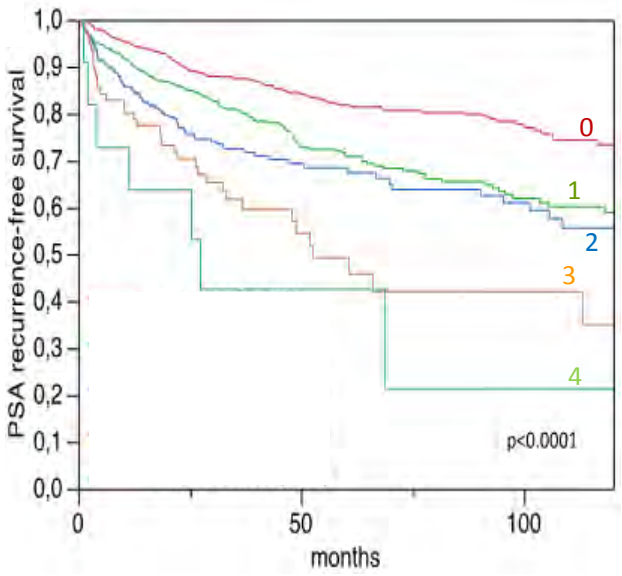



IR

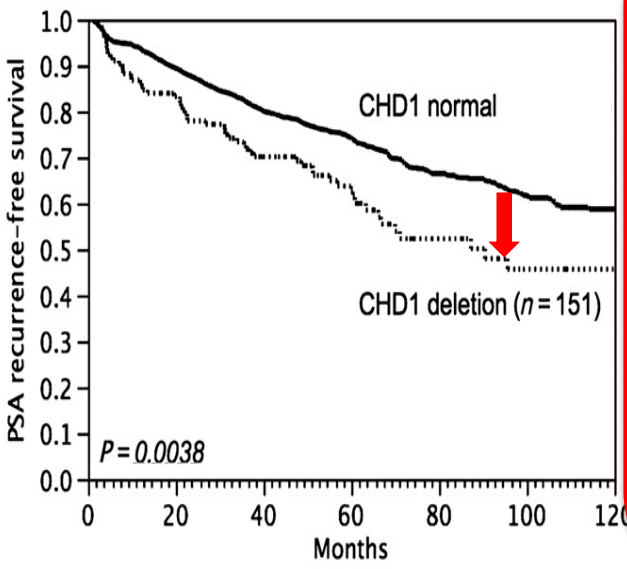
HR repair proteins

DSB Repair (HR)

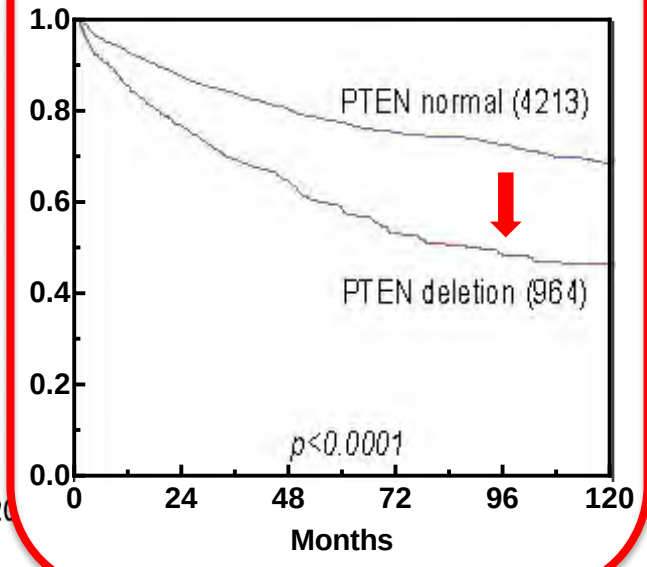
# Deletions affect survival of PCa patients




