EBERHARD KARLS
UNIVERSITÄT
TÜBINGEN

MEDIZINISCHE
FAKULTÄT

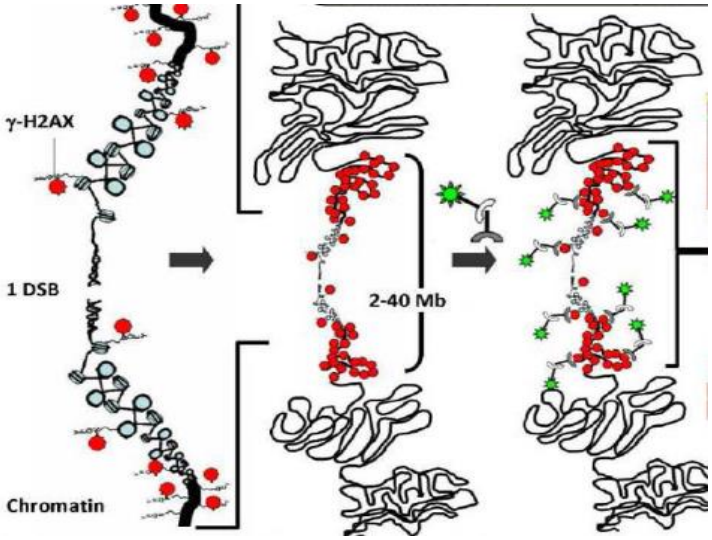
UNIVERSITÄTS
KLINIKUM
TÜBINGEN

Ex vivo γH2AX assay for determination of cellular radiation sensitivity in tumour biopsies

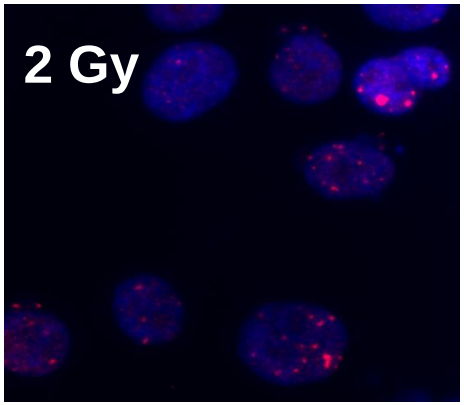
Chiara De Colle, Apostolos Menegakis, Arndt-Christian Mueller, Ala Yaromina, Joerg Hennenlotter, Arnulf Stenzl**,** Marcus Scharpf, Falko Fend, Umberto Ricardi**,** Michael Baumann and **Daniel Zips**

Background: γH2AX

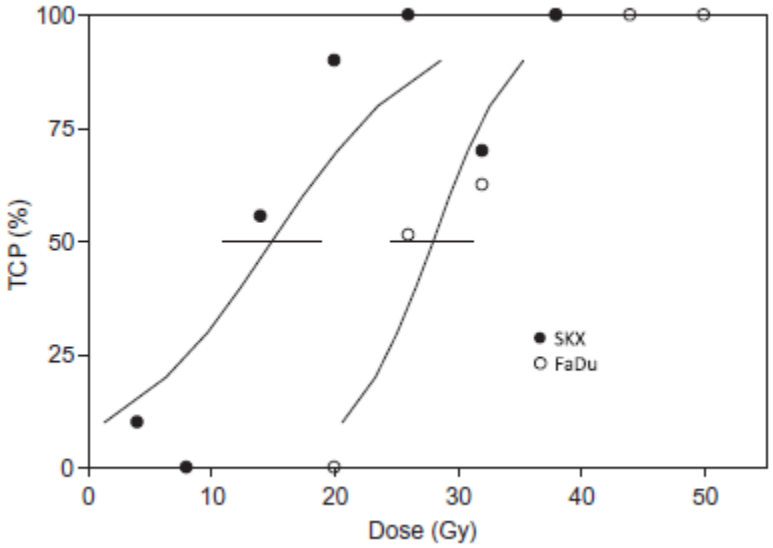
- The histone variant H2AX is rapidly phosphorylated (γH2AX) in the chromatin flanking DSBs and dephosphorylated upon repair has been completed.
- The number of residual γH2AX foci determined with phospho-specific antibodies has been shown to correlate with unrepaired DSB, chromosomal aberrations and cell death.
- Residual γH2AX foci or the rate of foci disappearance have extensively been used as biomarkers in translational cancer research.

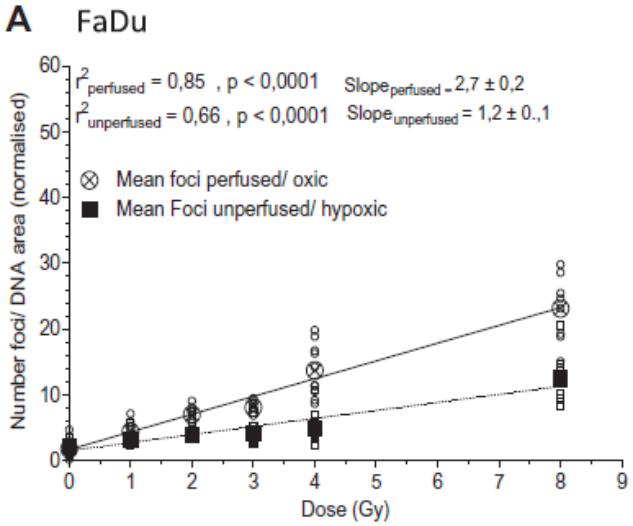


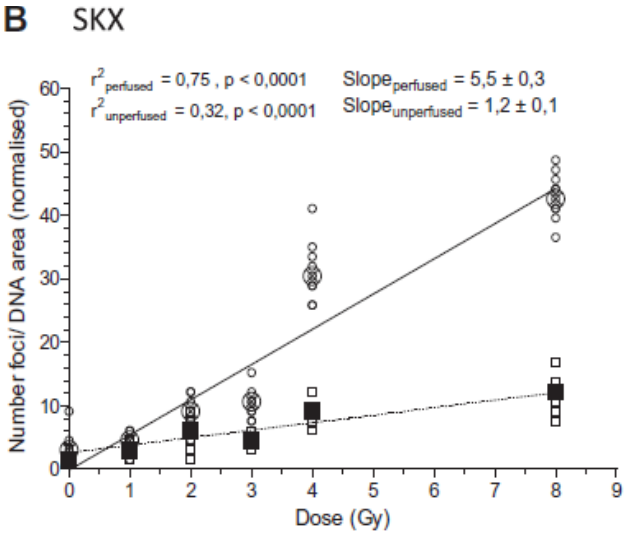
Redon et al. 2012



Residual γH2AX of *in situ* irradiated tumour xenografts as a marker of radiation sensitivity

