Ex vivo DNA damage response assays in tumor slices to predict therapy response

Dik C. van Gent

NWO











DNA damage response in tumors

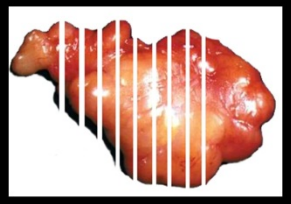
Tumors often have DDR defects

Can DDR in cells be compared to DDR in tissues?

-In vivo treatment followed by staining
-Ex vivo culturing, treatment and staining



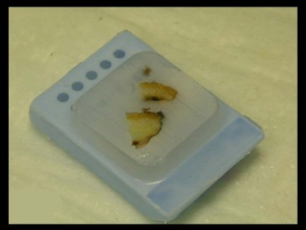
tissue



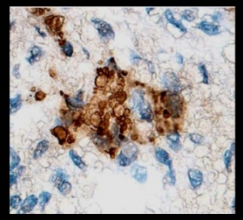
tissue slicing



tissue culture

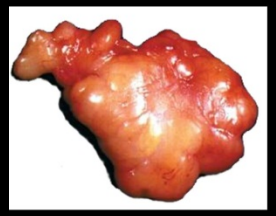


FFPE

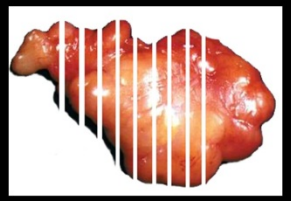


staining

DNA damage response in tumor slices



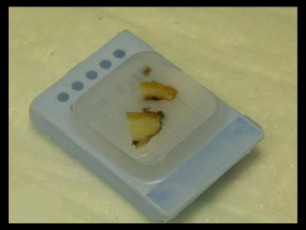
tissue



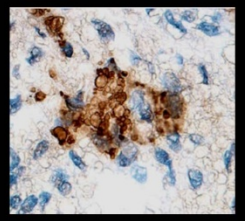
tissue slicing



tissue culture



FFPE



staining

- Optimise tissue viability:
  - Type of medium
  - Rotation
  - Automated tissue slicing

Tissue slicing

Leica Vibrotome 1200 S

Automated tissue slicer

300 µm slices



Rotation/ Shaker





Stuart mini orbital shaker

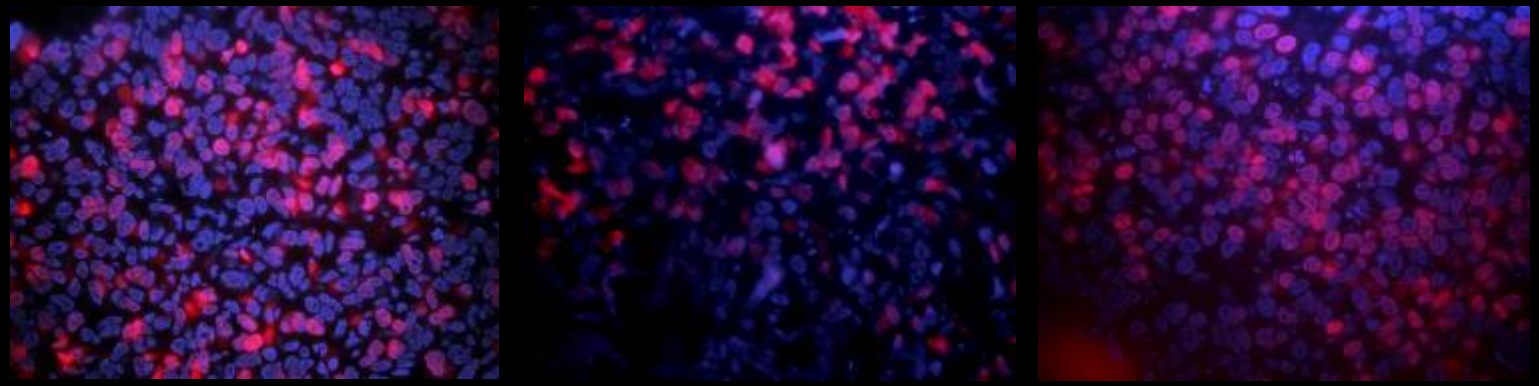
Placed in CO_2 incubator

60 rpm

Viability xenograft tumor culture

X13 Untreated

2hr *96hr* *144hr*

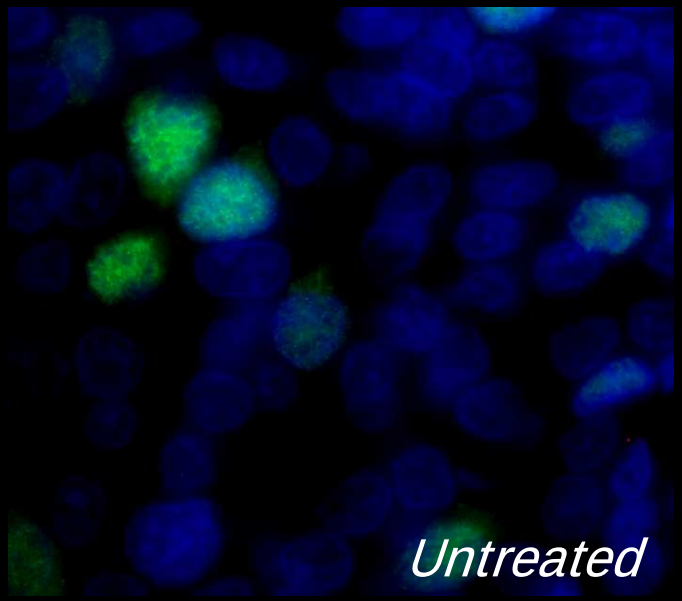


Dapi

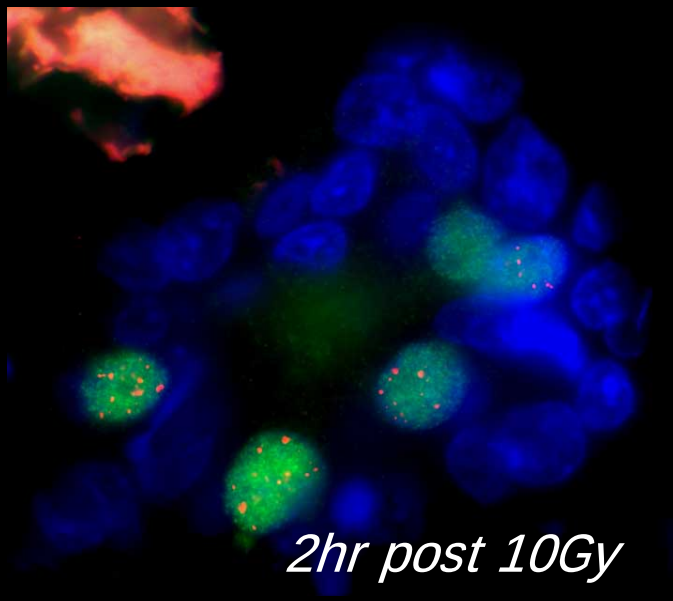
EdU (DNA synthesis)

% EDU positive cells remains constant for 6 days!