## CHD1 deletion

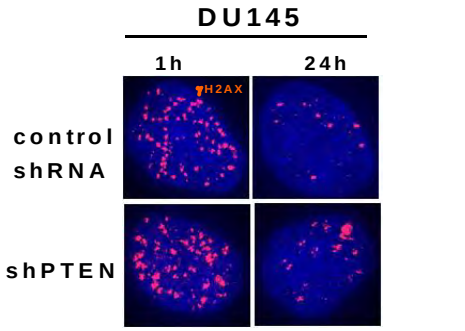


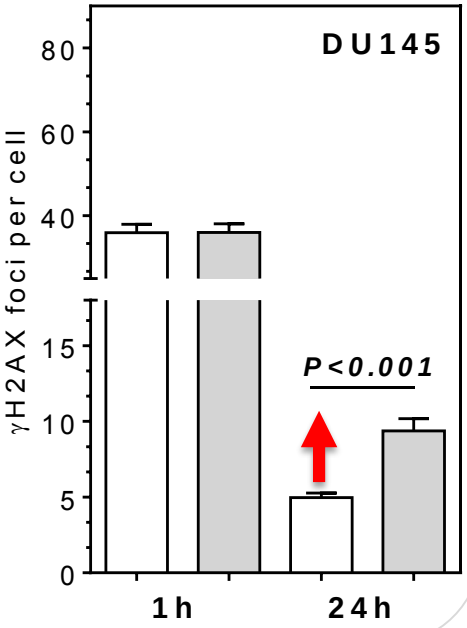

## PTEN deletion

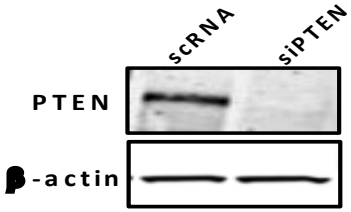



*Burkhardt et al. Ca Res, 2013*

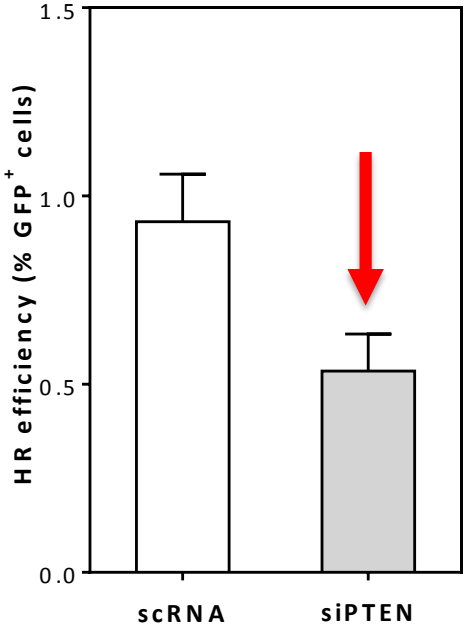