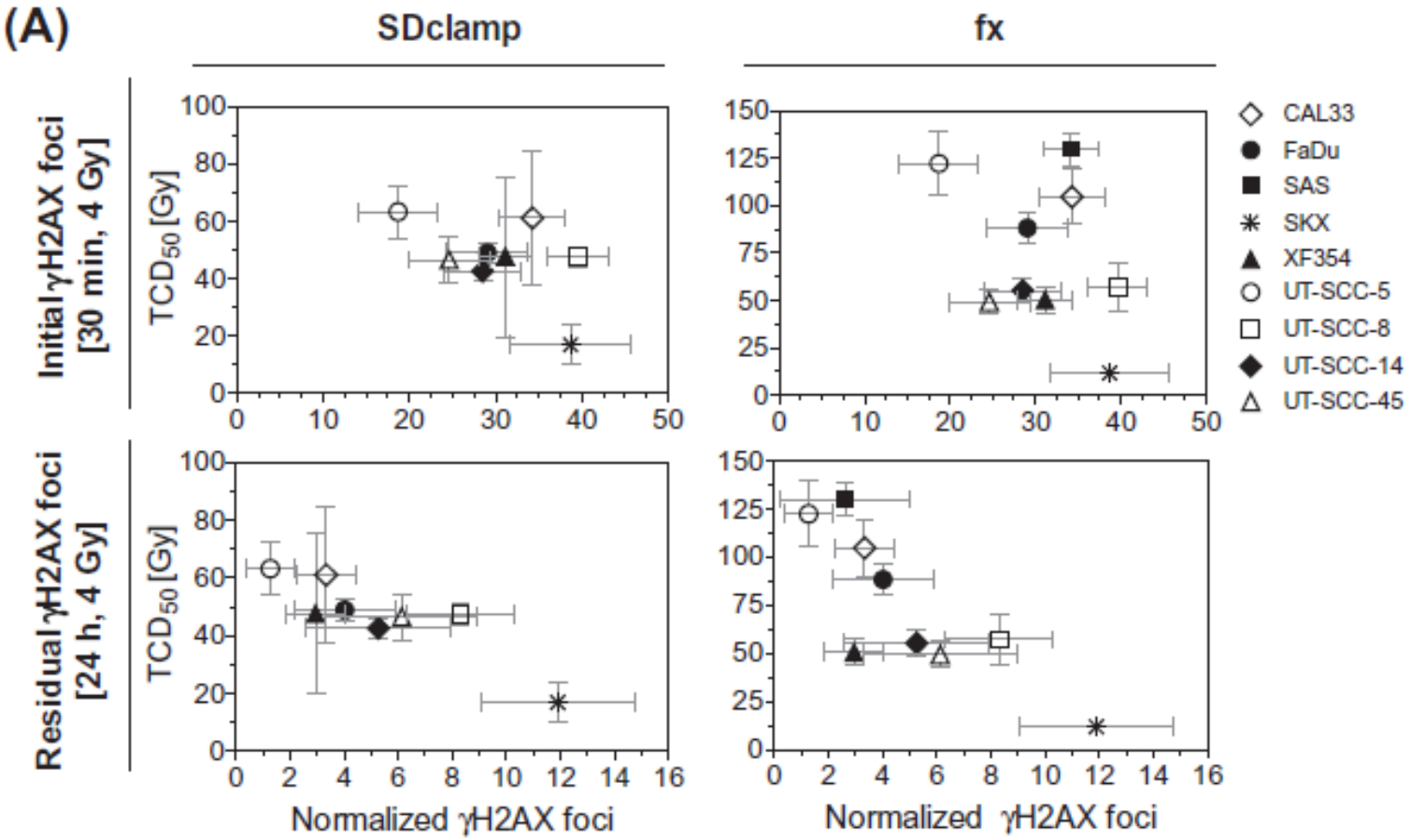






Menegakis et al. Radiother Oncol 2011;100: 137-144

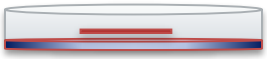
Residual γH2AX of *in situ* irradiated tumour xenografts as a marker of radiation sensitivity



Koch U et al. Radiother Oncol 2013;108: 434-439

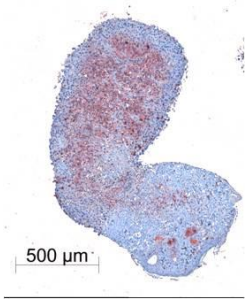
γH2AX assay in *ex vivo* irradiated tumour specimens

Taking of biopsy/ specimen

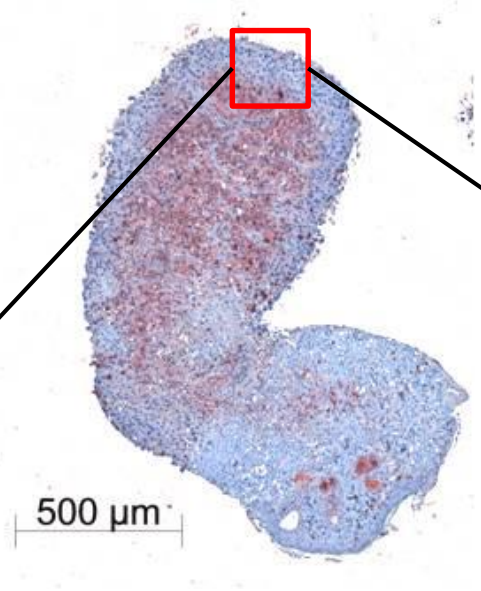


Ex vivo Irradiation of biopsies/ specimens

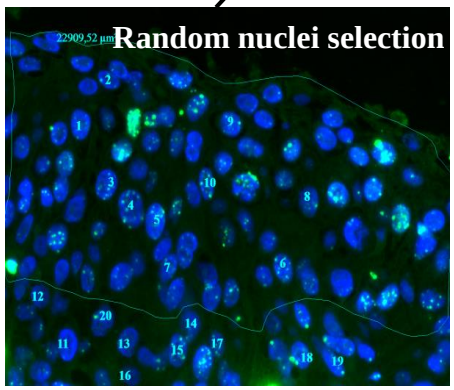
Formalin fixation/ paraffin embedment

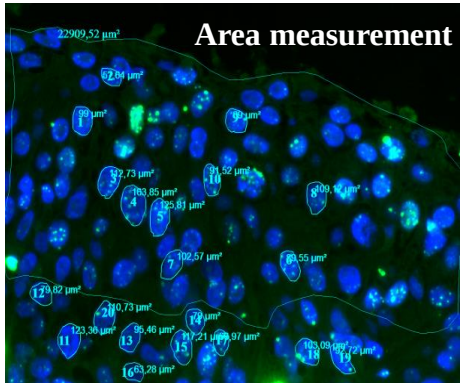


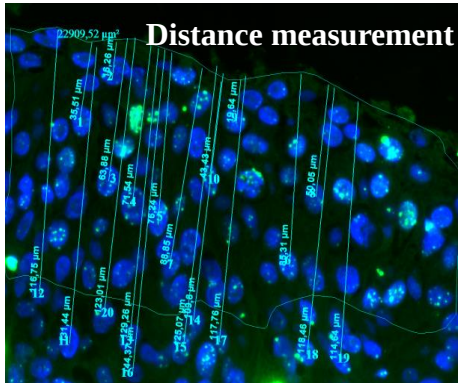
Evaluation procedure – FaDu biopsy cross-section

