Biomarkers from an experiemental point of view

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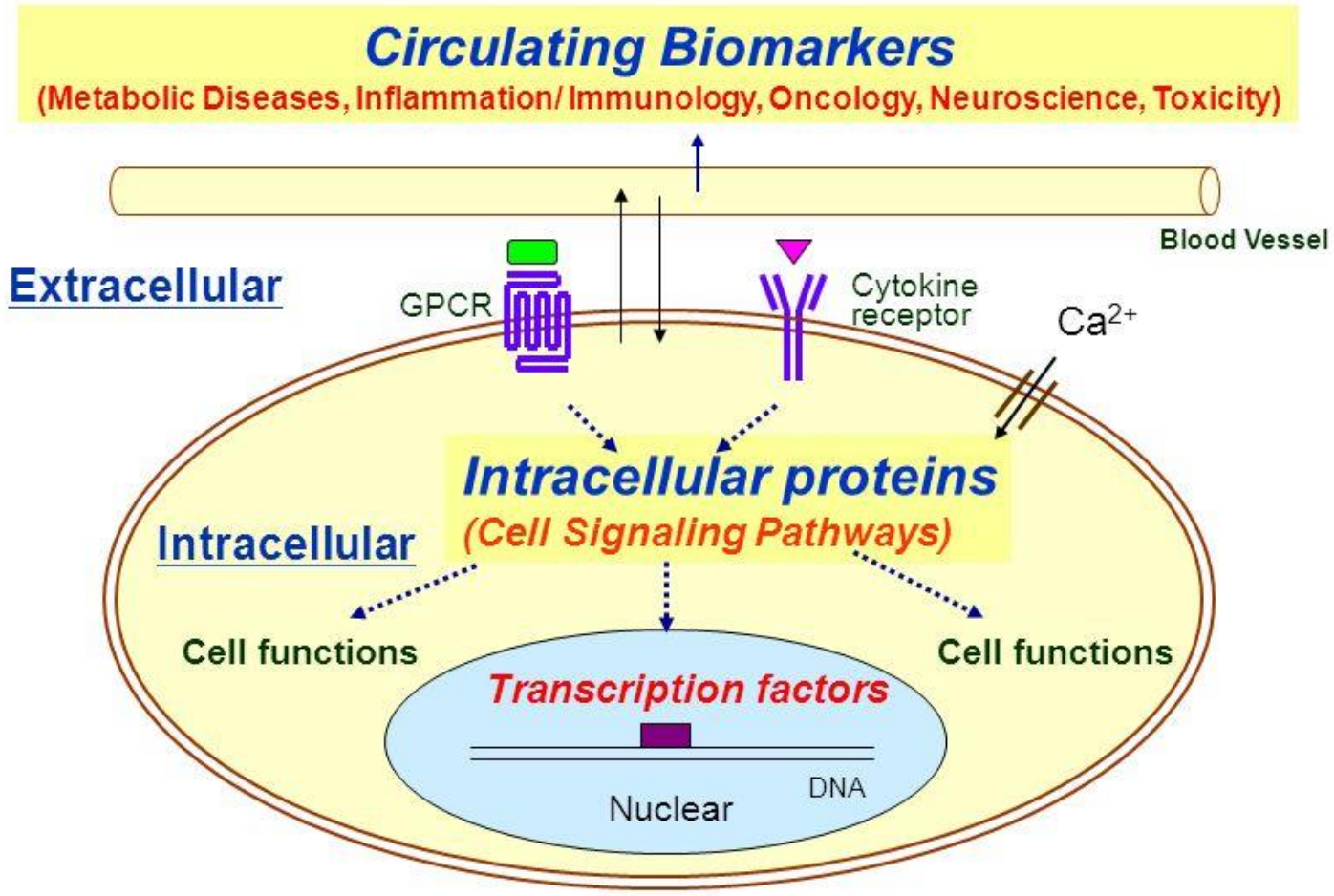
1:st July 2019, Enlight meeting, Caen