Rad51 foci formation



Untreated

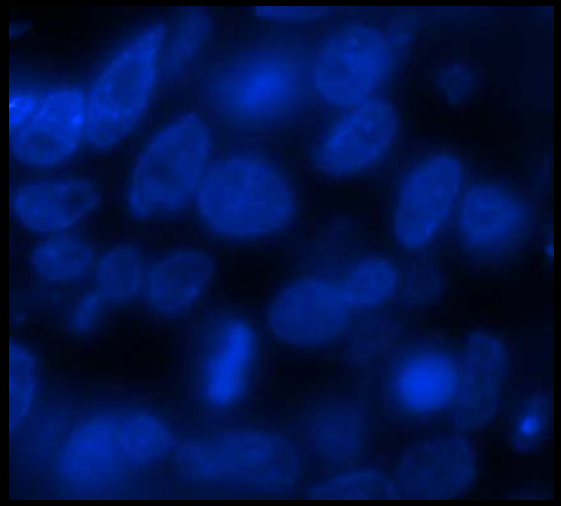


2hr post 10Gy

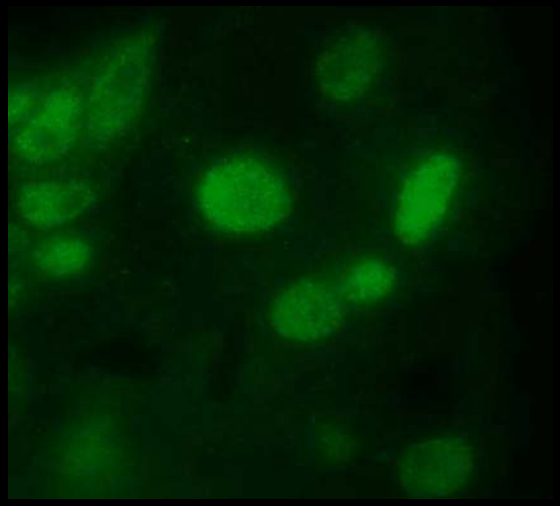
Blue=Dapi *Red= Rad51* *Green= Geminin*

Quantification: % of Geminin positive cells with Rad 51 foci

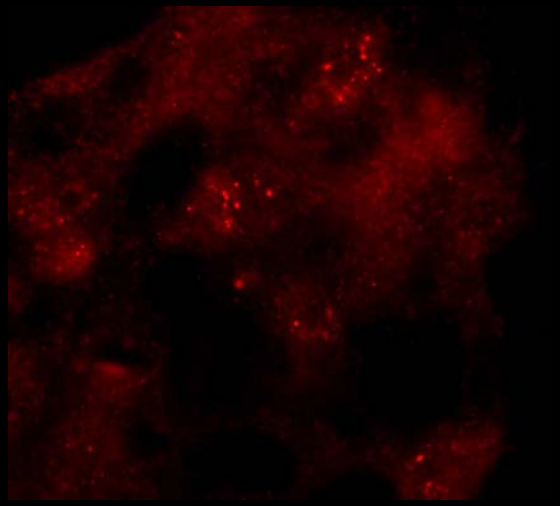
Xenograft 6: 5Gy 2hr



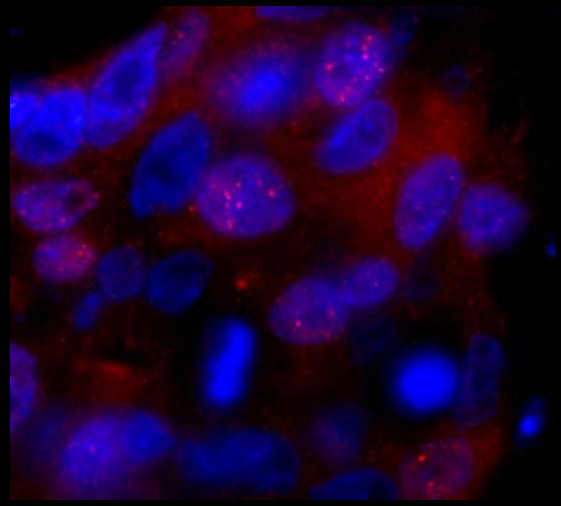
Dapi



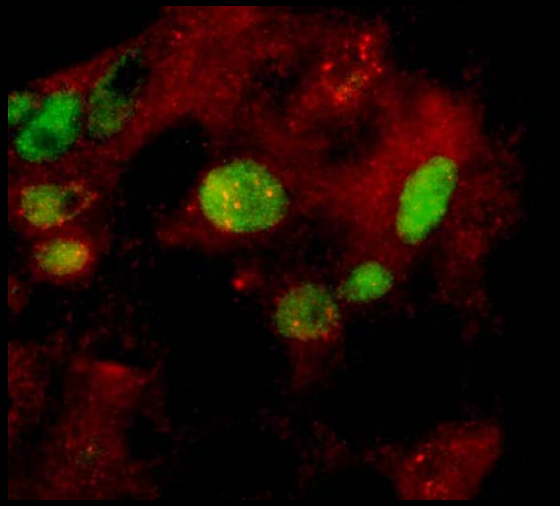
