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DESY.

State Committee of Science RA

***International Workshop 'Ultrafast Beams and Applications'***

*2-5 July 2019, Yerevan, Armenia*

# Differences in DNA damage and repair in human cancer and normal cells after ultrashort pulsed electron beam irradiation

## *Targeting the DNA Damage Response in CML cells*

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**Yerevan, 2019**

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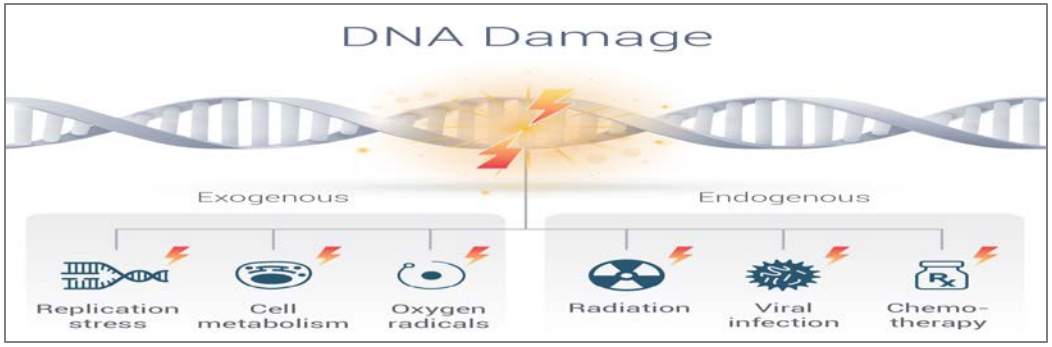
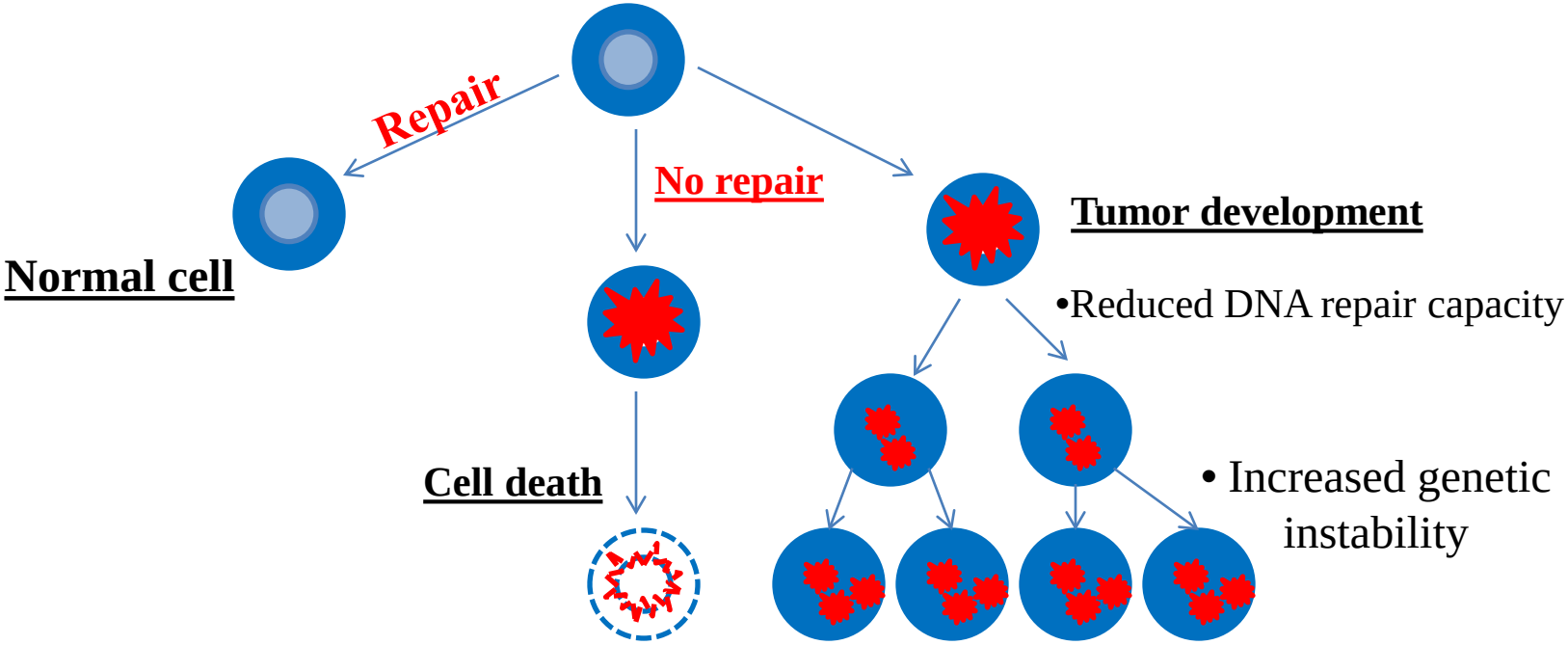
# *Research overview:* DNA damage response in cancer cells

Every cell experiences up to $10^5$ spontaneous or induced DNA lesions per day



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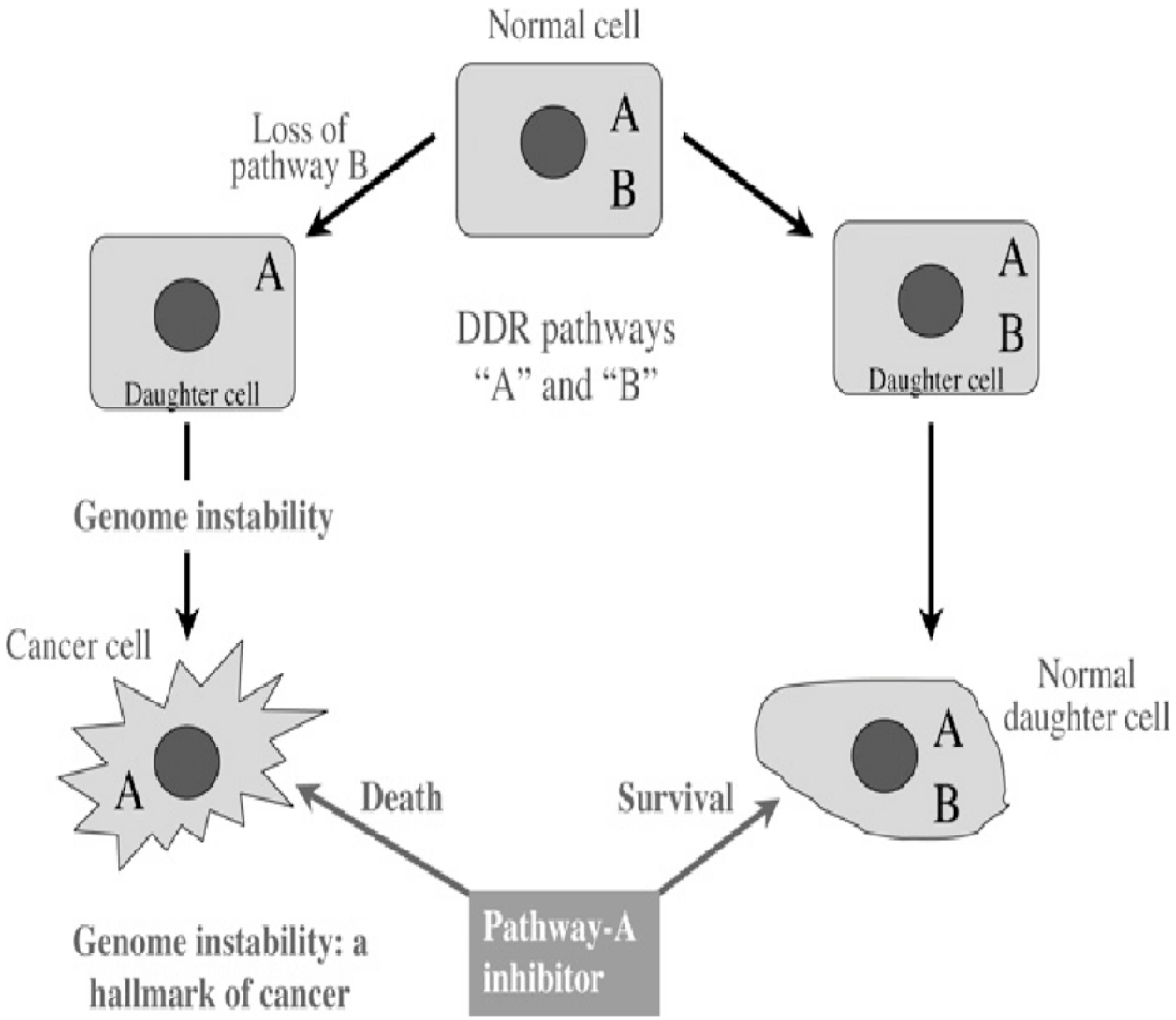
# Targeting DNA damage response in cancer therapy