# PTEN is required for HR



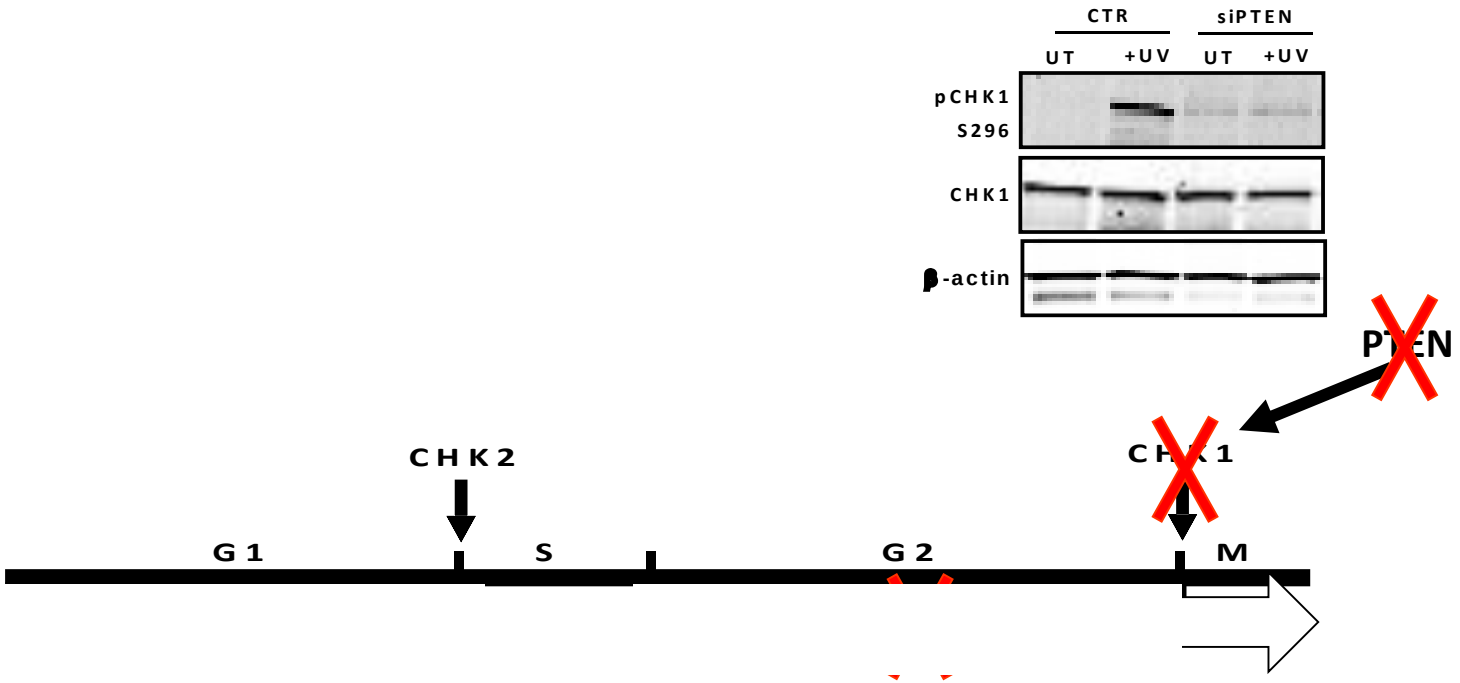




## Homologous Recombination


*Mansour et al., Sci Rep 2018*

# PTEN gives cells time to perform HR



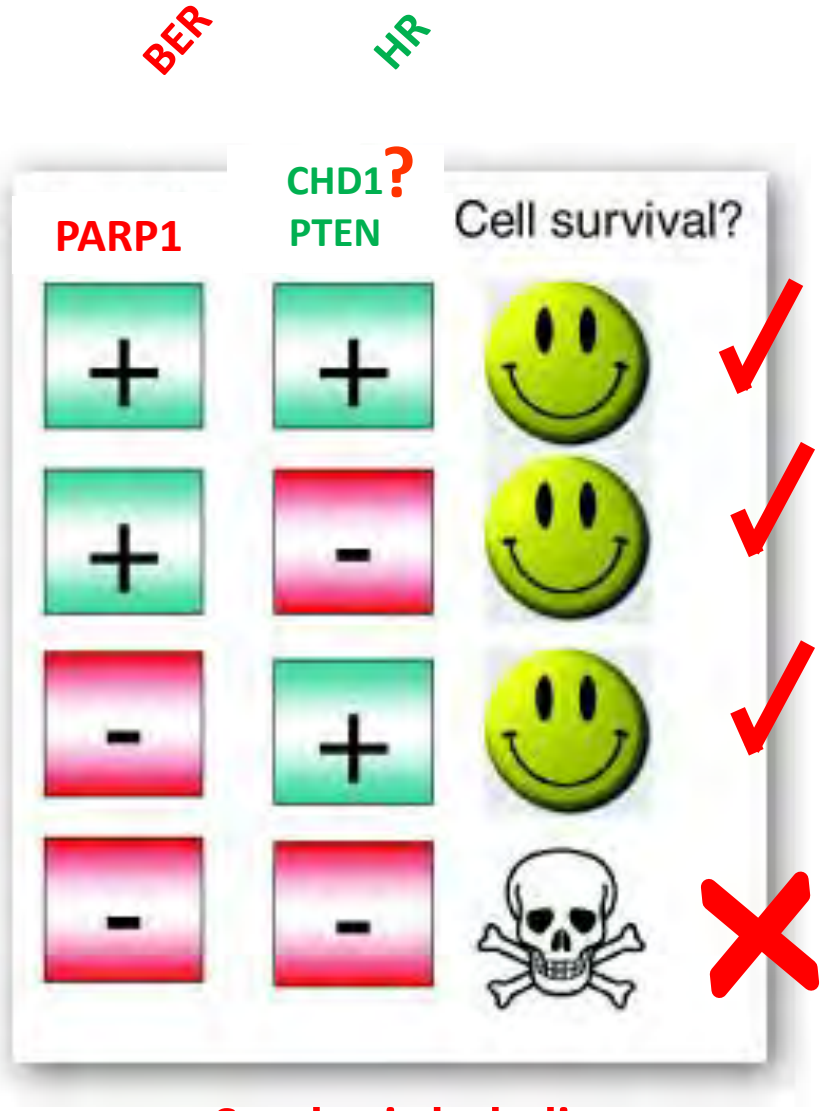

*Mansour et al., Sci Rep 2018*

# Targeting PCa genome:

- **Targeting deletions in PCa**