Geminin



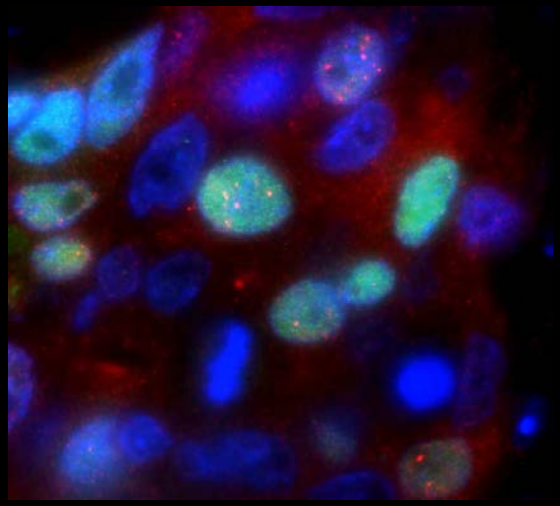
Rad 51



Dapi/ Geminin

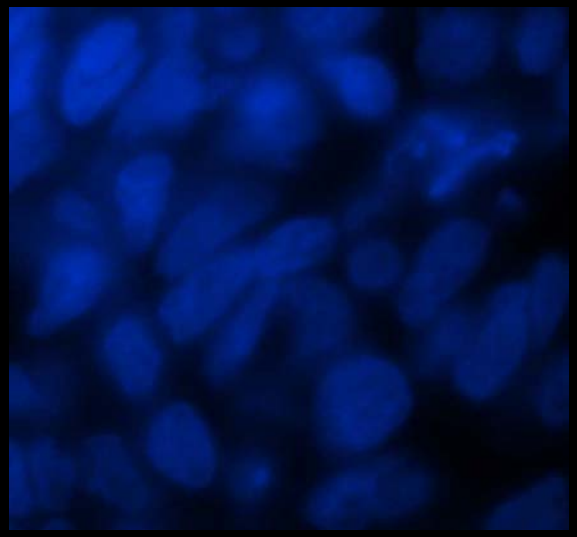


Geminin/ Rad 51

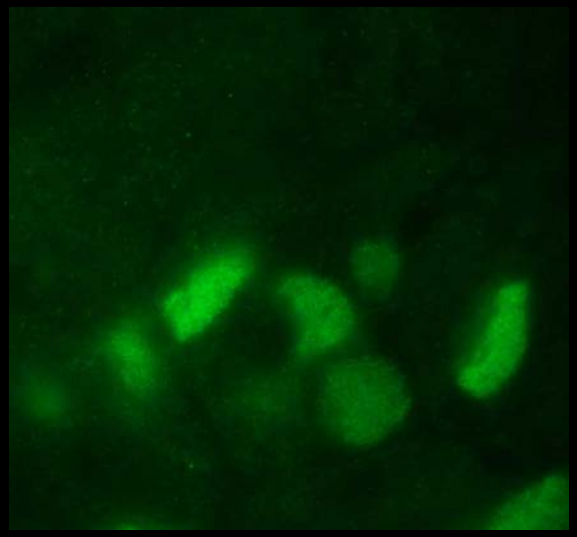


Dapi/ Geminin/ Rad51

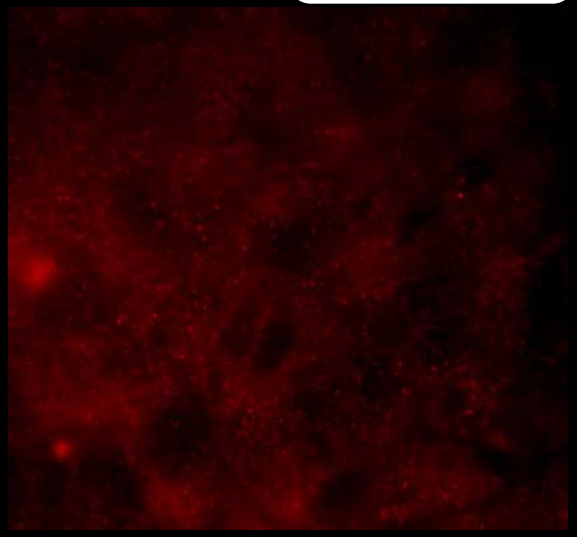
Xenograft 2: 5Gy 2hr



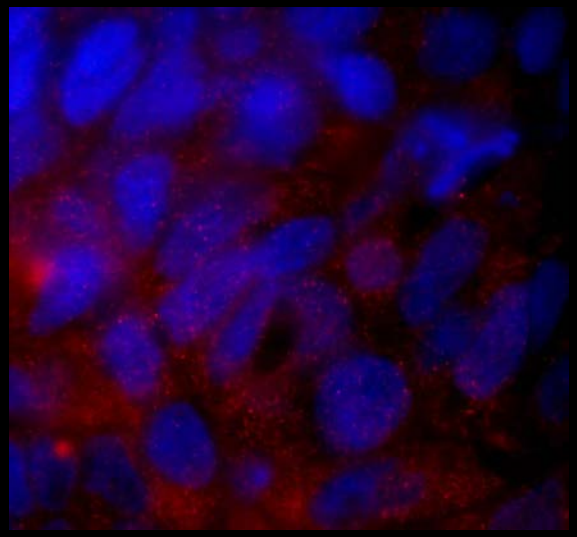
Dapi



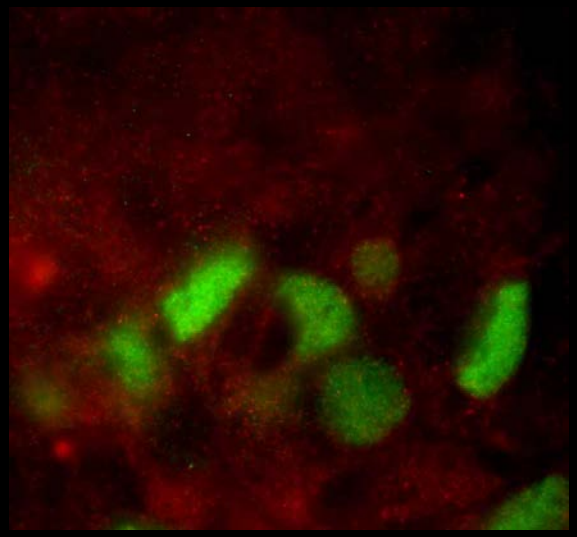
Geminin