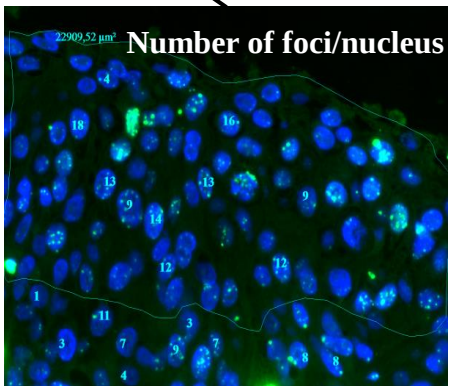


Pimonidazole – hypoxia marker
BrdU – proliferation marker
Hematoxylin



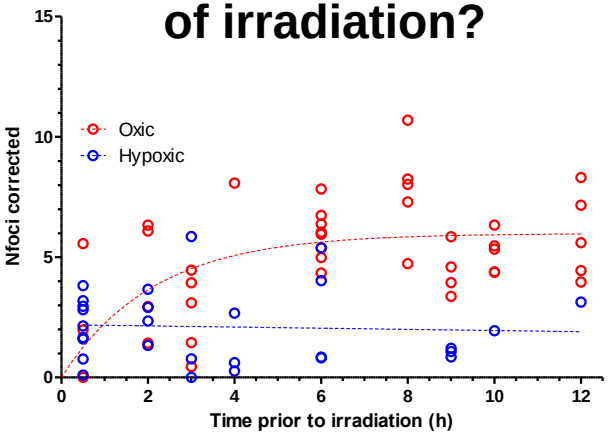






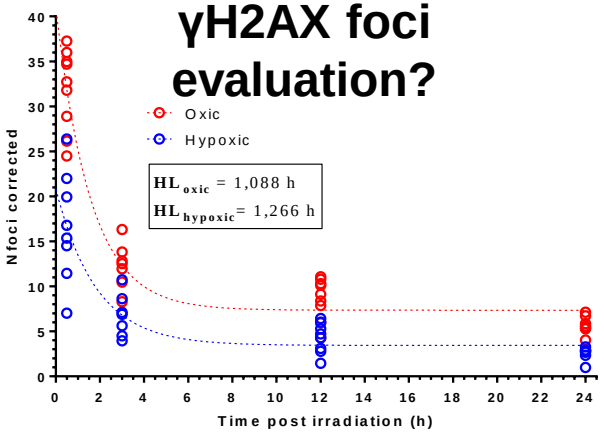
Establishment of assay in *ex vivo* irradiated FaDu tumour biopsies

Optimal time of irradiation?



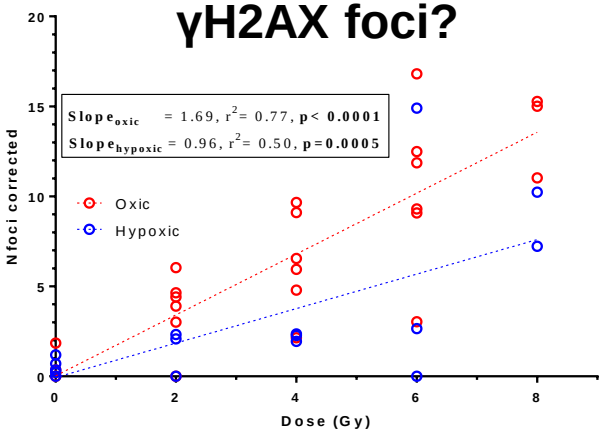
T ≥ 6 h

Optimal time of γH2AX foci evaluation?



T = 24 h

Dose response of γH2AX foci?