  **(HR-deficiency)**

  - **CHD1 deletion**
  - **PTEN deletion**



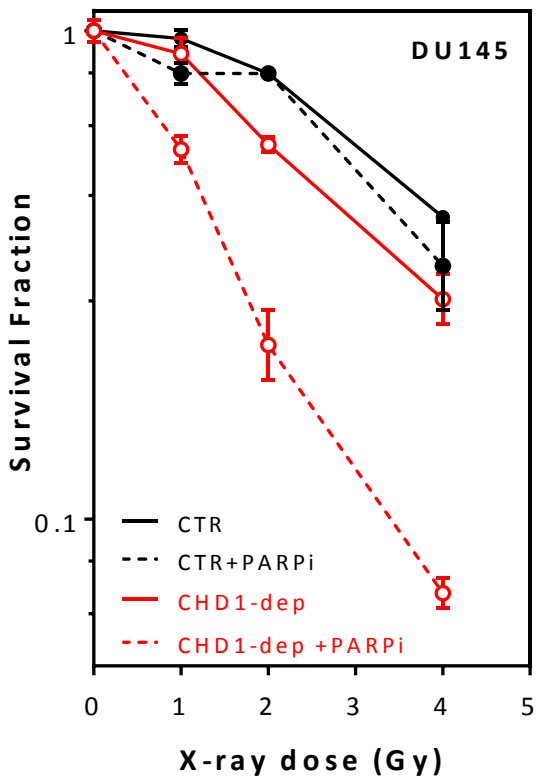

A and B

compensate each other

**Synthetic lethality**

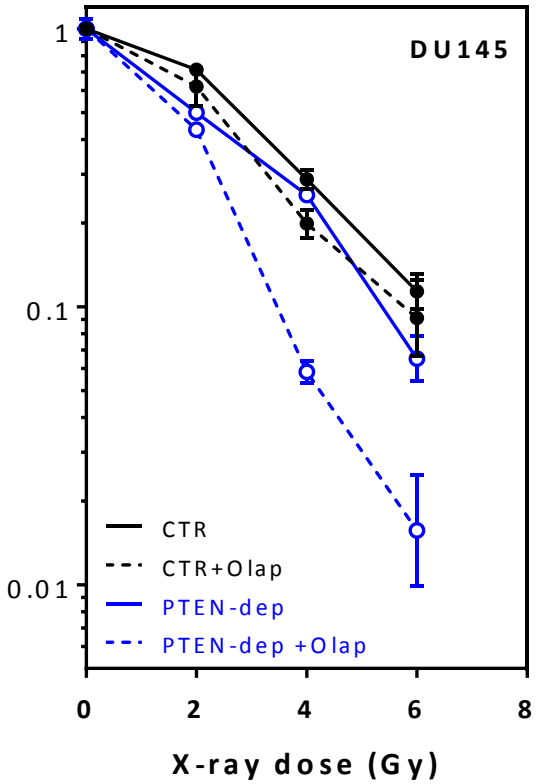
# Targeting PCa genome: *Proof of concept*