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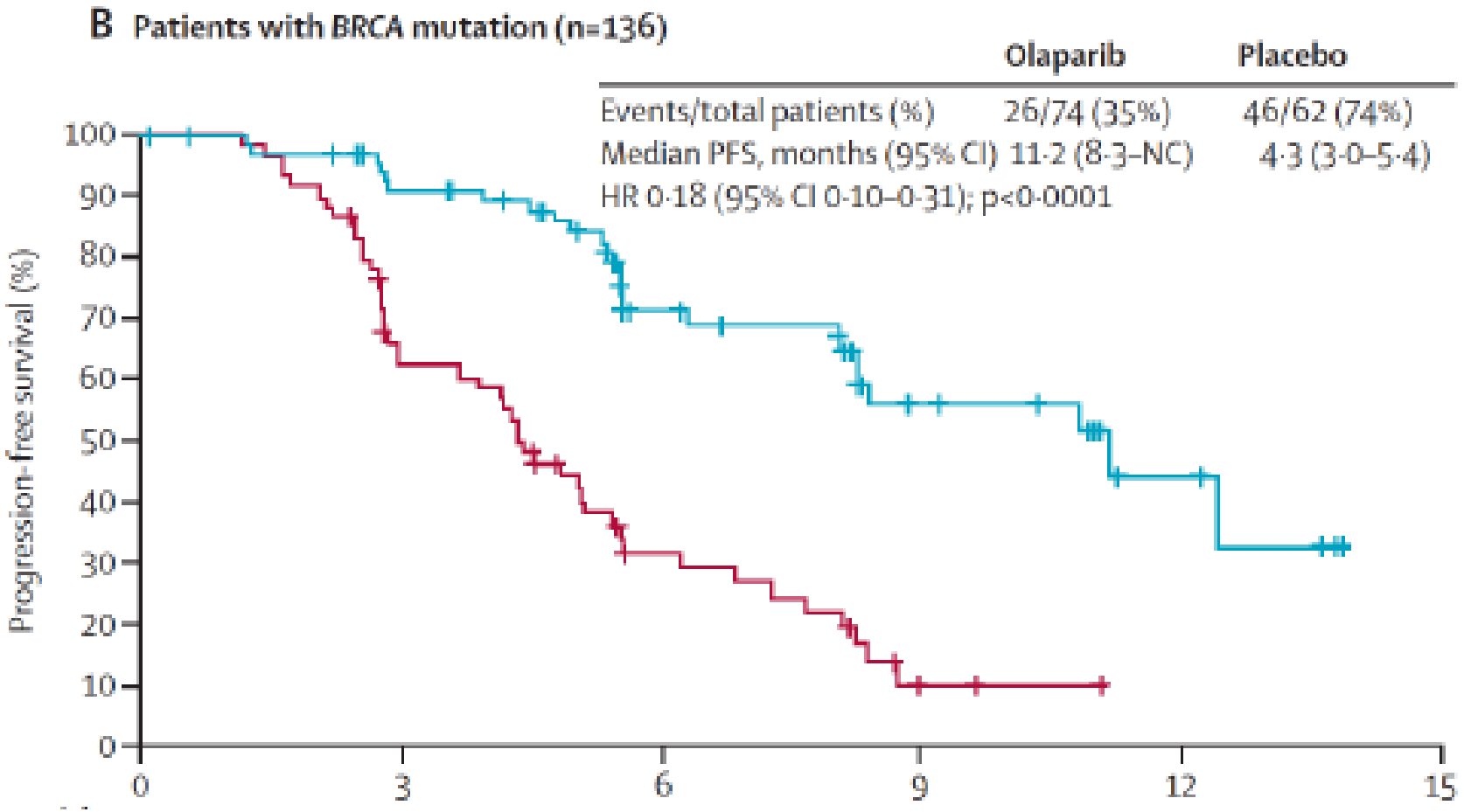
# Synthetic lethal interactions in DNA repair genes implicated in cancer

| Pathway | Protein | Syndrome | Primary cancers |
|---|---|---|---|
| **HR** | BRCA1 | | breast, ovarian |
| | BRCA2 | Fanconi's anemia | breast, ovarian |
| | AD54B | | non-Hodgkin lymphoma, colon cancer |
| **NHEJ** | | | |
| | MRE11 | Ataxia-telangiectasia- like disorder | colorectal cancer |
| | LIG4 | LIG4 syndrome | Leukemia |
| | Artemis | Omenn syndrome | Lymphoma |
| **NER** | | | |
| | XPA | Xeroderma pigmentosum | Skin cancers |
| | ERCC1 | cerebro-oculo-facio-skeletal syndrome | squamous cell carcinoma, head /neck |
| **Crosslink repair** | | | |
| | FANCA, B, C, D2, E | Fanconi's anemia | Various |
| ***etc.*** | | | |

# Olaparib treatment in BRCA-mut patients




- EMA approval: 16th December 2014
- FDA approval: 19th December 2014

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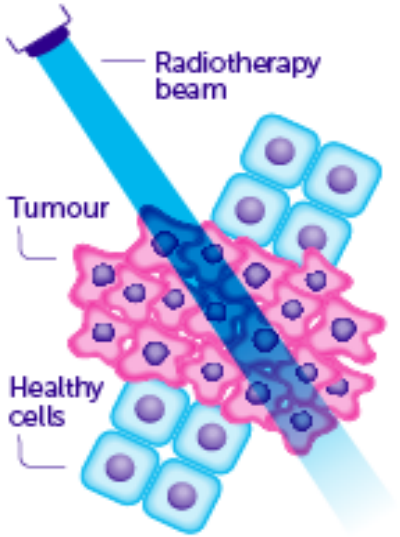
# Targeting DDR in cancer radiotherapy

## COMBINING RADIOTHERAPY WITH DRUGS

Drugs that stop cancer cells repairing their DNA could help make radiotherapy more effective.

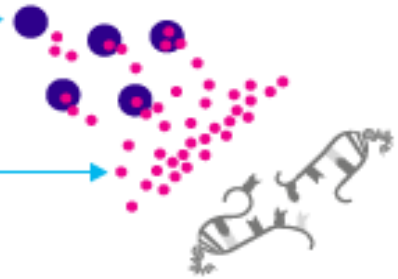
RADIOTHERAPY + DRUGS




1 Radiotherapy causes damage to cells' DNA. If cells can't repair the damage they die.

2 Healthy cells have lots of ways to repair DNA.

3 Cancer cells rely on certain repair molecules.



4 Drugs that block these molecules could leave the cancer cells unable to repair the damage, causing them to die.

LET'S BEAT CANCER SOONER
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# The Aim:

# DDR in cancer and normal cells after UPEB radiation

## Radiation Source: AREAL

**AREAL:**
Laser-driven radiofrequency gun-based linear pulsed electron accelerator

**Electron beam:**
- UHPDR - **$1.6 \times 10^{10}$ Gy/sec**
-pulse duration - **$0.04 \times 10^{-12}$ s**
- Electron energy – **3.6 MeV**

**Application**
- Potential alternative acceleration technology for ion radiotherapy
- More precise investigation of damage mechanisms