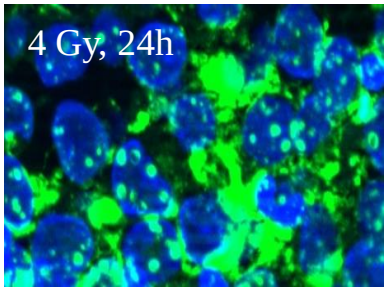


Linear dose response of residual γH2AX foci

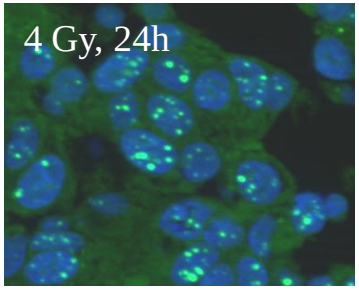
Menegakis et al. Radiother Oncol 2015

Residual γH2AX foci in different tumour types

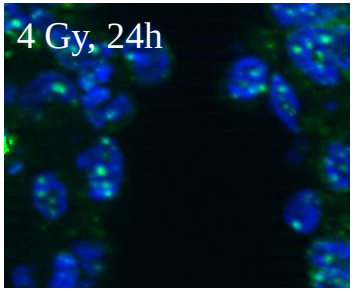
Seminoma



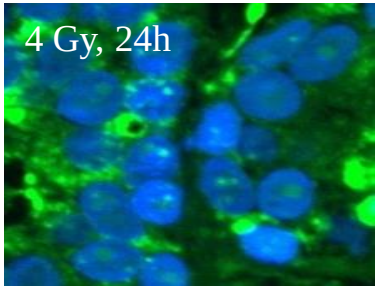
Bladder Ca



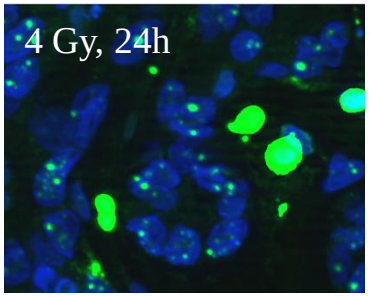
Colo-rectal Ca



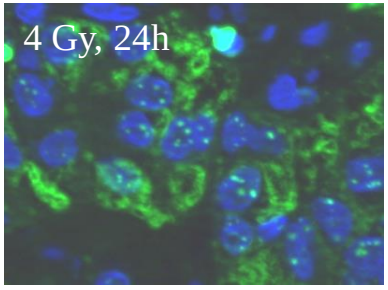
Breast Ca



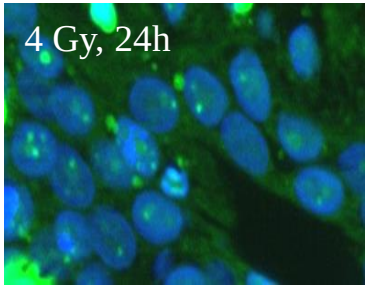
HCC



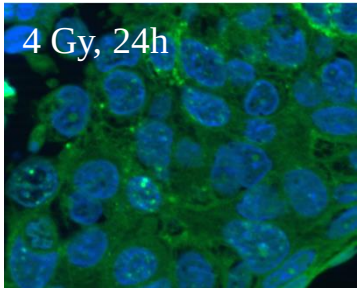
RCC



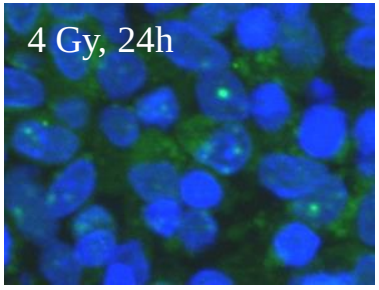
Prostate Ca



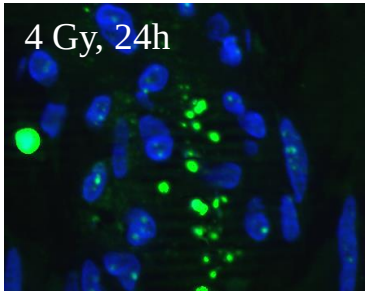
Cervix Ca



GBM

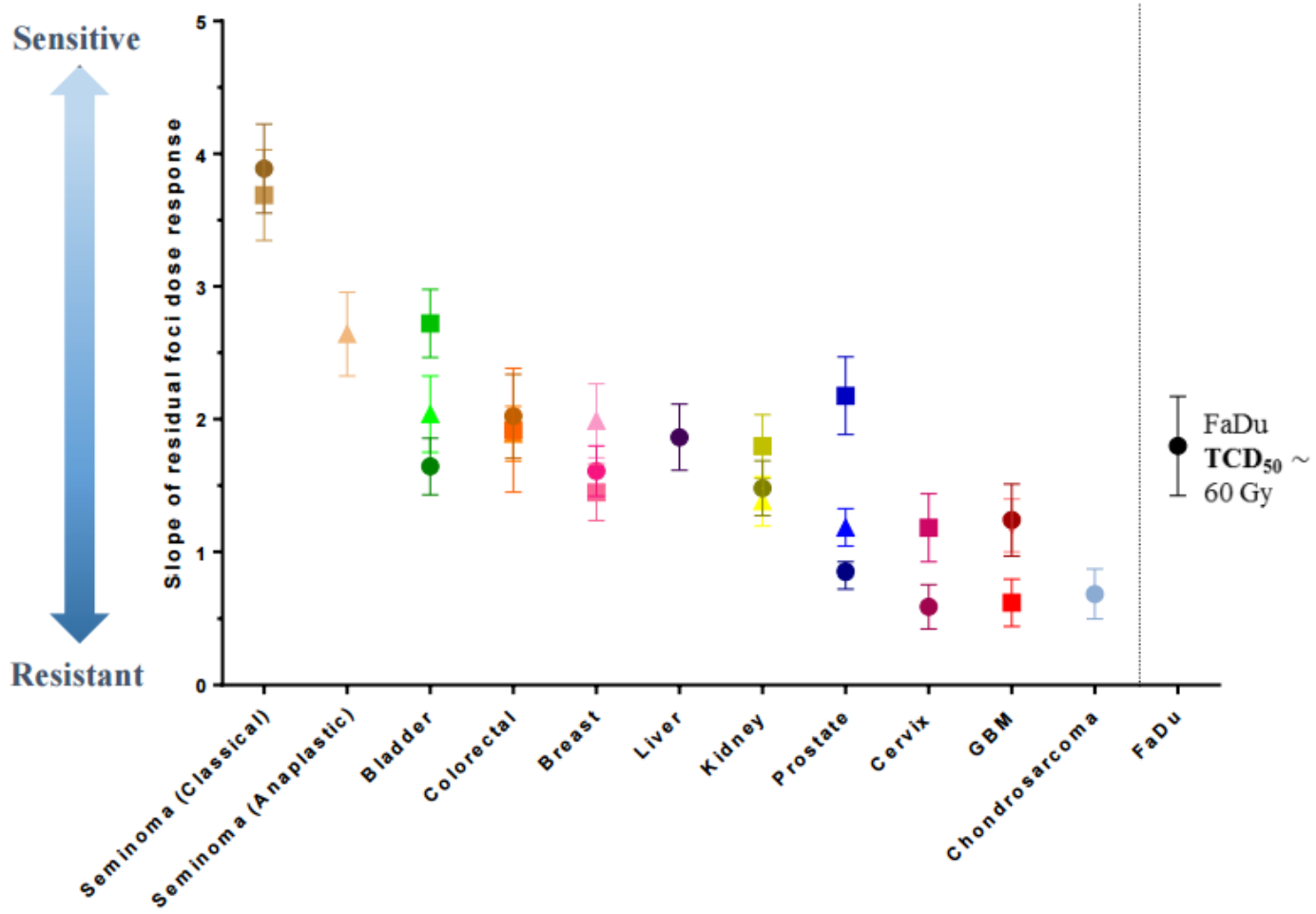


Chondrosarcoma



Menegakis et al. Radiother Oncol 2015

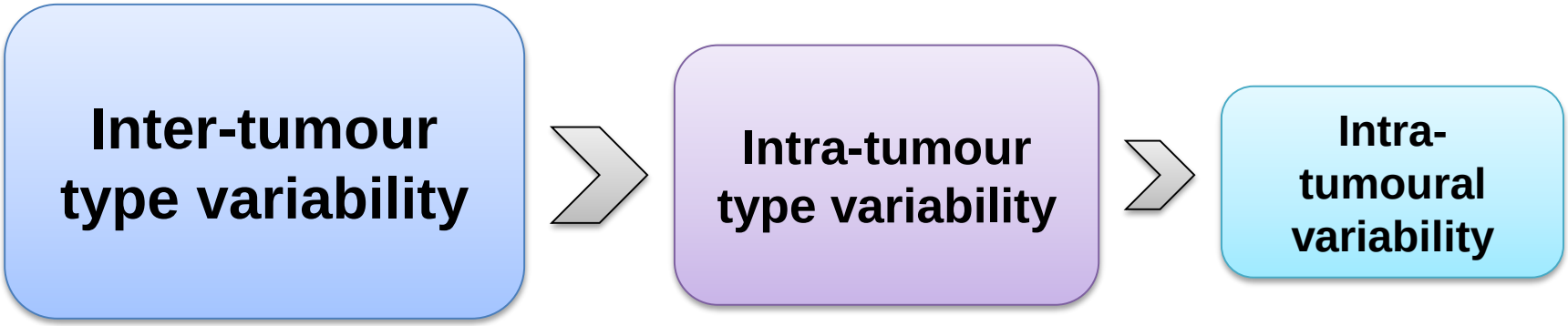
Slope of the residual γH2AX foci dose response discriminates sensitive and resistant tumour types



Menegakis et al. Radiother Oncol 2015

Inter- and intratumoural variability

- Evaluate the γH2AX *ex vivo* assay in tumours of the same histology (inter-tumoural variability)
- Evaluate the potential sampling error in the same tumour (intra-tumoural variability)