## Targeting CHD1 deletion in PCa



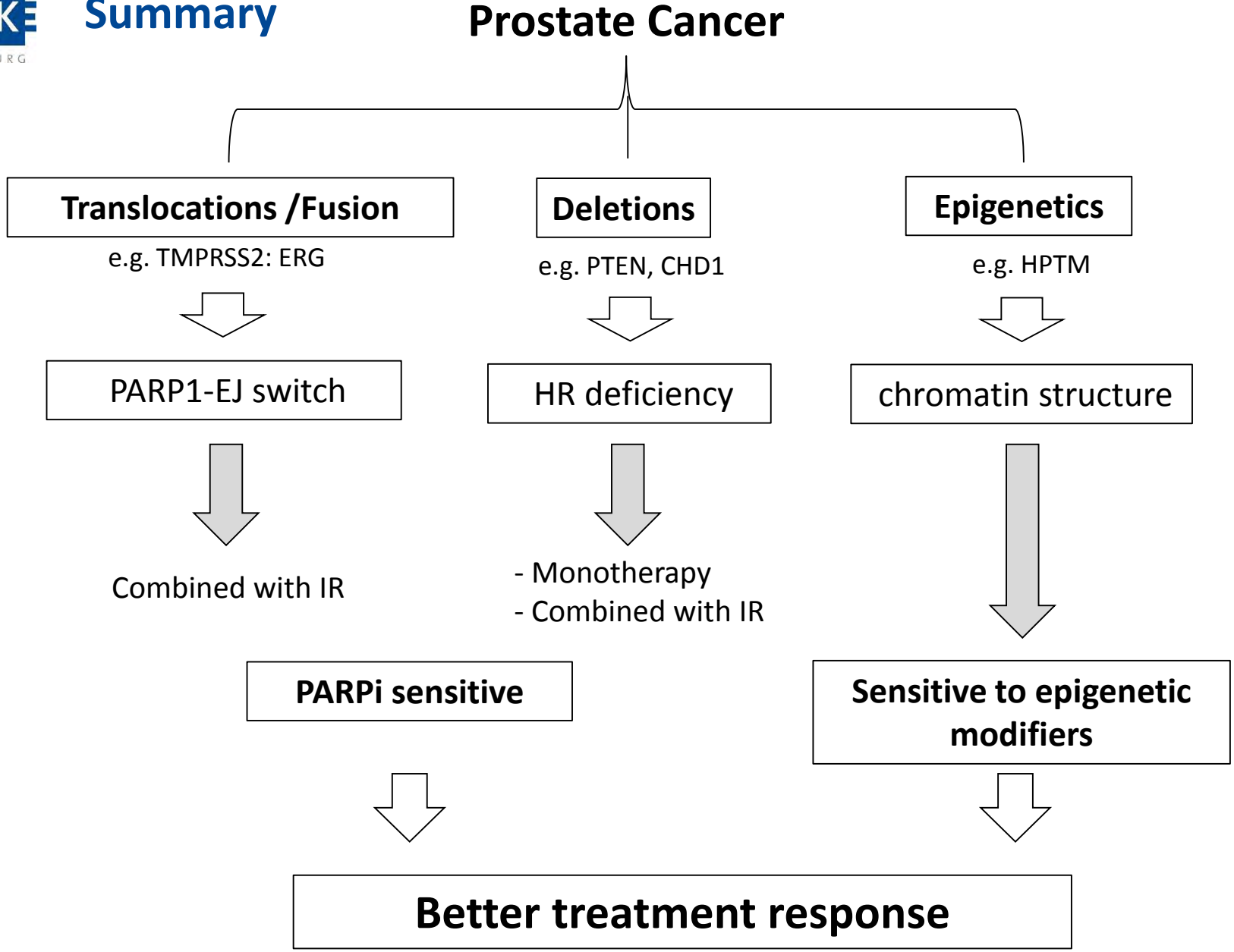

*Kari & Mansour et al., EMBO Rep, 2016*

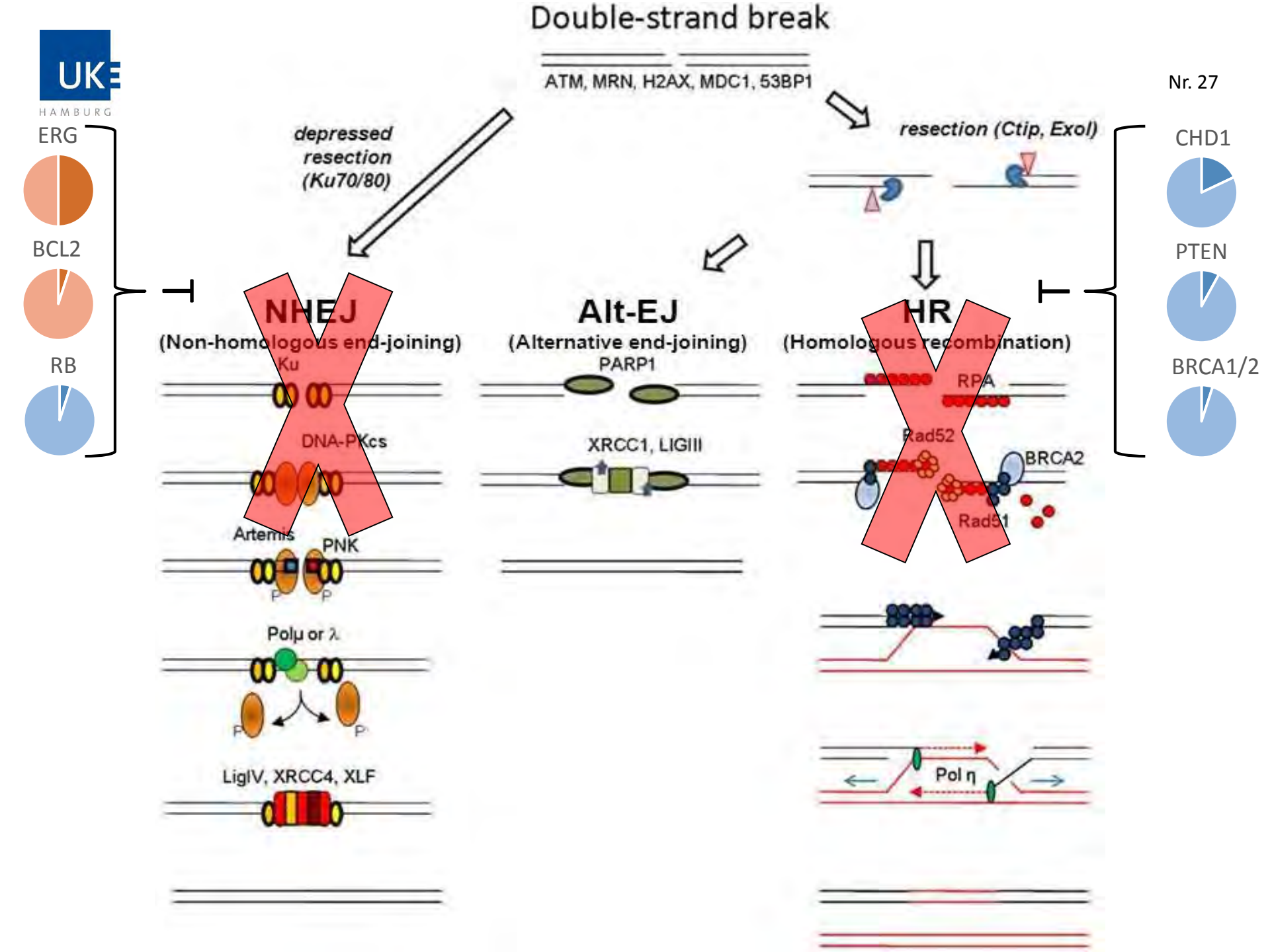
## Targeting PTEN deletion in PCa




*Mansour et al., Sci Rep, March 2018*

# Summary


Prostate Cancer
Translocations /Fusion
e.g. TMPRSS2: ERG
Deletions
e.g. PTEN, CHD1
Epigenetics
e.g. HPTM
PARP1-EJ switch
HR deficiency
chromatin structure
Combined with IR
- Monotherapy
- Combined with IR
PARPi sensitive
Sensitive to epigenetic modifiers
Better treatment response


Double-strand break
ATM, MRN, H2AX, MDC1, 53BP1
depressed resection (Ku70/80)
resection (Ctip, Exol)
ERG
BCL2
RB
CHD1
PTEN
BRCA1/2
NHEJ
(Non-homologous end-joining)
Alt-EJ
(Alternative end-joining)
HR
(Homologous recombination)