## Radiobiological Endpoints

DNA damage and repair

Comparison of DNA damage response (DDR) in cancer and normal cells

Identification of target DNA repair genes/proteins for synthetic lethality therapy

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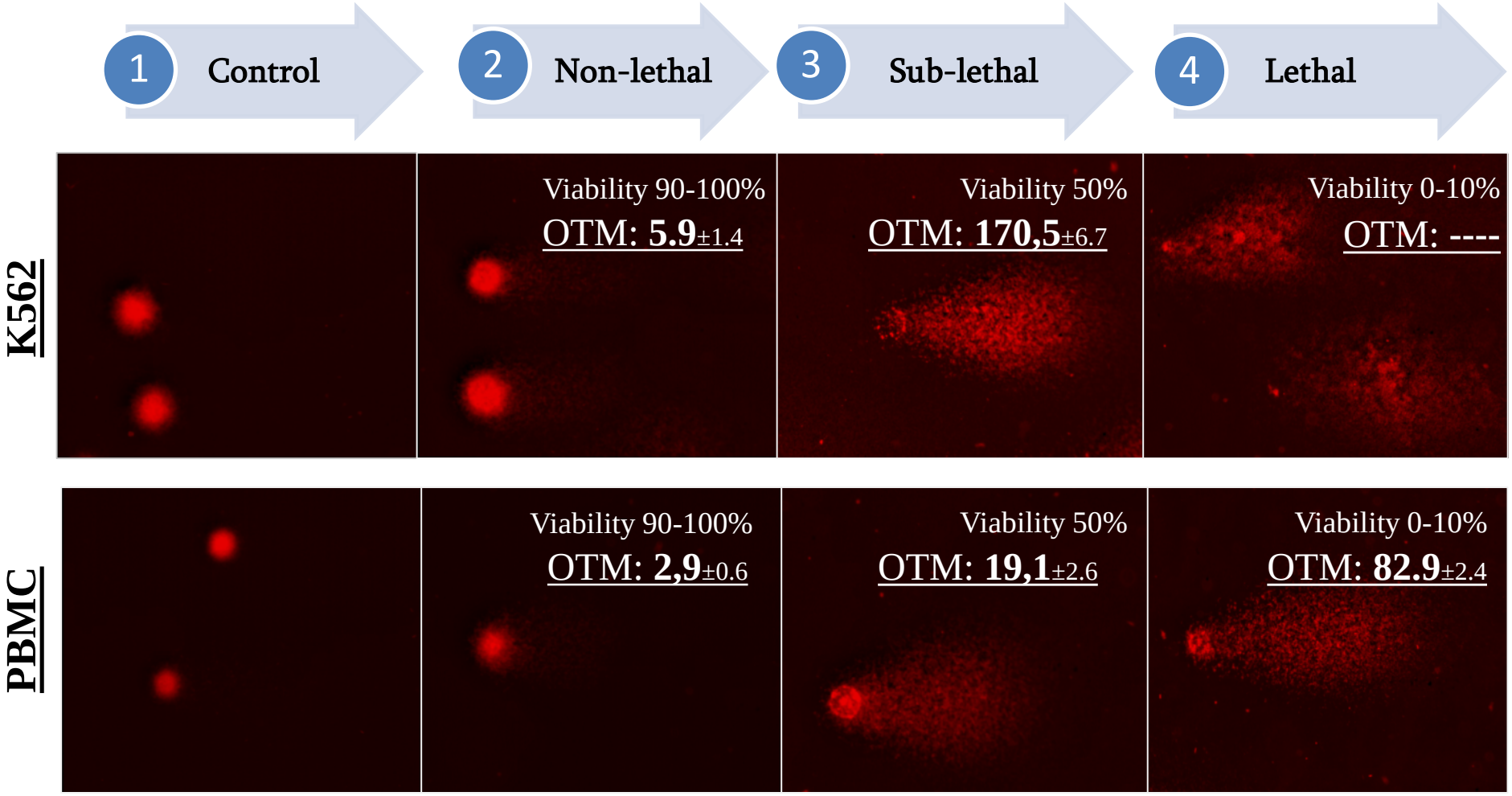
# Endpoint:

## The level of **DNA damage** in cancer/normal cells

**Method: Comet assay**




Level of DNA damage in human normal (PBMCs, blood normal cells, female) and cancer (K562, blood cancer cells, female) cells after irradiation (0 h) at the non-lethal, sub-lethal and lethal doses

**OTM** - Olive Tail moment is defined as the product of the tail length and the fraction of total DNA in the tail

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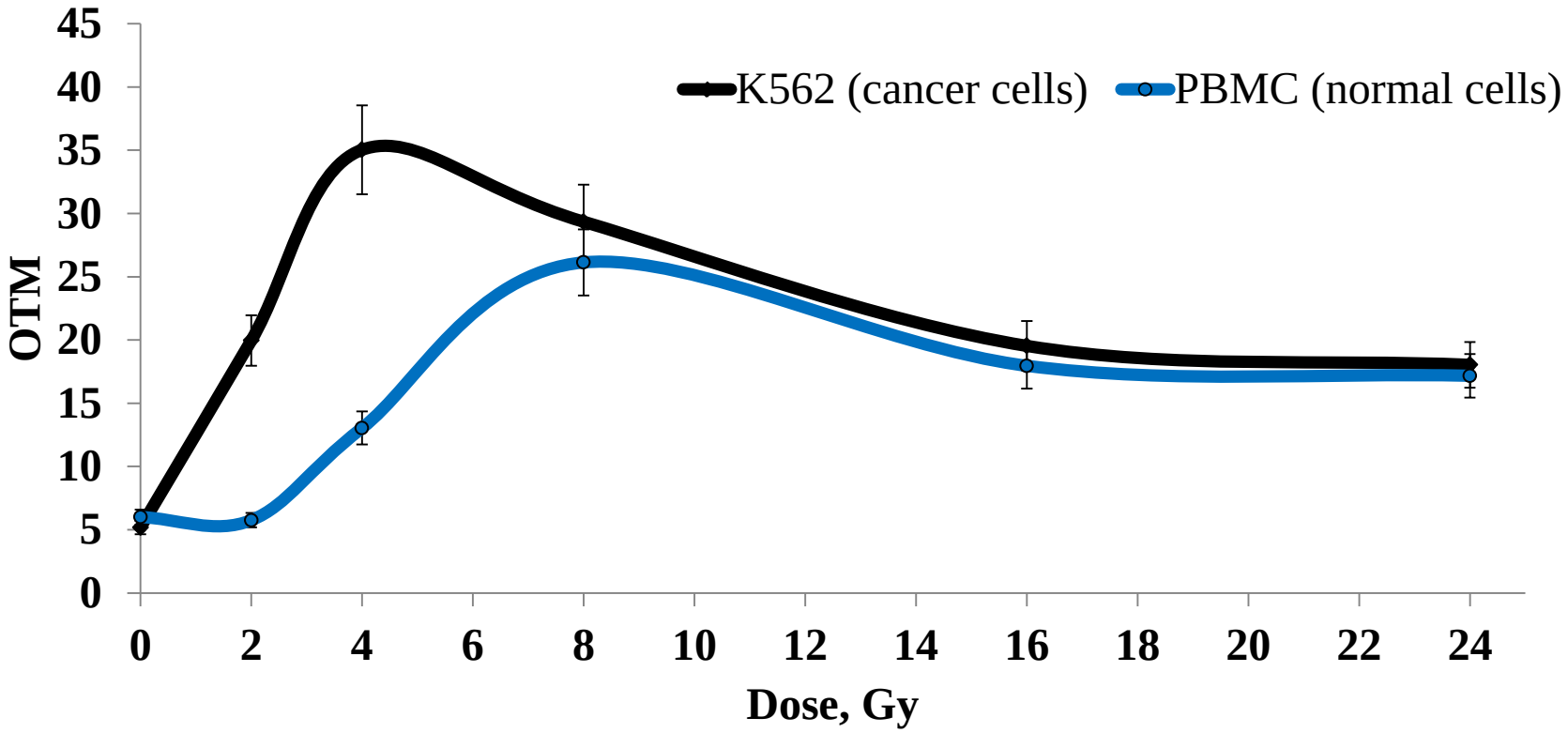
# Endpoint:

## **DNA repair** in cancer/normal cells

**Method: Comet assay**




The level of DNA-damage in PBMC (normal) and K562 cell line (cancer) cells **after 3 hours of irradiation**

**OTM** - Olive Tail moment is defined as the product of the tail length and the fraction of total DNA in the tail

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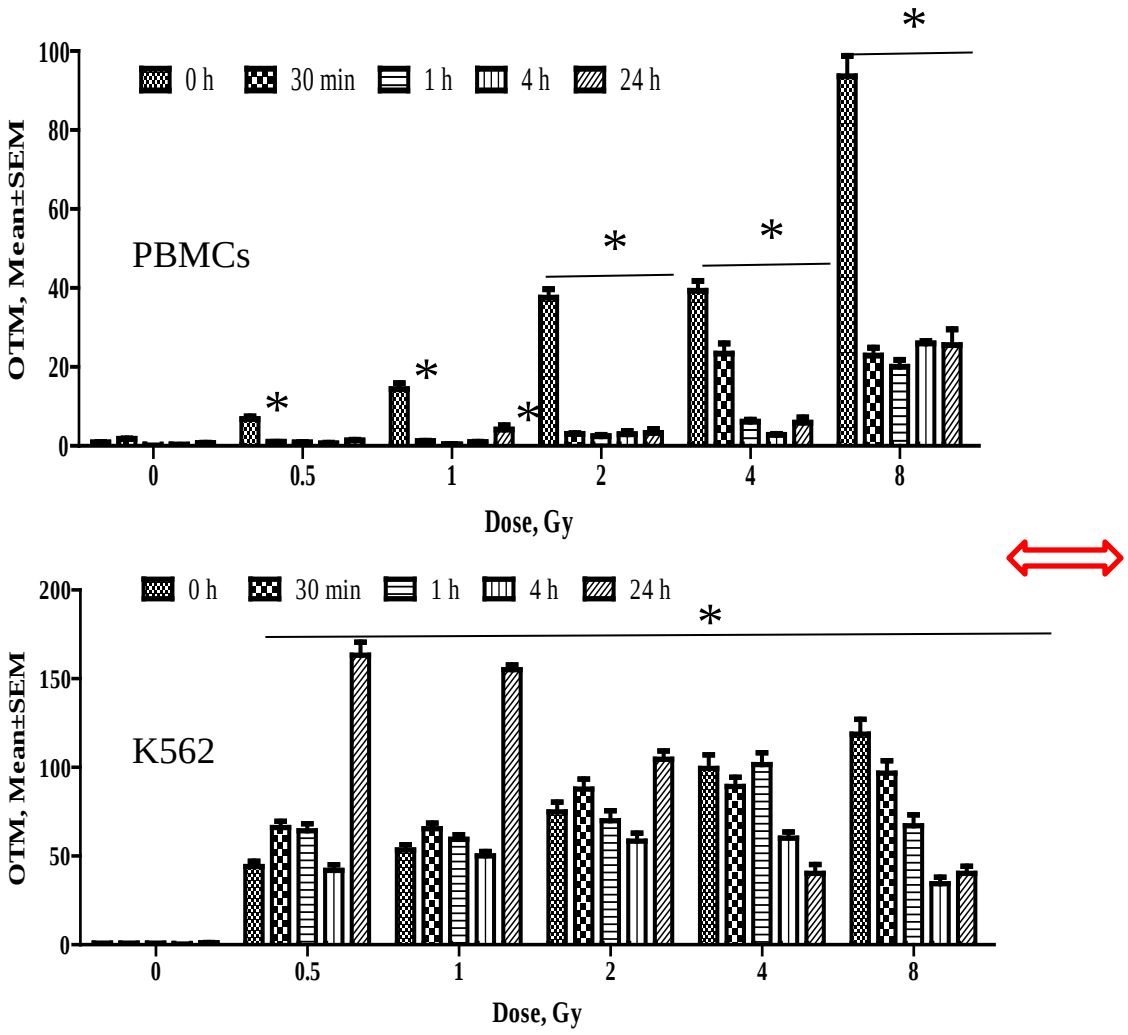
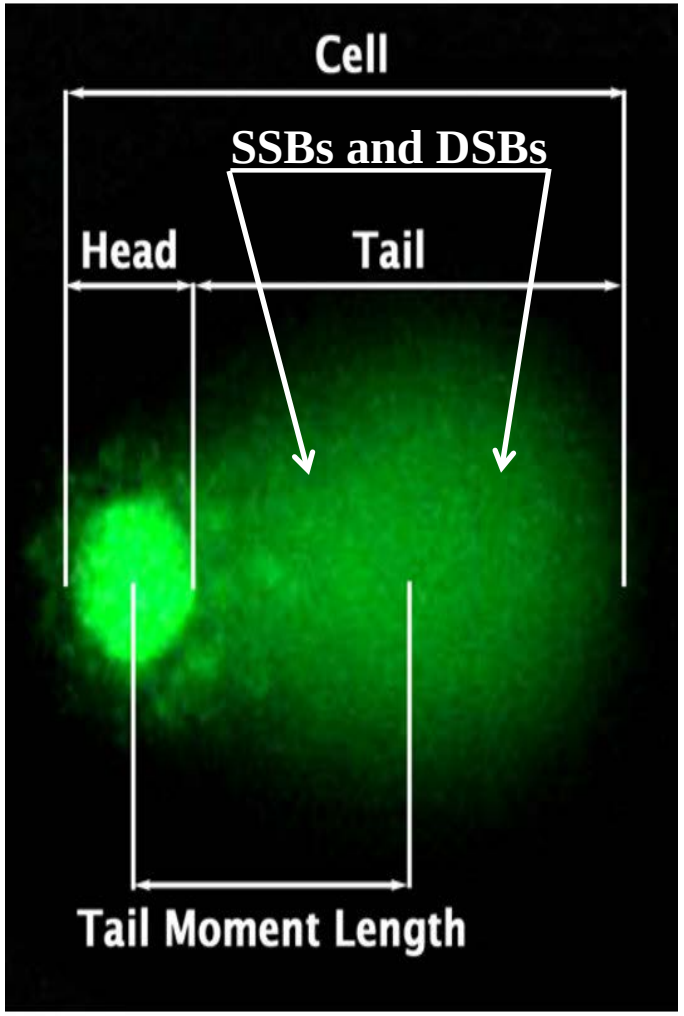
# Endpoint:

## DNA repair kinetics in cancer/normal cells

Method: Comet assay




Comparison of the induced primary DNA-damage level and repair kinetics in PBMCs (a) and K562 (b) cells. *p<0.05 in comparison to corresponding control