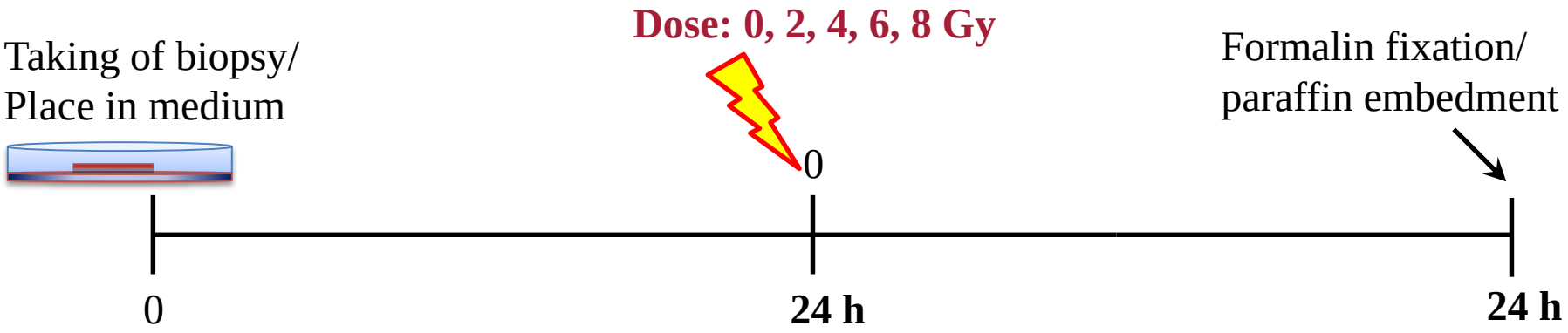
Material & Methods

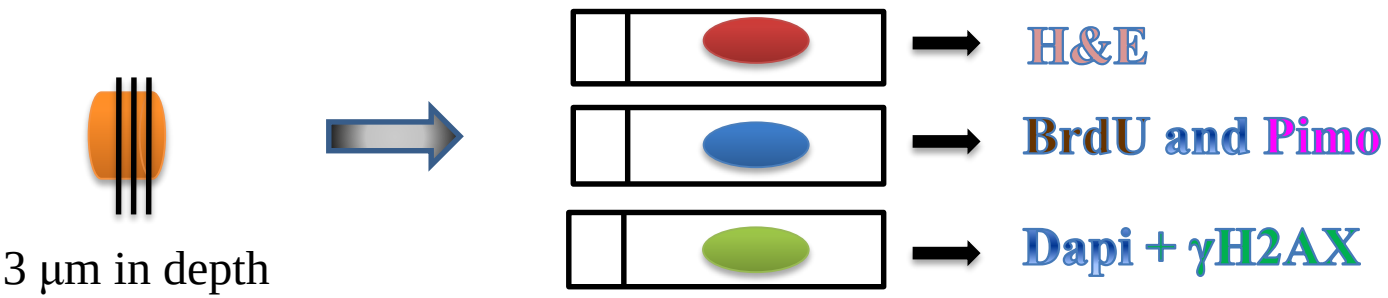
1. n=30 patients with prostate cancer undergoing prostatectomy

2. **Inter-tumoural variability:** 21 *ex vivo* irradiated tumour samples, full dose response (0, 2, 4, 6, 8 Gy irradiation)

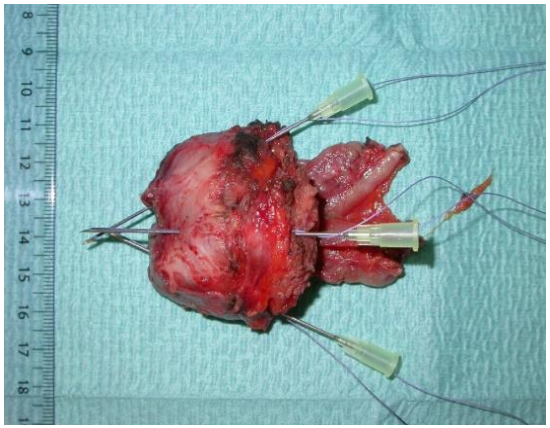
3. **Intra-tumoural variability:** *ex vivo* irradiated tumour biopsies collected from 9 patients (3-4 biopsies/ location, 2-3 locations/ patient), 4 Gy irradiation

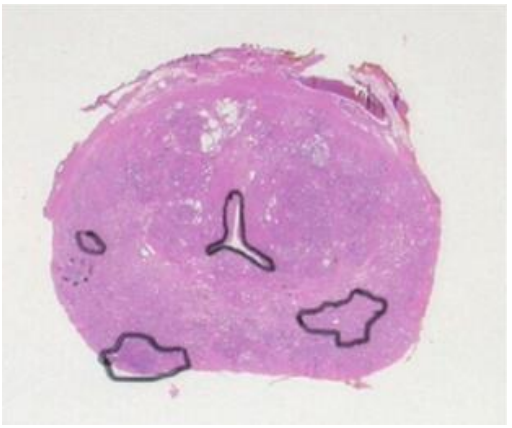
Material & Methods: samples collection and staining

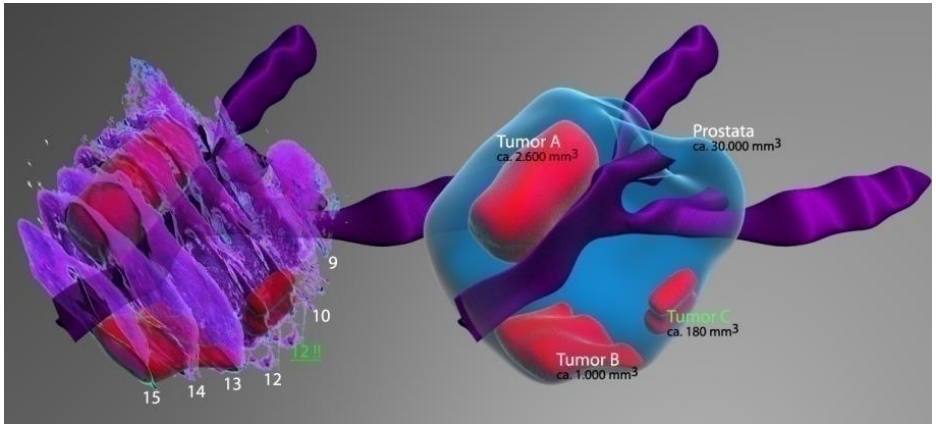




Material & Methods: samples collection (intratumoural variability)