Ku
DNA-PKcs
Artemis
PNK
Polμ or λ
LigIV, XRCC4, XLF
PARP1
XRCC1, LIGIII
RPA
Rad52
BRCA2
Rad51
Pol η

# Treatment options exploiting synthetic lethality/sensitivity in an (oligo)metastatic setting

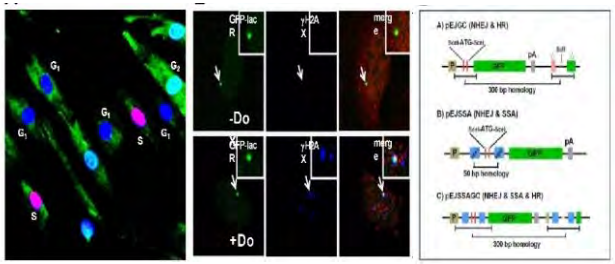


| | ERG+ | BCL2+ | CHD1- | PTEN- | BRCA1/2- | RB- |
|---|---|---|---|---|---|---|
| PARPi monotherapy |  |  |  |  |  |  |
| SBRT/RNT/CT |  |  |  |  |  |  |
| PARPi + SBRT/RNT/CT |  |  |  |  |  |  |

# Work flow for research towards PCa therapy individualisation



Cancer patient




DDR and DSB repair analysis

WGS/WES, expression (WB, qPCR), recruitment (fractionation, IF), chromatin structure (WB, FACS, IF, FAIRE), interactions (CoIP, ChIP), repair efficiency (IF, plasmid assay), cell cycle (WB, FACs,..)