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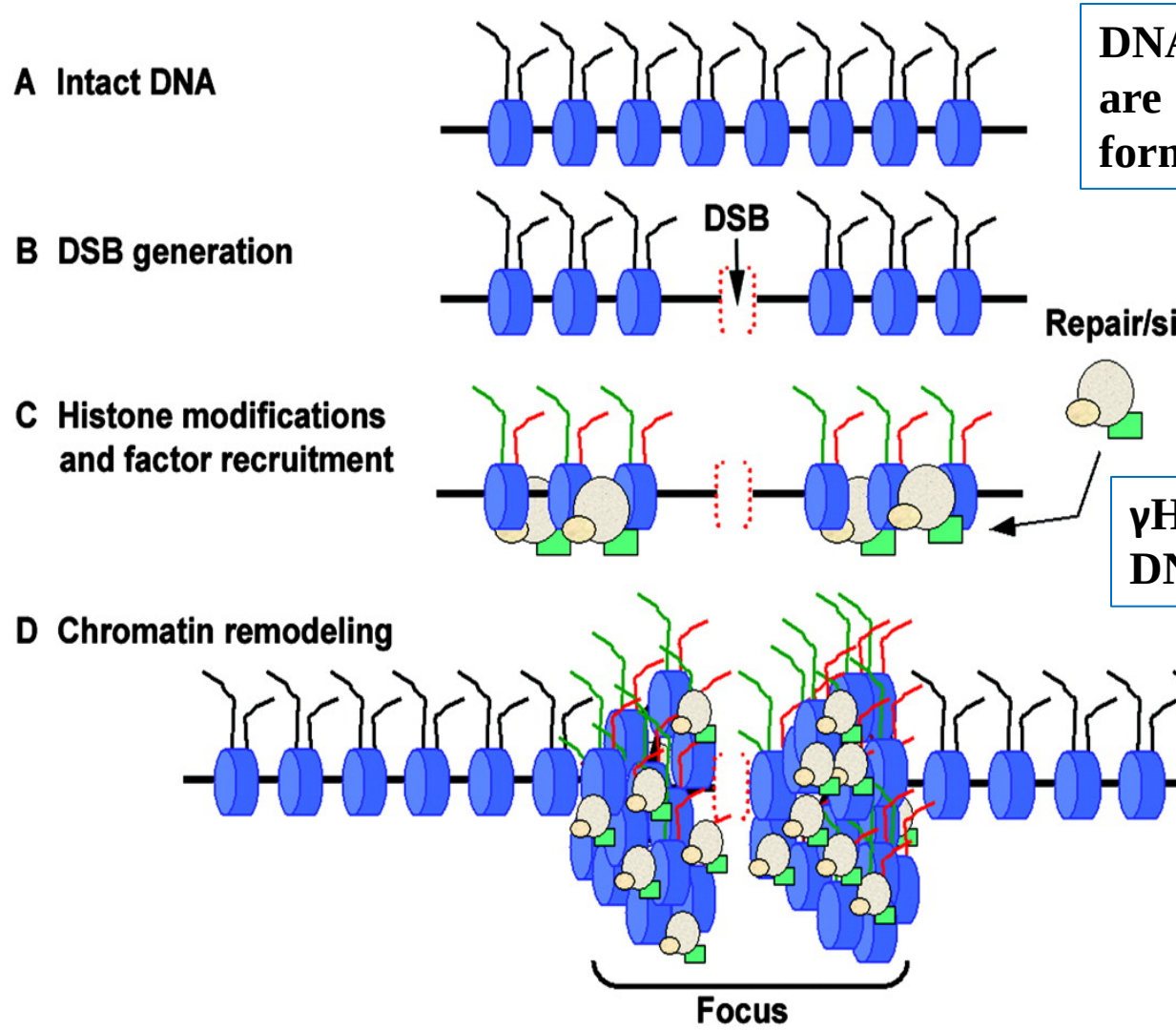
# Phosphorylation of Histone H2AX at DNA Double-Strand Breaks



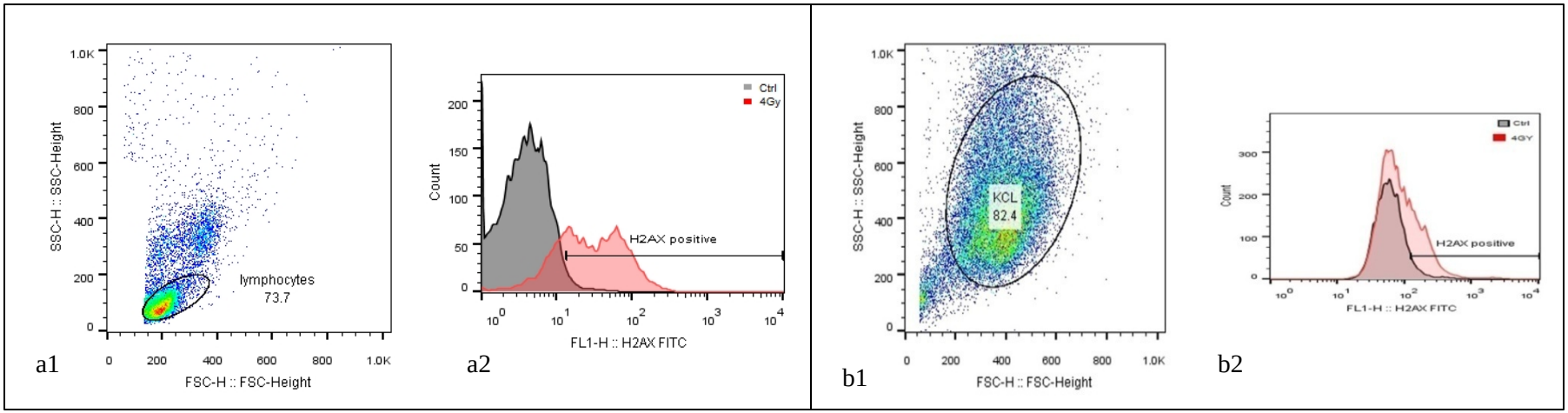

**DNA double strand breaks (DSB) are considered the most lethal form of DNA damage**

**γH2AX is a key regulator of the DNA damage response**

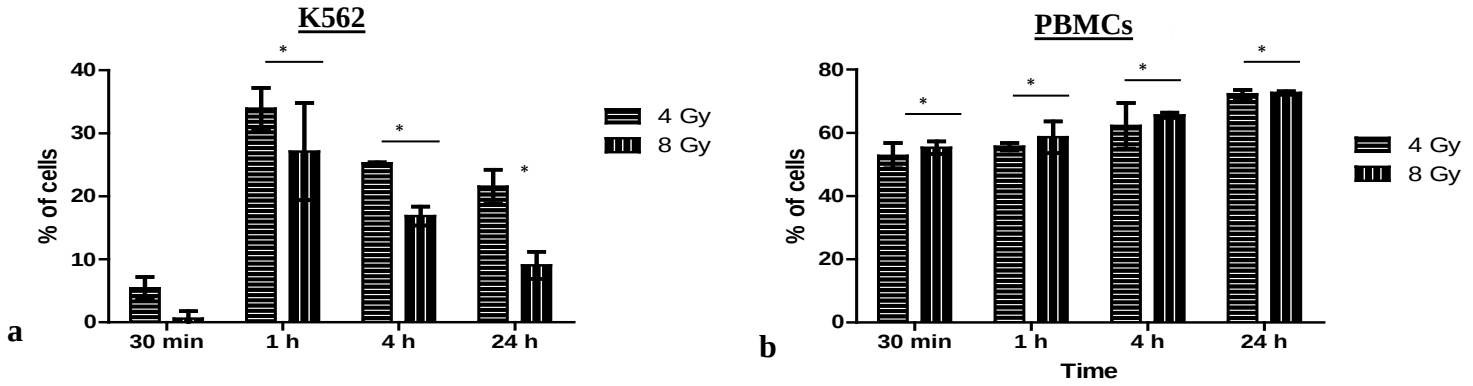
# Endpoint:

## DNA DSBs and repair kinetics in cancer and normal cells

### Method: Flow cytometric analysis of γ-H2AX




The representative dot-plot (a1, b1) and histogram (a2, b2) of the level of γ-H2AX in PBMCs (a) and K562 cells (b) after sub-lethal (4 Gy) dose of irradiation




The kinetics of γ-H2AX foci formation in cancer (a) and normal (b) cells after lethal (8 Gy) and sub-lethal (4 Gy) doses of irradiation.