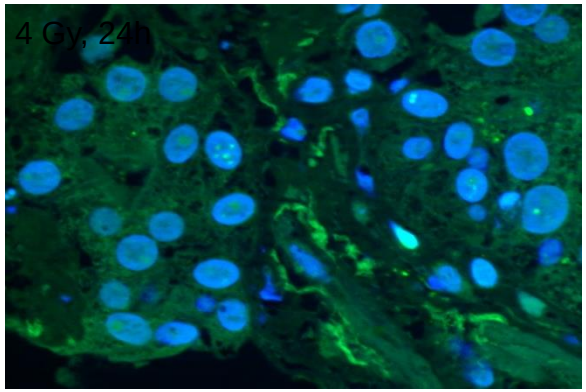




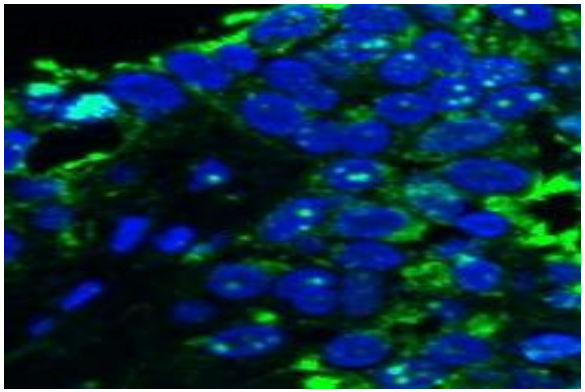


Results: residual γH2AX foci in patients with prostate adenocarcinoma (inter-tumoural variability)

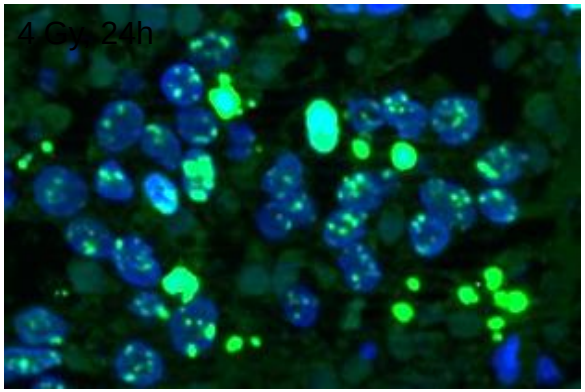
Resistant



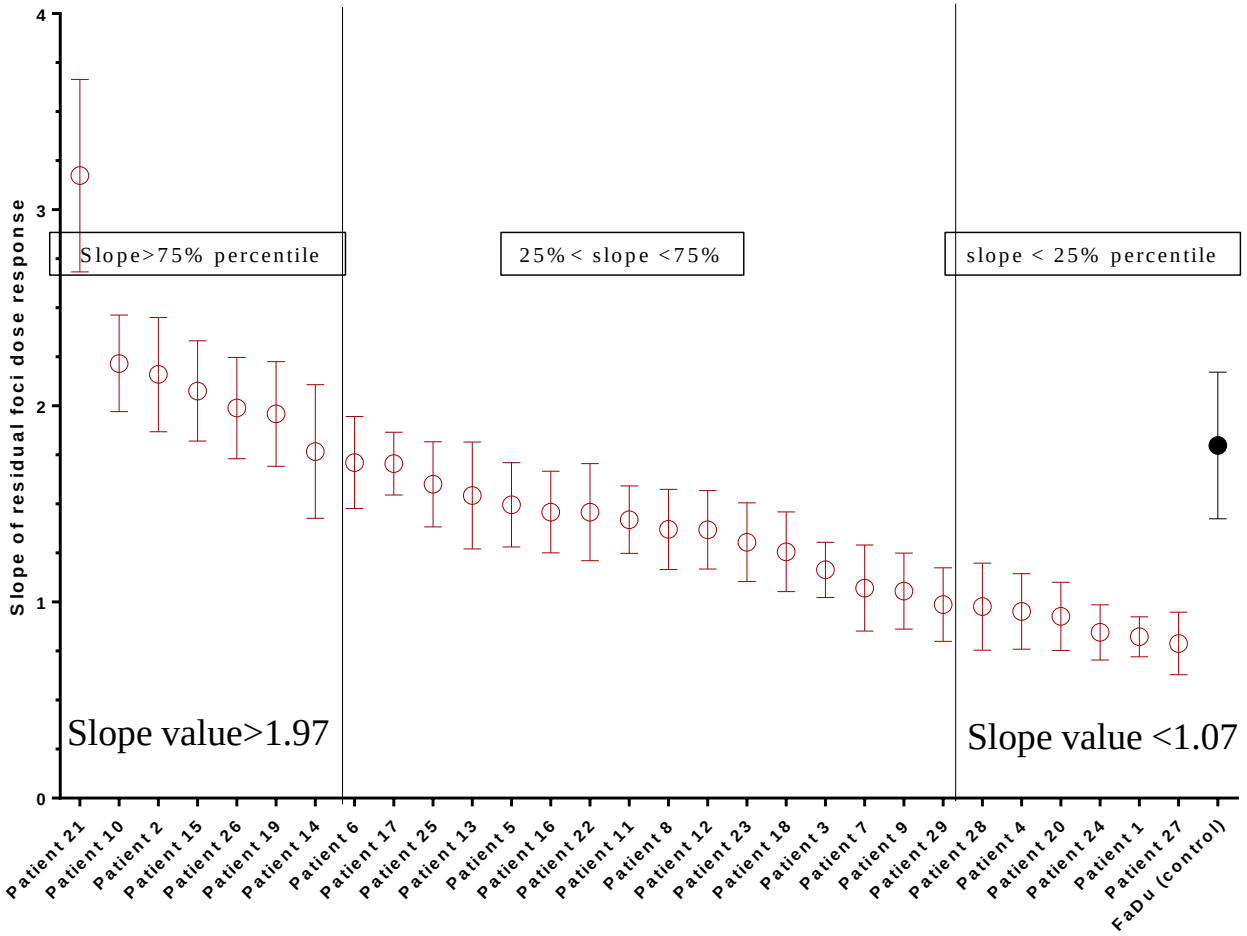
Moderate



Sensitive



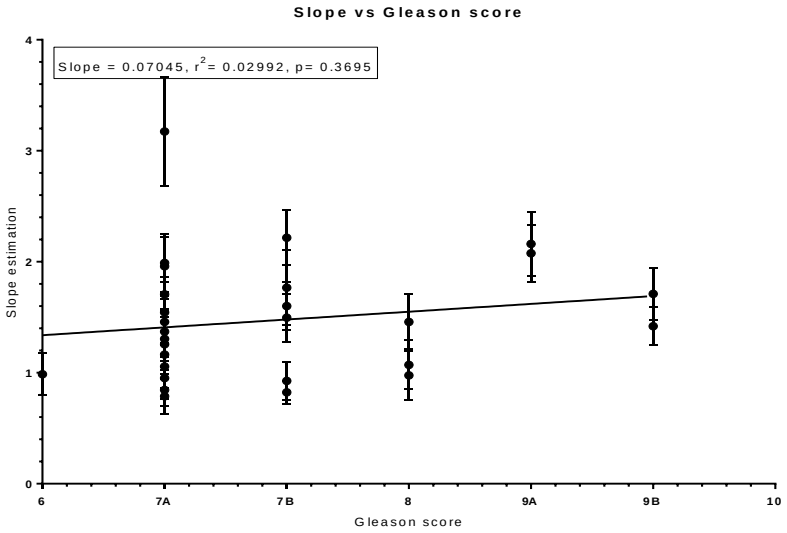
Results: residual γH2AX foci in patients with prostate adenocarcinoma (inter-tumoural variability)