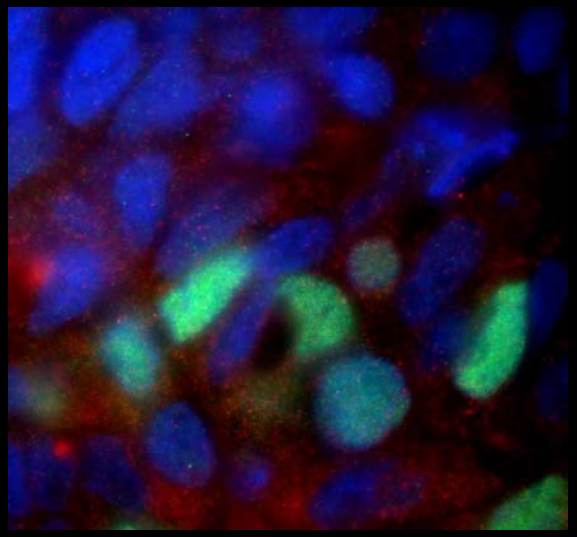
Rad 51



Dapi/ Geminin

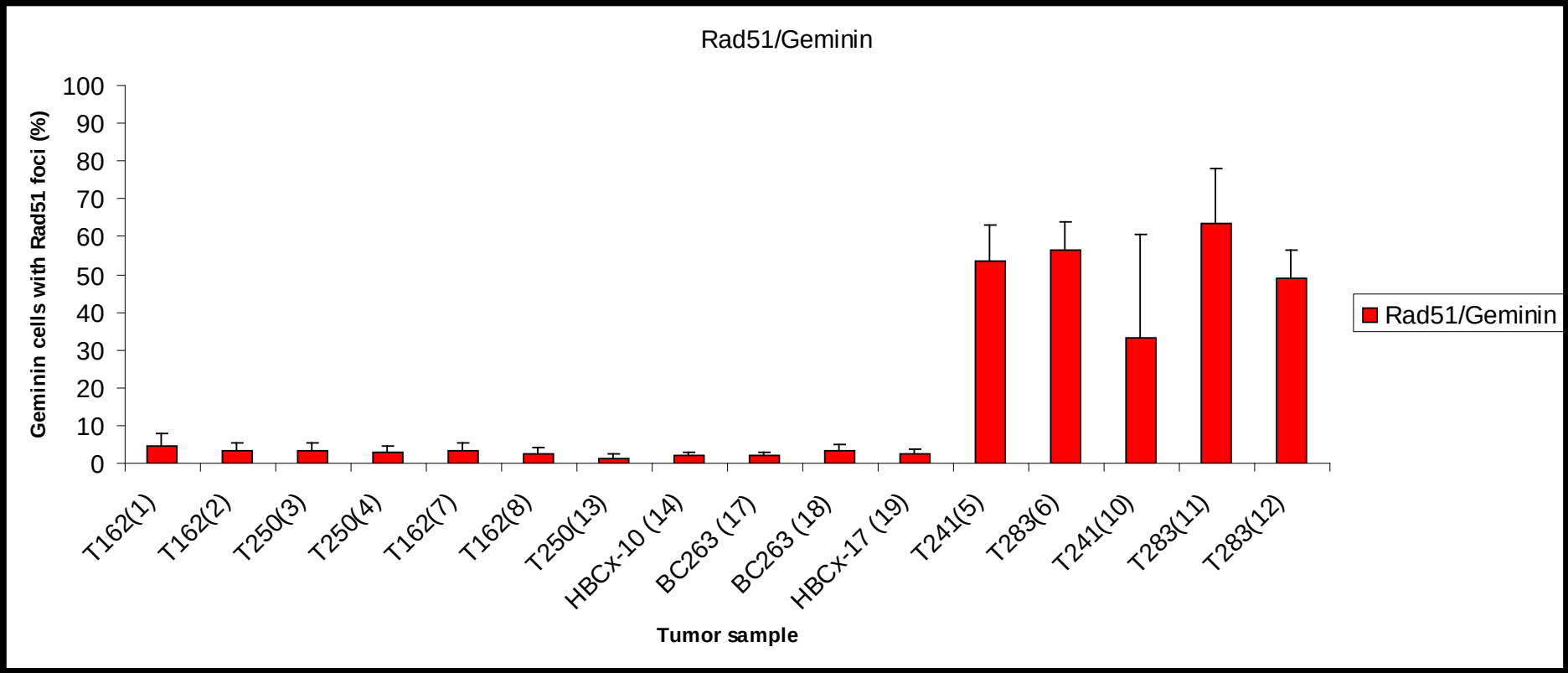


Geminin/ Rad 51



Dapi/ Geminin/ Rad51

Rad51 focus formation



BRCA 1 def

BRCA 2 def

Non BRCA

RAD51 foci in tumors

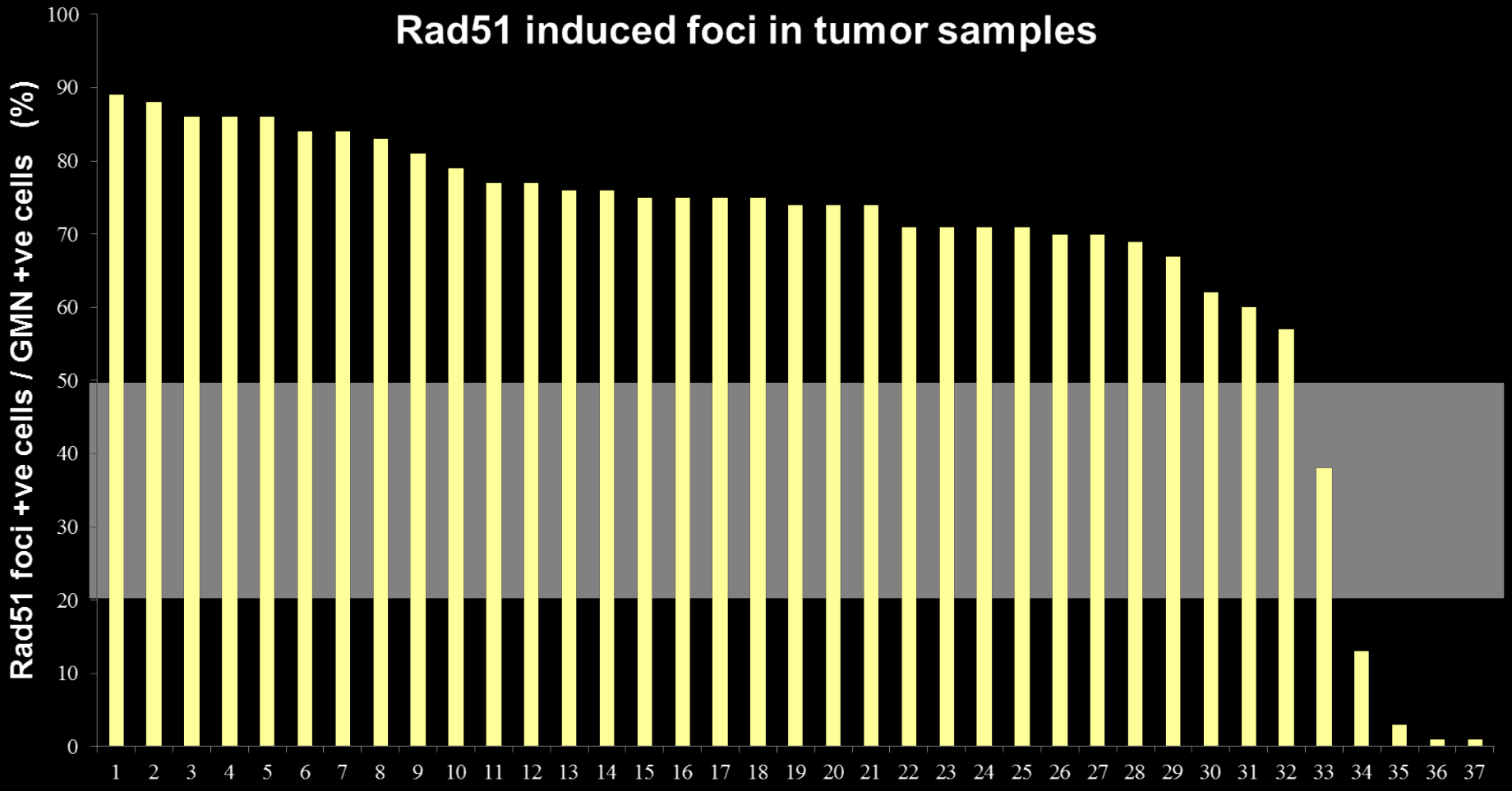
Total number of tumors: 75

No tumor/ no irradiation: 22

Rad51 assay performed: 44
No/ few GMN cells: 7

37 tumors could be analyzed