*- $p<0.05$ in comparison with non-irradiated cells

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# Ionizing radiation induced-DNA SSBs/DSBs repair pathways

Non-Homologous End Joining
**NHEJ**

**FAST**
**Error-prone**
**Core protein: DNA-PK**

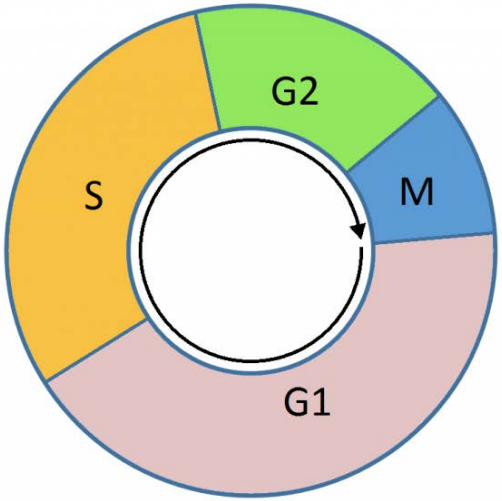
Homologous Recombination Repair
**HRR**

**Slow**
**Free of errors**
**Core protein: MRE11**

Base Excision Repair
**BER**

**FAST**
**Free of errors**
**Core protein: APEX1**

# DNA SSBs/DSBs repair pathway activation accompanied with cell cycle arrest

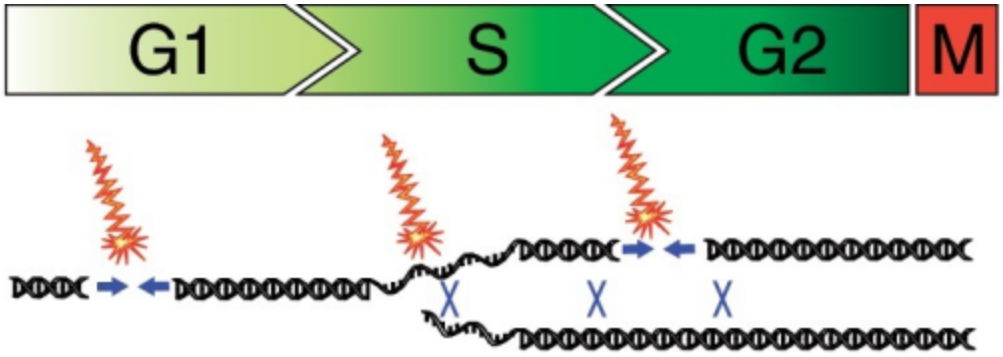



G1 - Growth

S - DNA synthesis

G2 - Growth and preparation for mitosis

M - Mitosis (cell division)

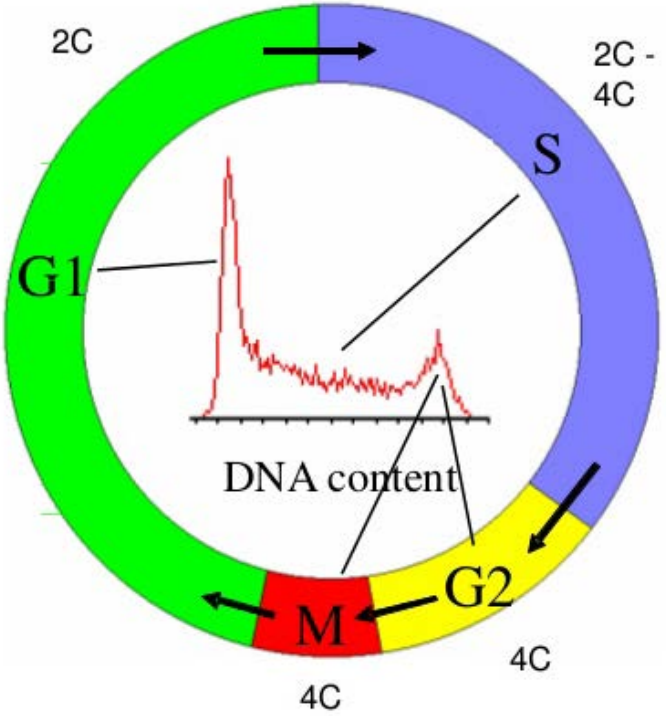



No **HR**

**HR** in repair of one-ended DSBs

**HR** in repair of two-ended DSBs

**NHEJ** in repair of two-ended DSBs



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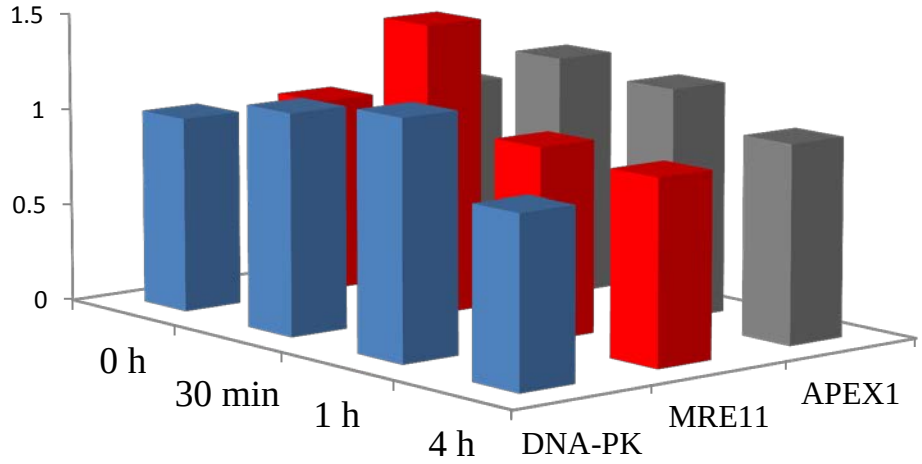
# Endpoint:

The activation of repair pathways in cancer/normal cells at the **non-lethal** level of DNA damages