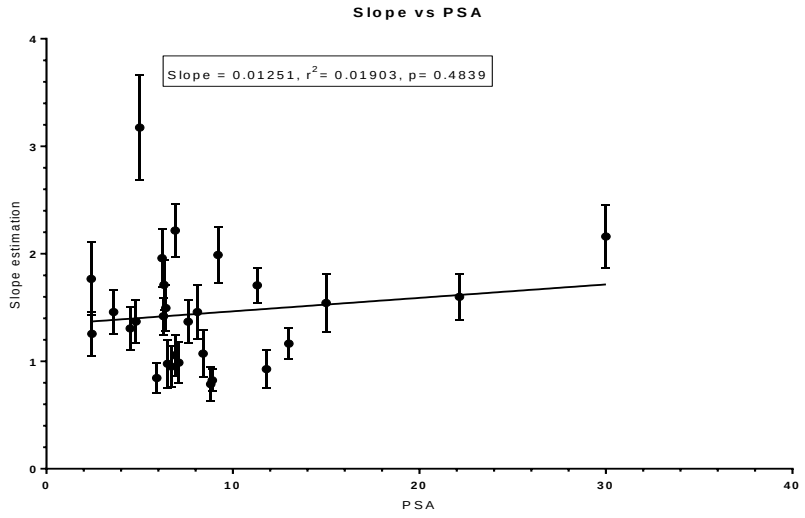


Slope values range:
0.82 (95% C.I.: 0.72-0.92) – 3.17 (95% C.I.: 2.68-3.67)

> factor 3 fold difference

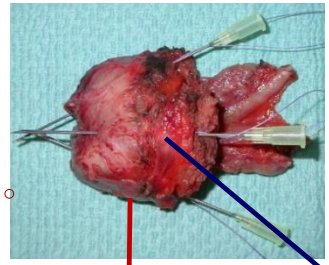
Results: no correlation with known risk factors



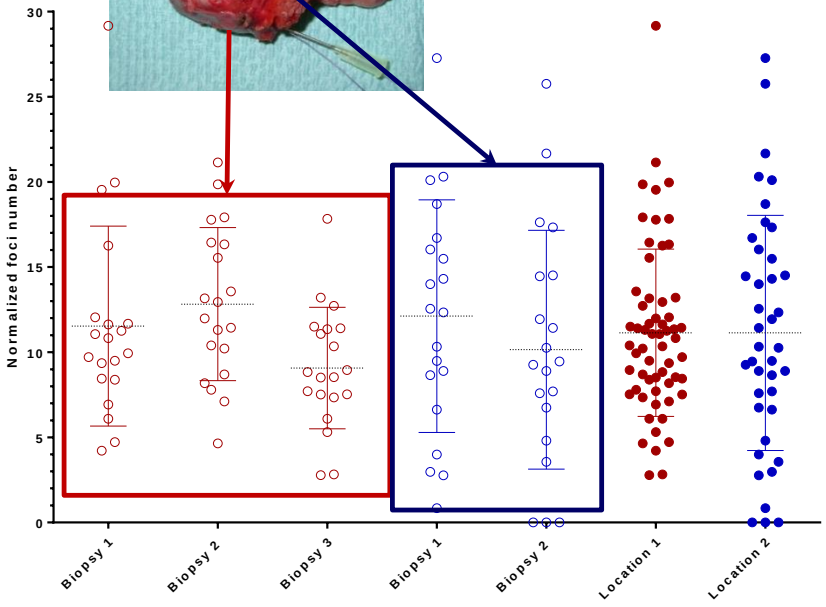


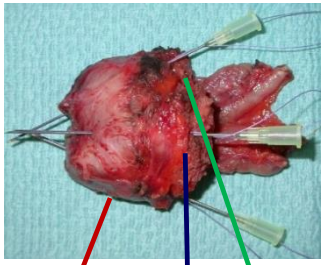
No correlation between the slope values either with the with GS (p=0.37), PSA (p=0.48) or T stage (p=0.89).

Results: residual γH2AX foci in biopsies from the same patient (intra-tumoural variability, 4 Gy, 24h)

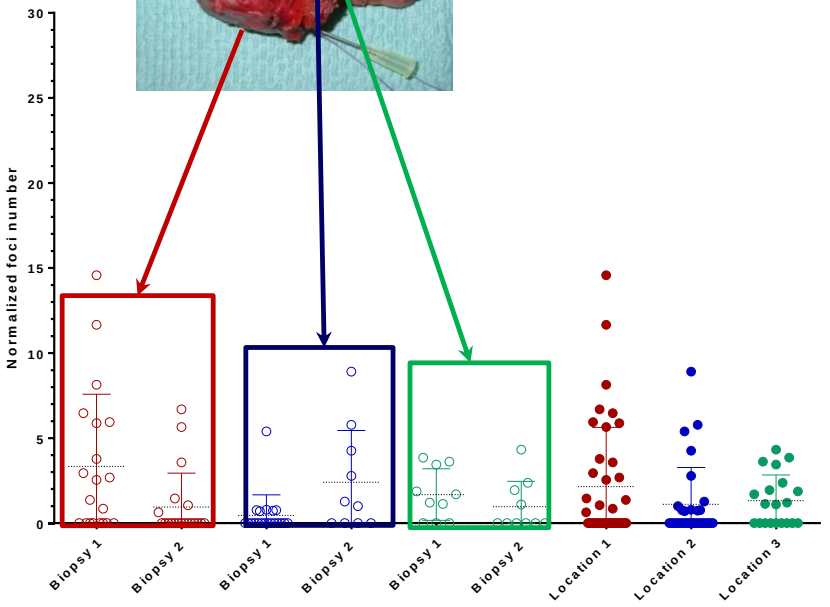


Patient 1 –
Sensitive tumour





Patient 9 –
Resistant tumour

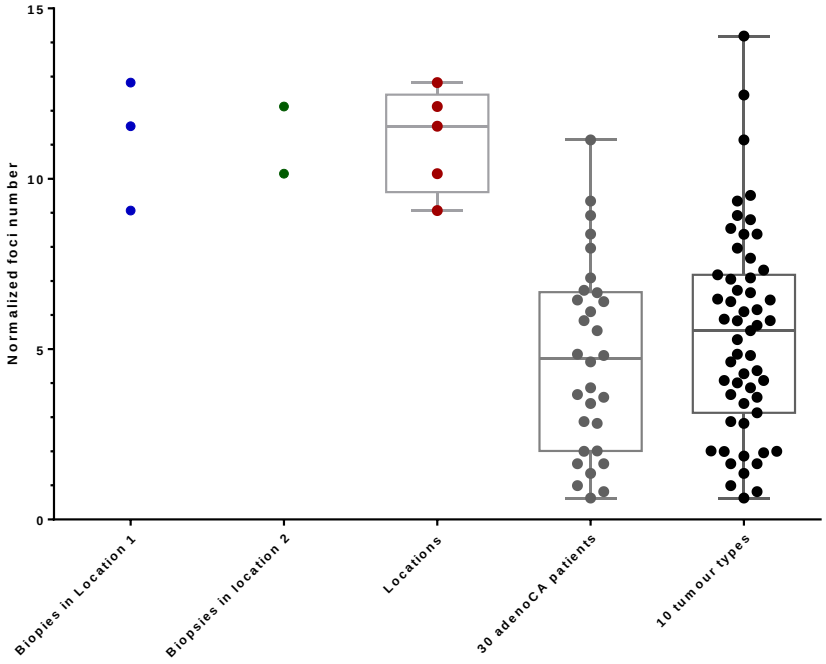


ANOVA analysis: no difference across biopsies.
In all but one patient (1/9) no difference between biopsies from different tumour locations.