To be analyzed: 9

RAD51 foci in tumors



Sequencing of tumors

Three tumors have been sequenced for 68 DDR genes

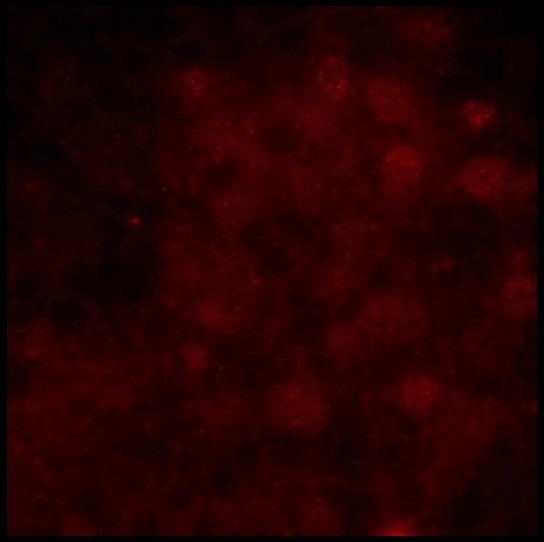
Result:

1 BRCA2 mutation (ER+)

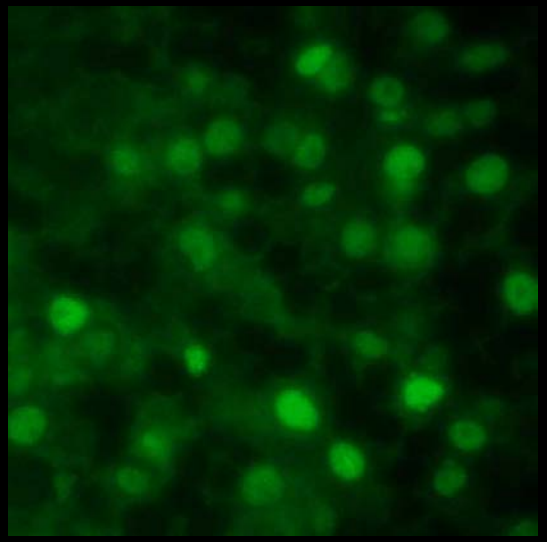
2 without BRCA mutations

An interesting human tumor

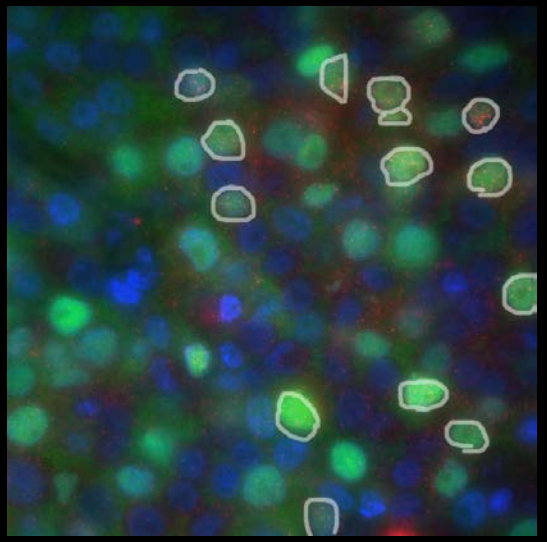
- Breast cancer sample from Havenziekenhuis
- 5Gy 2hr:



Rad51



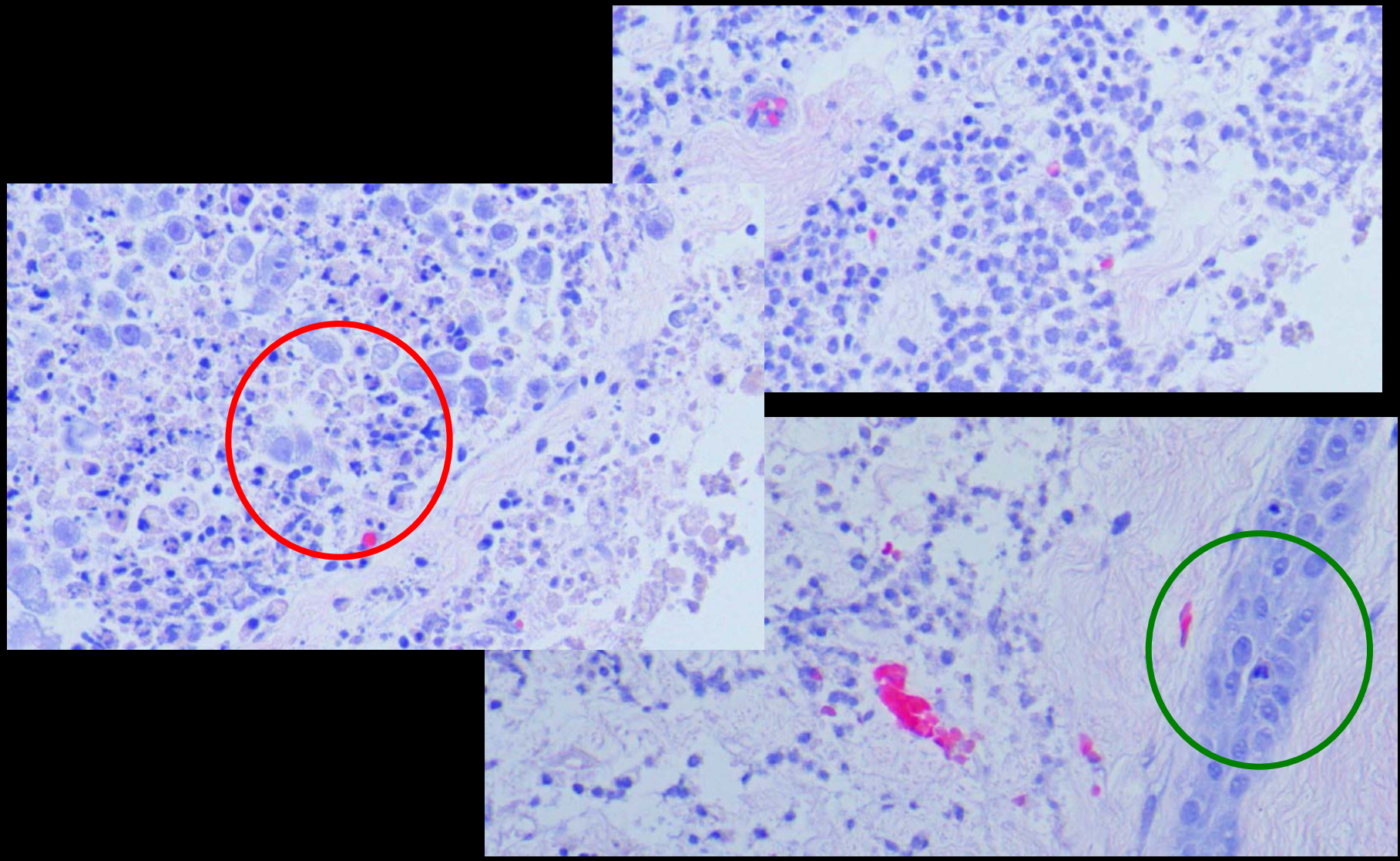
Geminin



Dapi/ Geminin/ Rad51

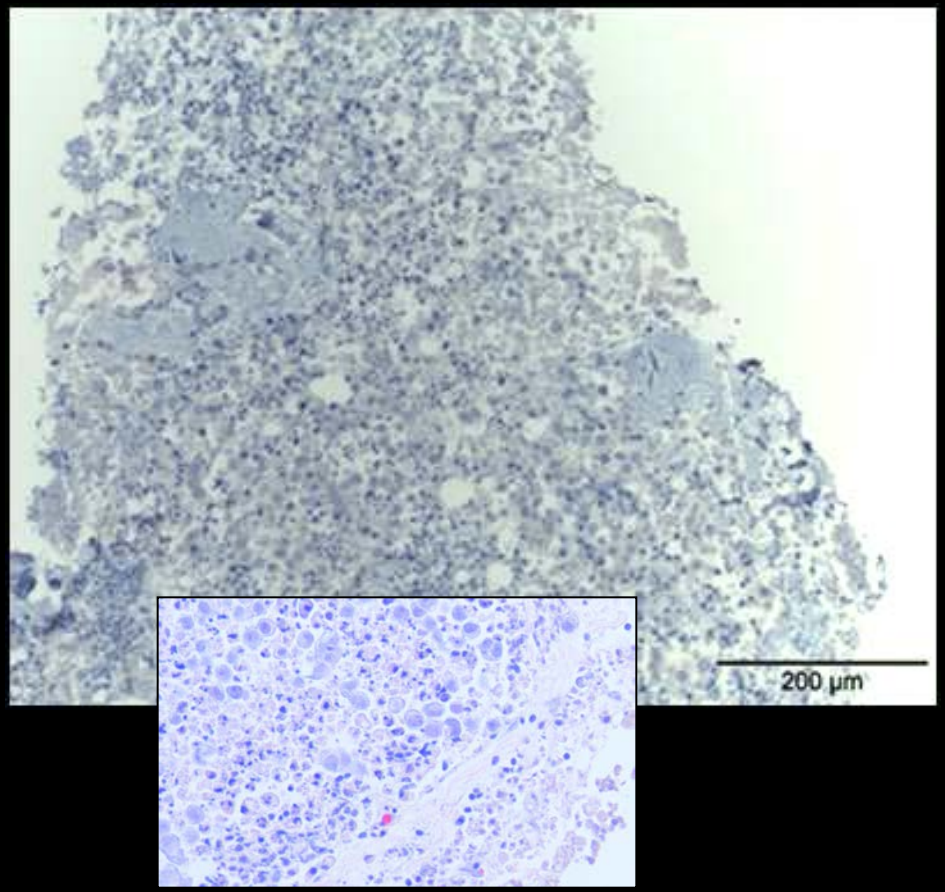
- Extremely high % Geminin+ cells
- No clear induction of Rad51 foci