DDR/repair defects

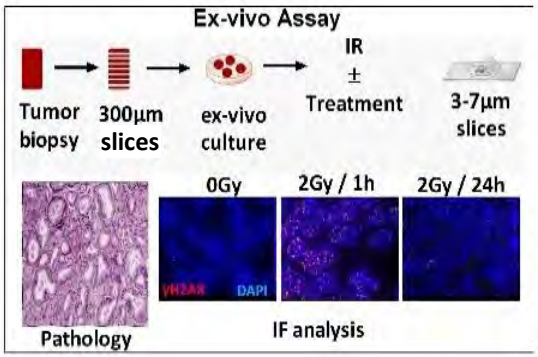
e.g. ATM-def SKX, CHD1/PTEN-deletion, ERG-overexp., EZH2/Tip5 overexp., ….

Synthetic lethality/ sensitivity, targeting

CHD1/PTEN-deletion, ERG-overexp., EZH2/Tip5 overexp., ….

*In vitro* validation i.e. cell lines

*In vivo* validation xenograft

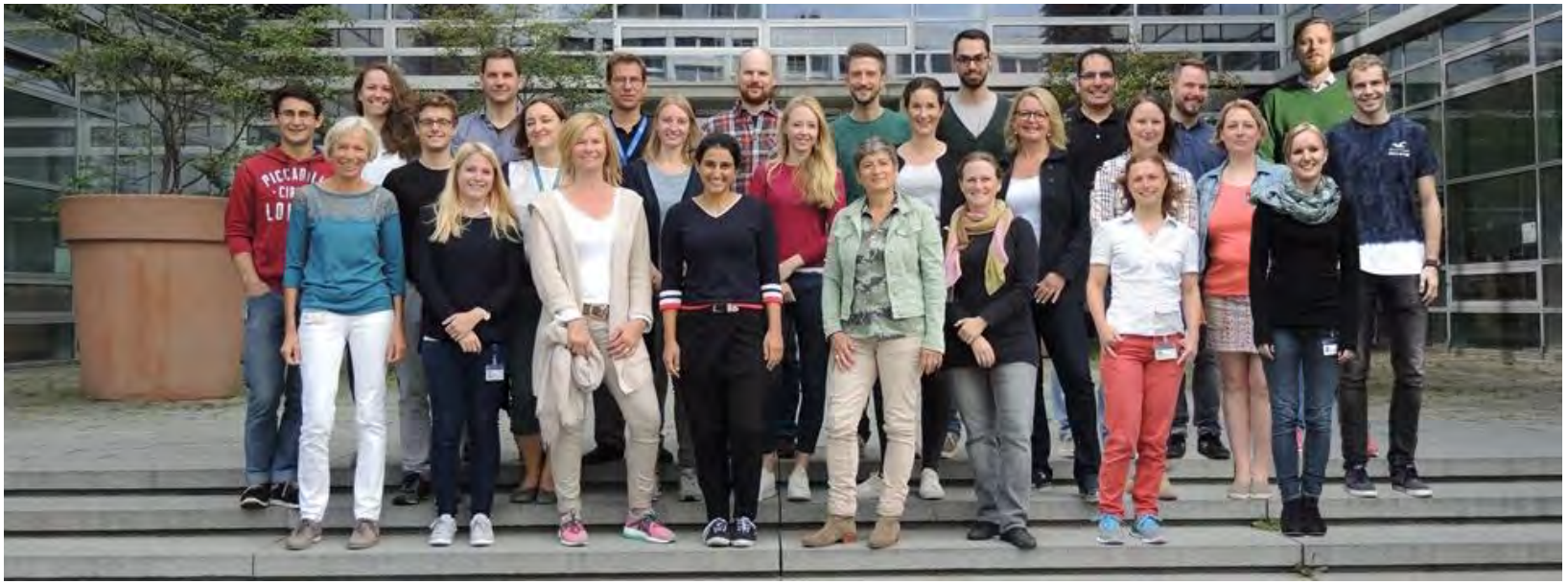



*Ex vivo* validation Patients tissues

Clinical trials

UKE HAMBURG

# Thank you!

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DFG Deutsche Forschungsgemeinschaft

Hamburger Krebsgesellschaft e.V.

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