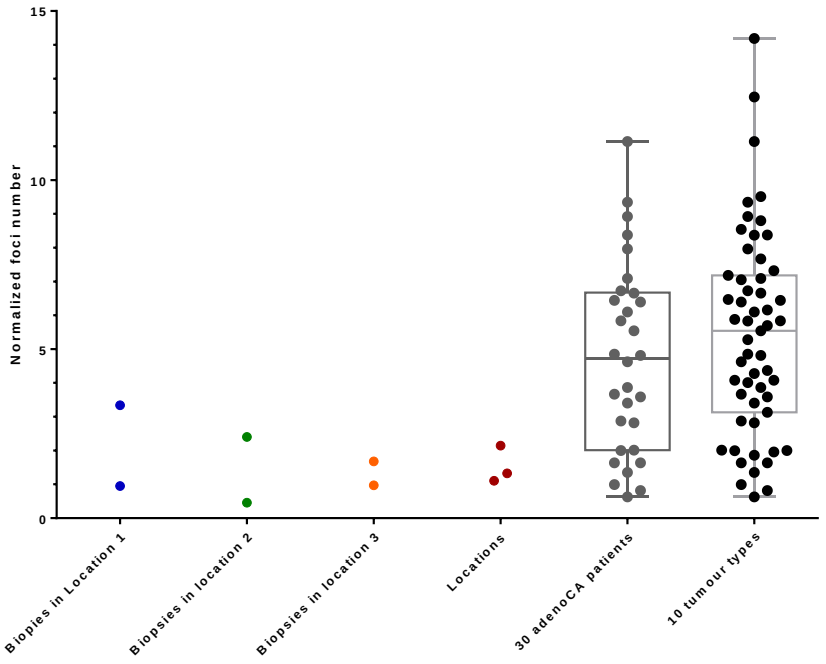
Intra-tumoural, inter-patient, inter-tumour type variability of nfoci values 24h post 4 Gy

Box plot of mean nfoci values 24h post 4Gy

Patient 1 – Sensitive tumour



Patient 9 – Resistant tumour



Summary

Intertumoural variability

- linear dose response in all the tumour samples
- results consistent with slope values for very sensitive (> 2) and resistant (~1) tumour types
- factor >3 fold radiosensitivity difference within prostate cancer, no correlation with known risk factors

Intratumoural variability

- no major differences between biopsies from the same tumour in the majority of patients

Acknowledgements

Dr. Apostolos Menegakis
Dr. Chiara De Colle

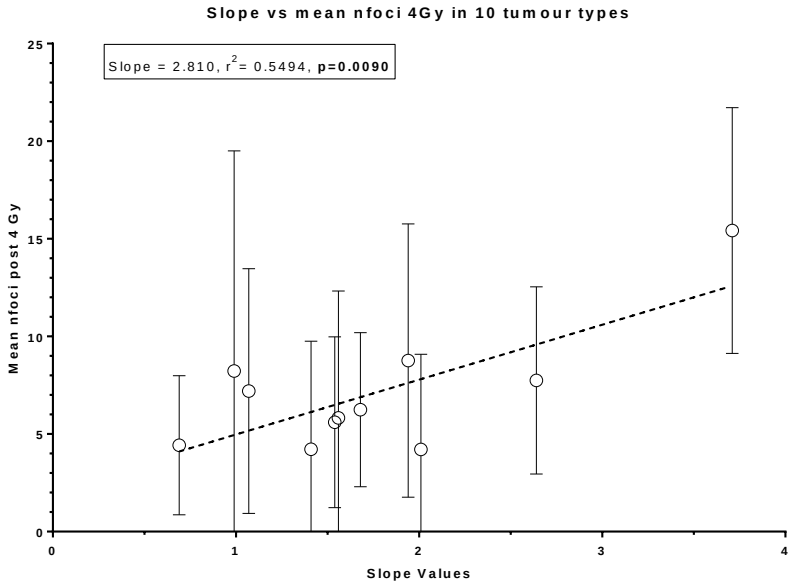
Prof. Dr. Michael Baumann
Prof. Dr. Mechthild Krause
Dr. Ala Yaromina
Prof. H.D. Thames

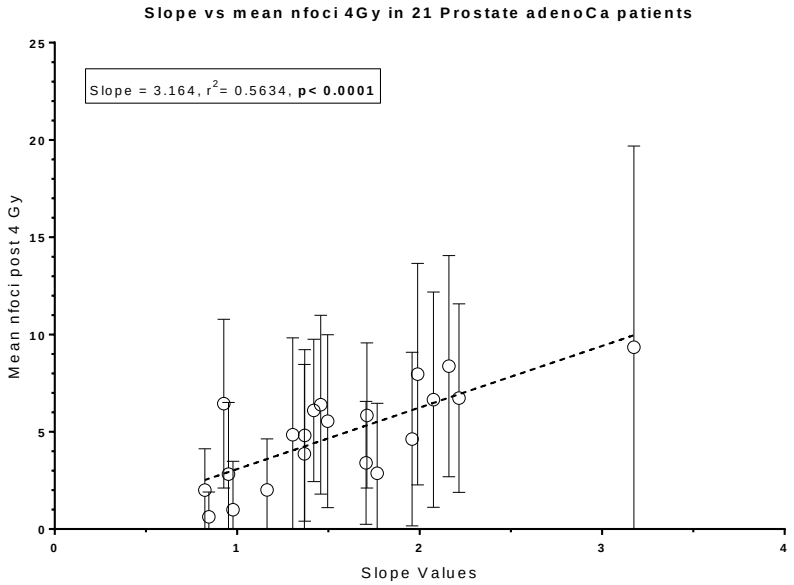
PD Dr. Müller
Prof. Dr. Stenzl
Prof. Dr. Fend
Prof. Dr. Ricardi
Dr. Hennenlotter
Dr. Scharpf

Funded by AKF, Medical Faculty of Eberhard-Karls Universität Tübingen


PERSONALISED
MEDICINE
DATA – DIAGNOSIS – DECISIONS
18. – 20. MAY 2016
IN TÜBINGEN

Slope values correlate with mean nfoci 24h post 4 Gy





3D reconstruction of prostate gland with marked positions of tumour and biopsies (blue, black, yellow)

Patient 10