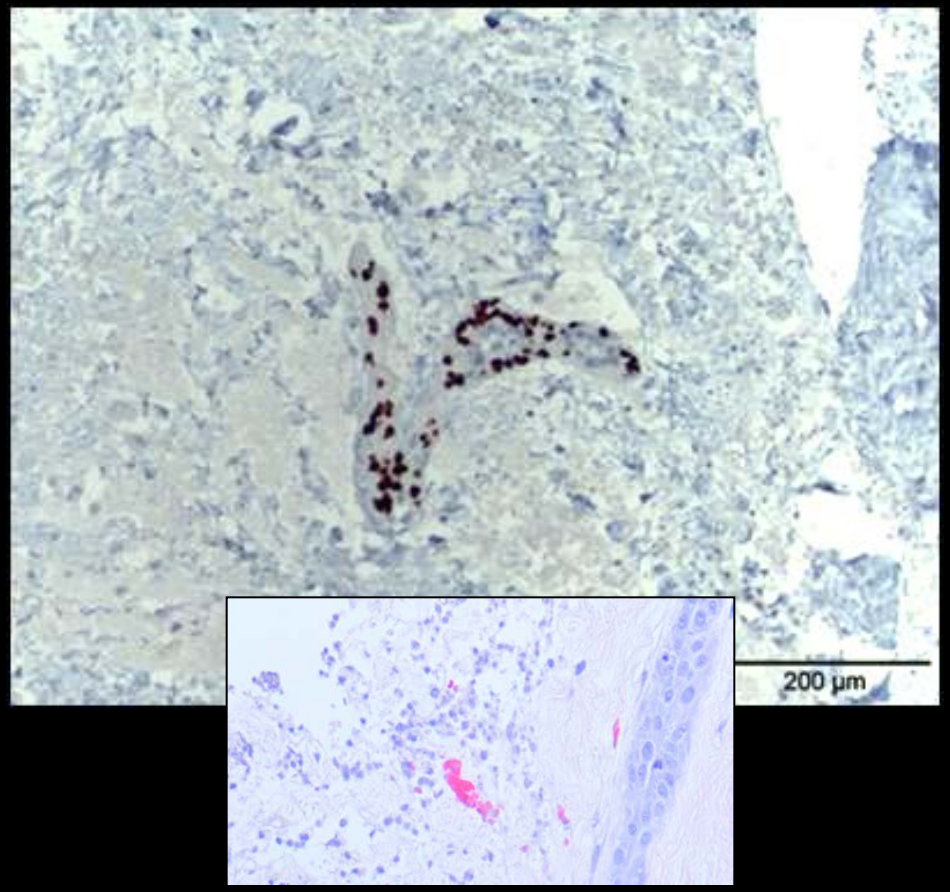
M028 – 4 days Olaparib 10 µM



M028 – 4 days Olaparib 10 µM

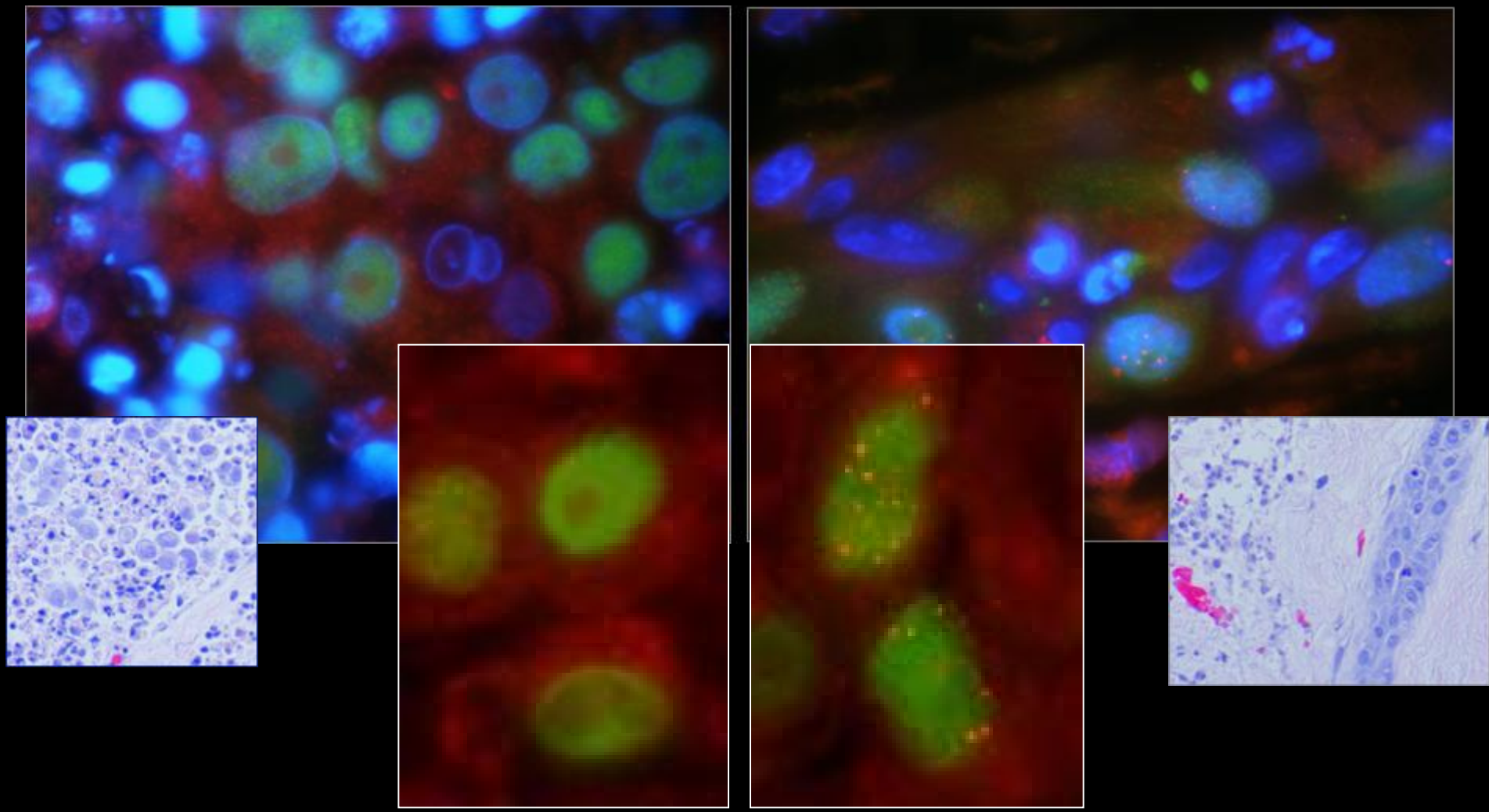
p63: specific marker for myo-epithelial cells, only present in normal mammary glands.