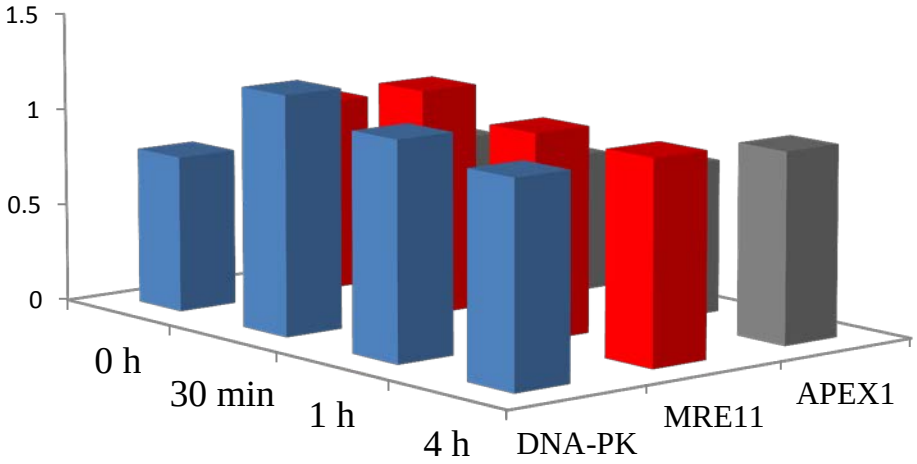
Method: ELISA

## Normal cells

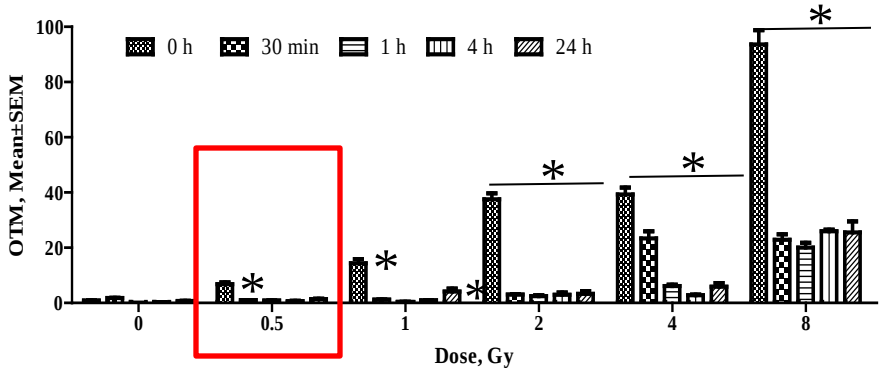


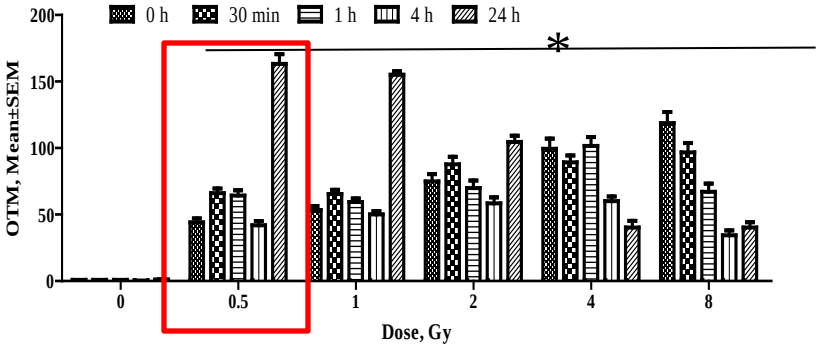

**HRR** >> **BER** >> **NHEJ**

## Cancer cells




**NHEJ** >> **HRR** >> **BER**



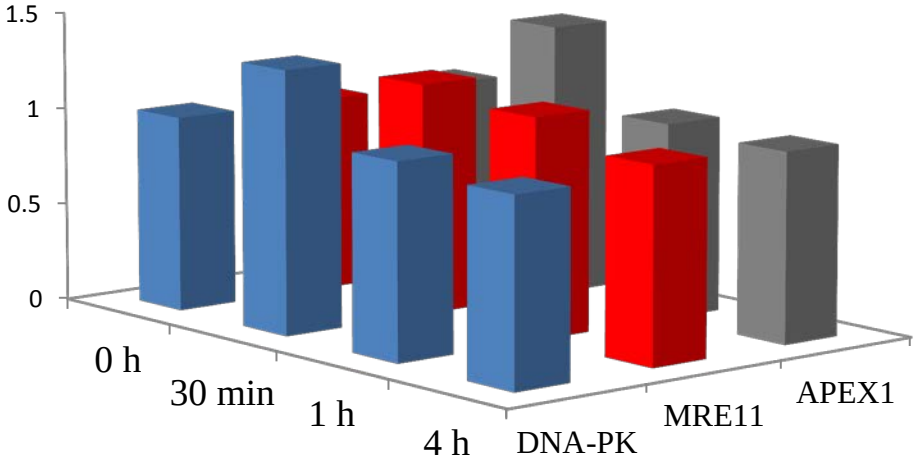



# Endpoint:

The activation of repair pathways in cancer/normal cells at the **sub-lethal** level of DNA damages

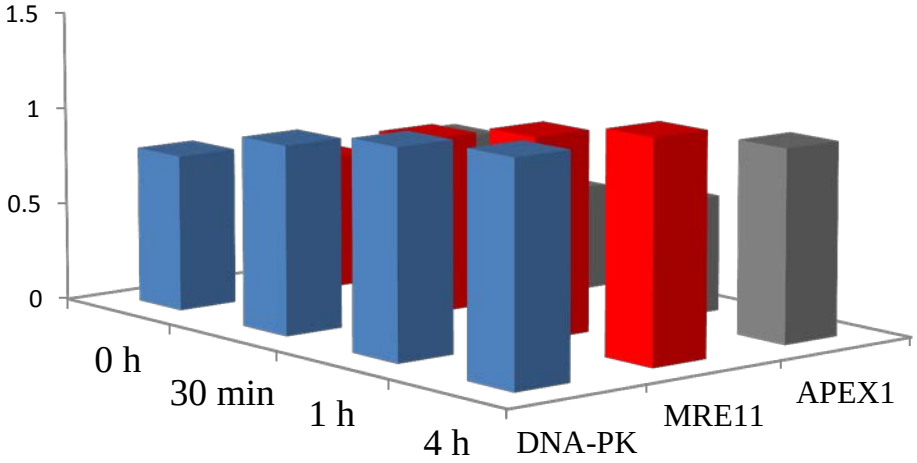
Method: ELISA

## Normal cells

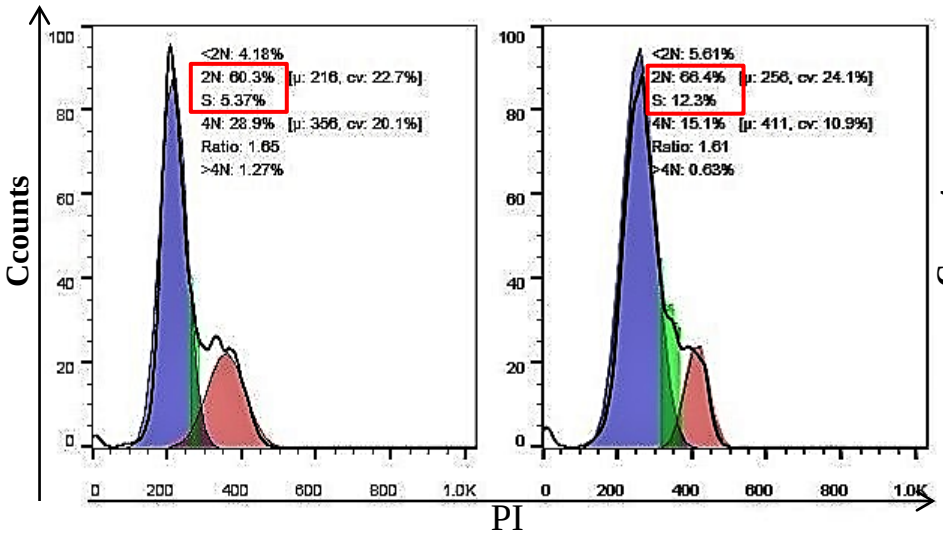


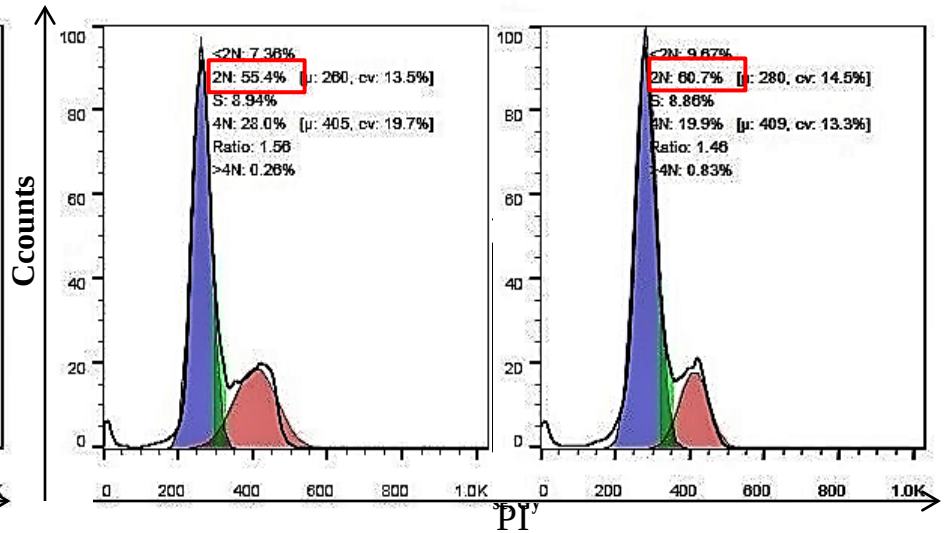

**BER >> NHEJ >> HRR**

## Cancer cells




**NHEJ = HRR >> BER**



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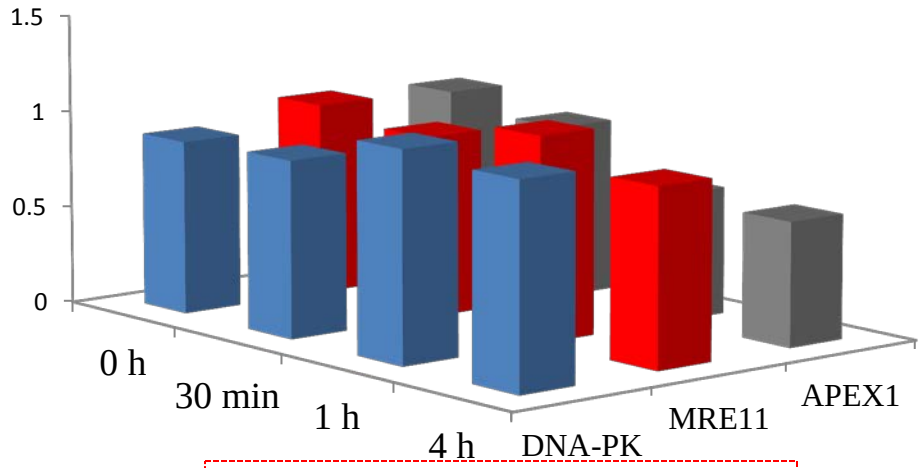
# Endpoint:

The activation of repair pathways in cancer/normal cells at the **lethal** level of DNA damages

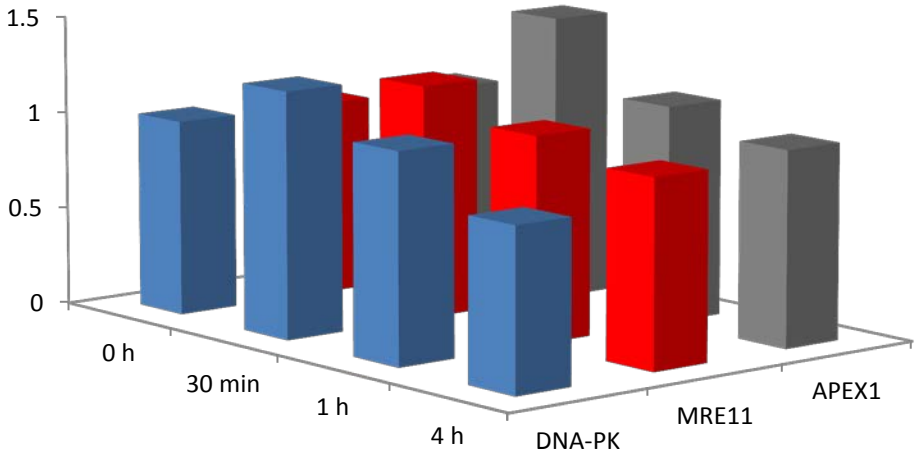
Method: ELISA

## Normal cells

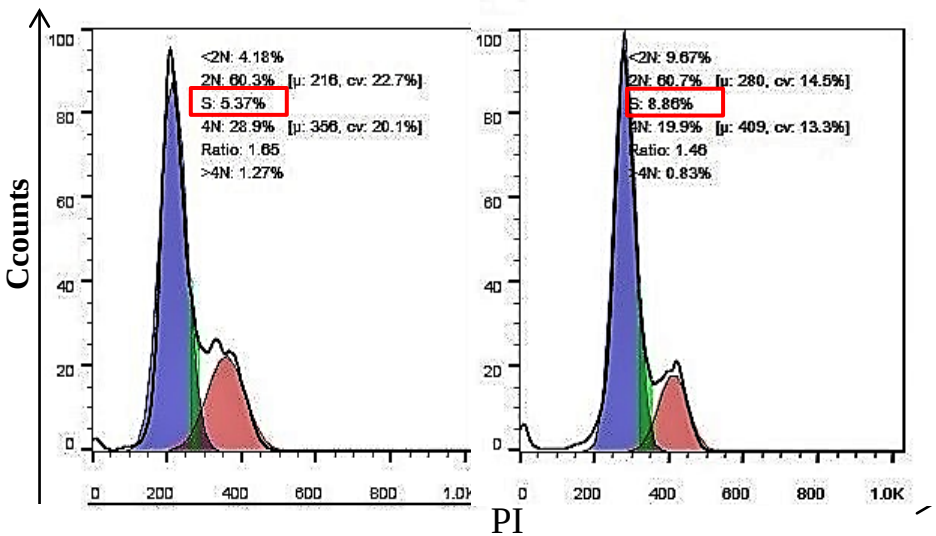


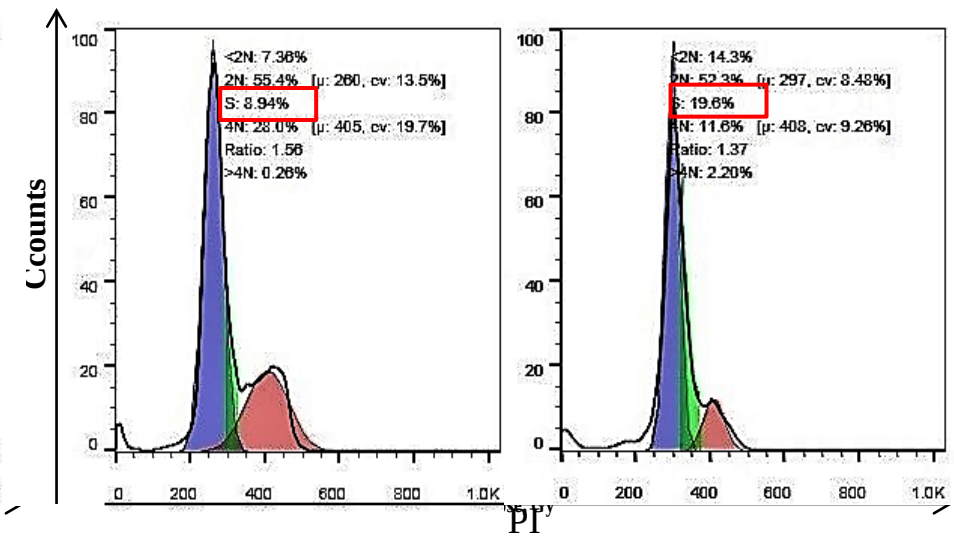

**NHEJ >> HRR >> BER**

## Cancer cells




**BER >> NHEJ = HRR**



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# Conclusions/Recommendations

## To increase the sensitivity of cancer cells (CML) *vs.* normal cells

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**Target for synthetic lethality**

**Inhibition of DNA-PK (NHEJ pathway)**

**Non-lethal dose of irradiation**

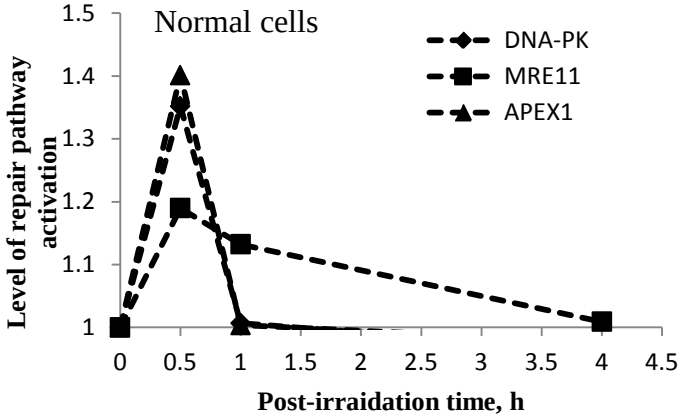
**Normal cells**
HRR >> BER >> NHEJ

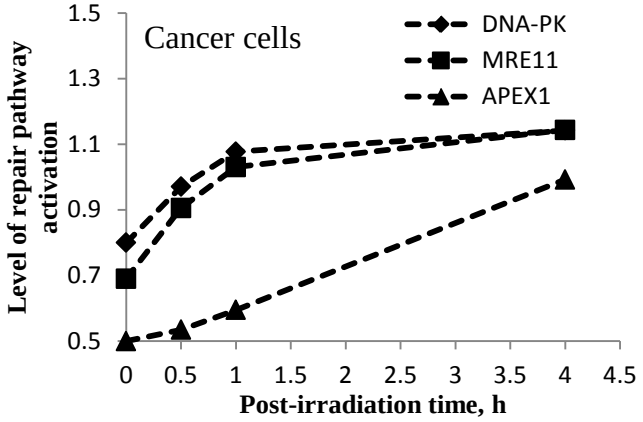
**Cancer cells**
NHEJ >> HRR >> BER

Effective DNA repair

Non-effective DNA repair

**Decrease the time of dose delivery or fractionation of sub-lethal dose**



# Acknowledgements

Dr., Prof. V. Tsakanov and Dr. B. Grigoryan – “CANDLE” Synchrotron Research Institute
Dr., Prof. A. Osipov - A.I. Burnazyan Federal Medical and Biophysical Center, Moscow, RF
Dr. Prof. R. Aroutiounian – Yerevan State University

# Thanks for Your Attention !!!