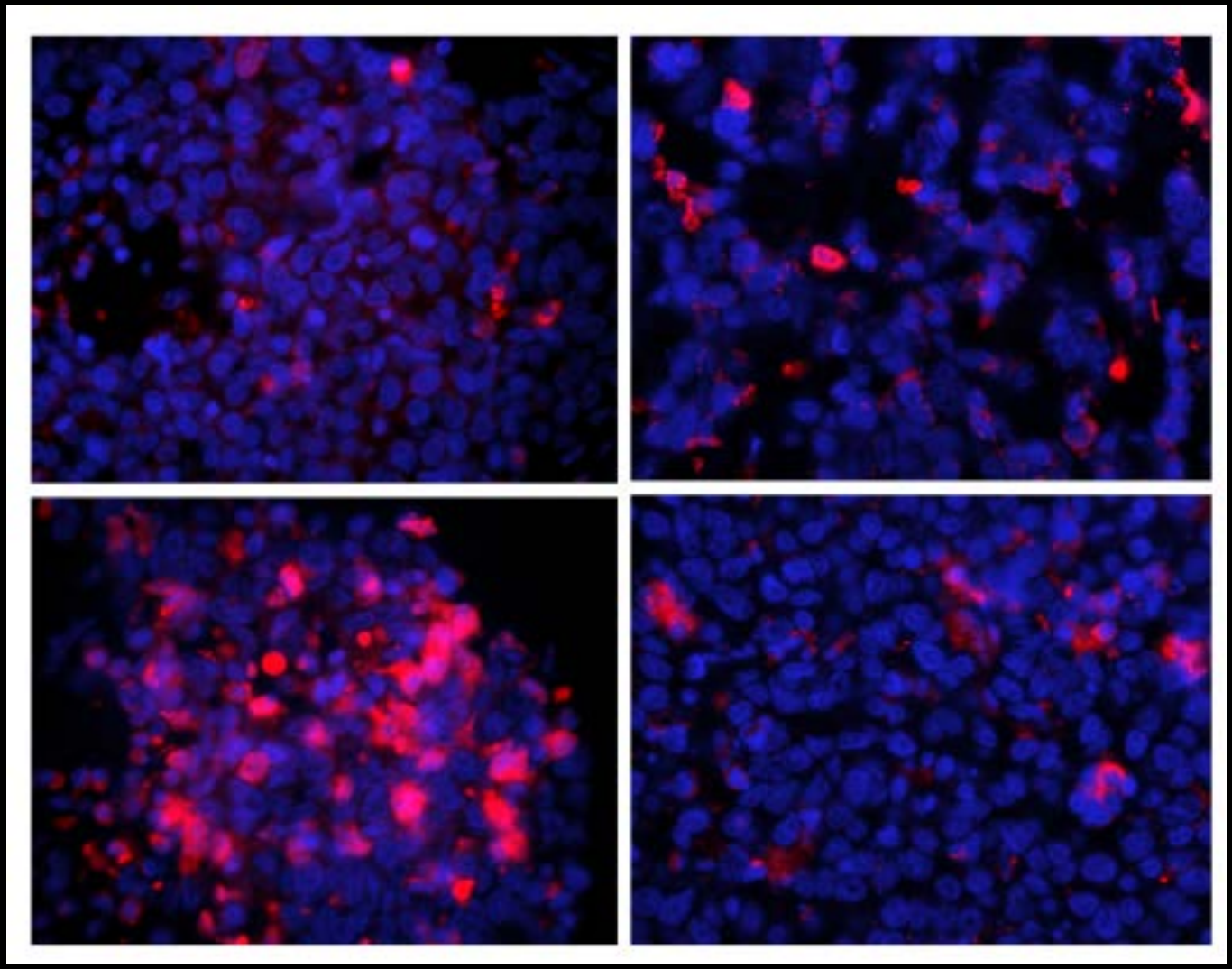


M028 – 4 days Olaparib 10 µM

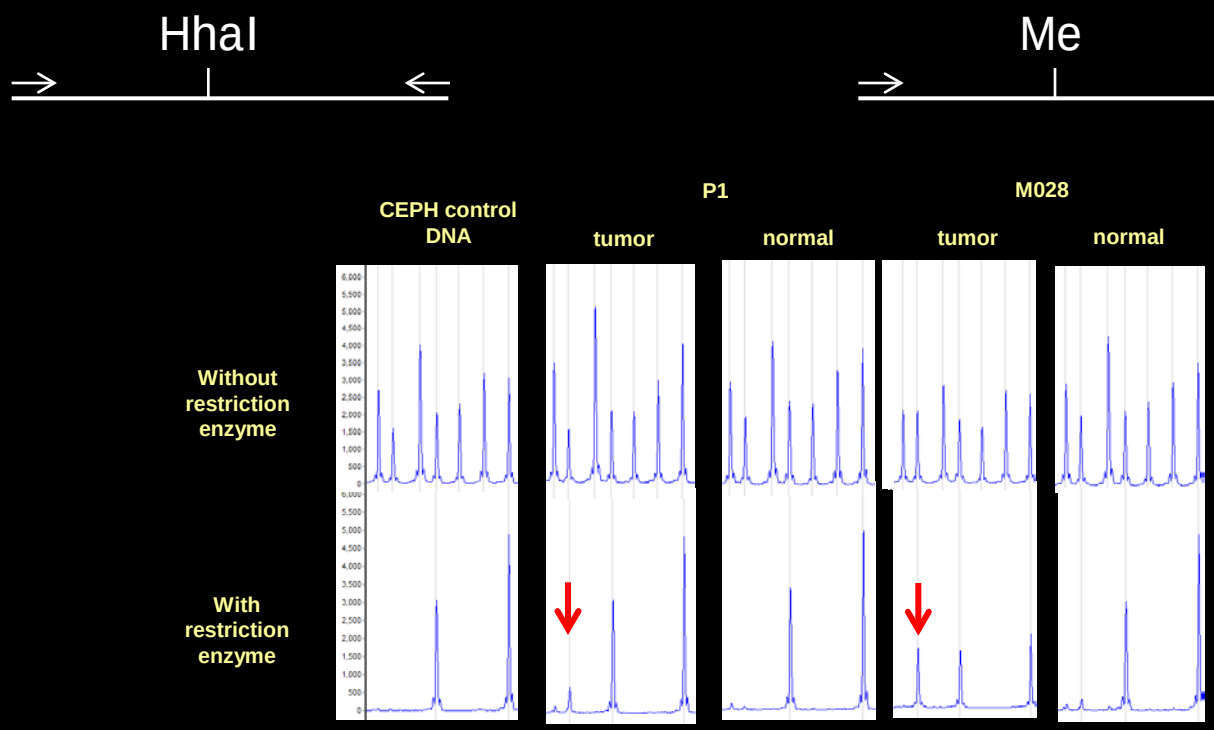
Blue= Dapi Red= Rad51 Green= Geminin



Cleaved Caspase 3 (Apoptosis)



Why was this tumor sensitive?



Analysis: BRCA1 promoter methylation (no protein expression)

Conclusions

- Tumor slices can be cultured for at least a week
- They still continue their cell cycle
- RAD51 foci are a read out for HR capacity
- BRCA1 deficient tumors do not form RAD51 foci
- 10% of random mammary tumors is foci negative
- Analysis: 1 x BRCA2 mutation, 2 x BRCA1 promoter methylation (no protein expression), 1 x not yet analyzed

Future challenges

- Find similar defects in other tumors
- Develop novel therapeutic strategies
- Predict optimal therapy for each individual tumor
- Will cancer be a chronic disease soon?



Erasmus MC
University Medical Center Rotterdam

Czaplinski

Kishan Naipal
Nicole Verkaik
Inger Brandsma
Roland Kanaar
Jan Hoeijmakers
Dik van Gent
Dept. of Genetics

Agnes Jager (clinic)
Carolien van Deurzen (Pathology)
John Martens (Medical Oncology)

Petra ter Brugge
Jos Jonkers
NKI Amsterdam

Johan de Winter
VU medical center, Amsterdam