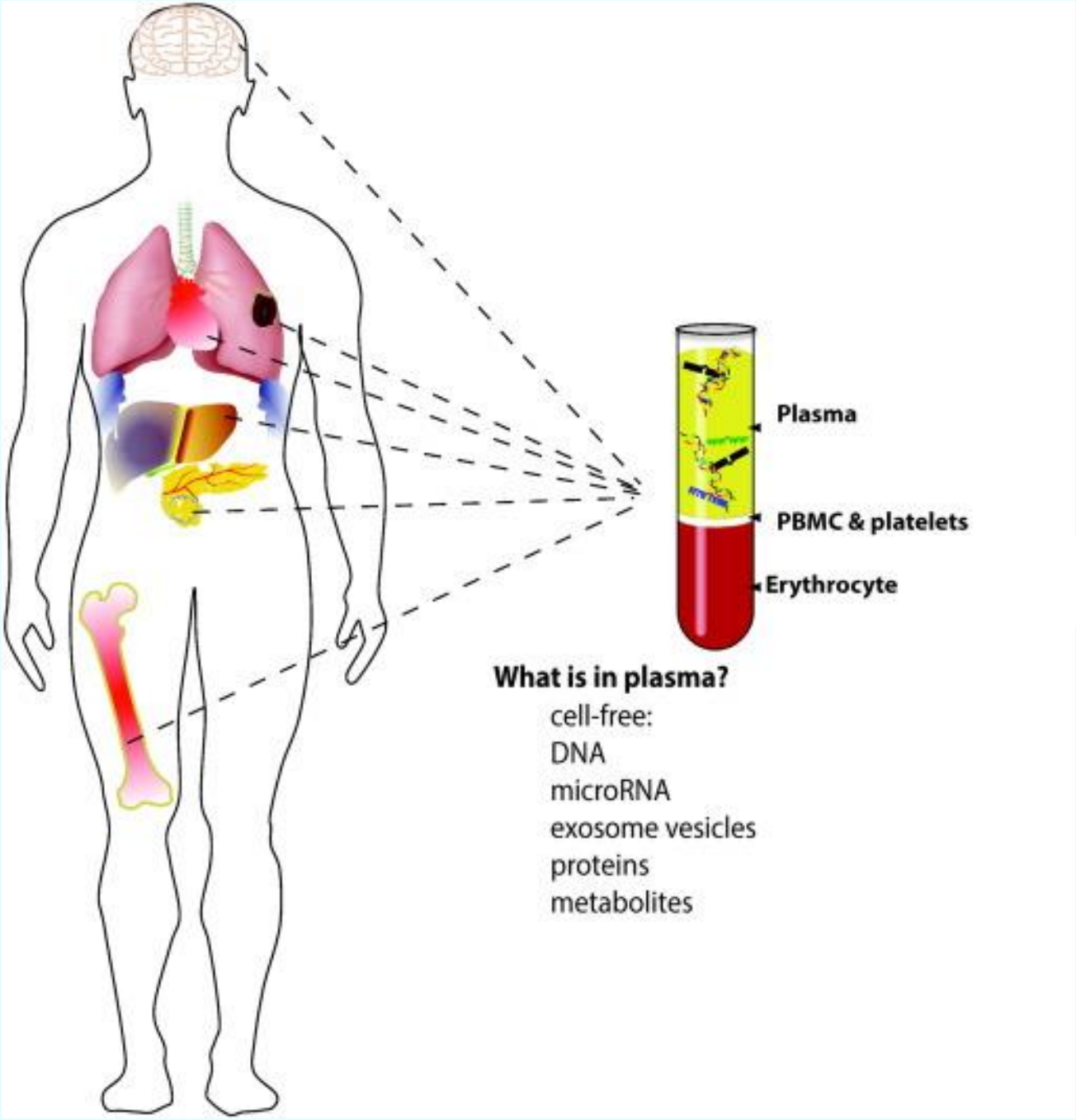
Biomarker:

Any measurement reflecting an interaction between a biological system and an environmental agent, which may be chemical, physical or biological.

Considering radiation:

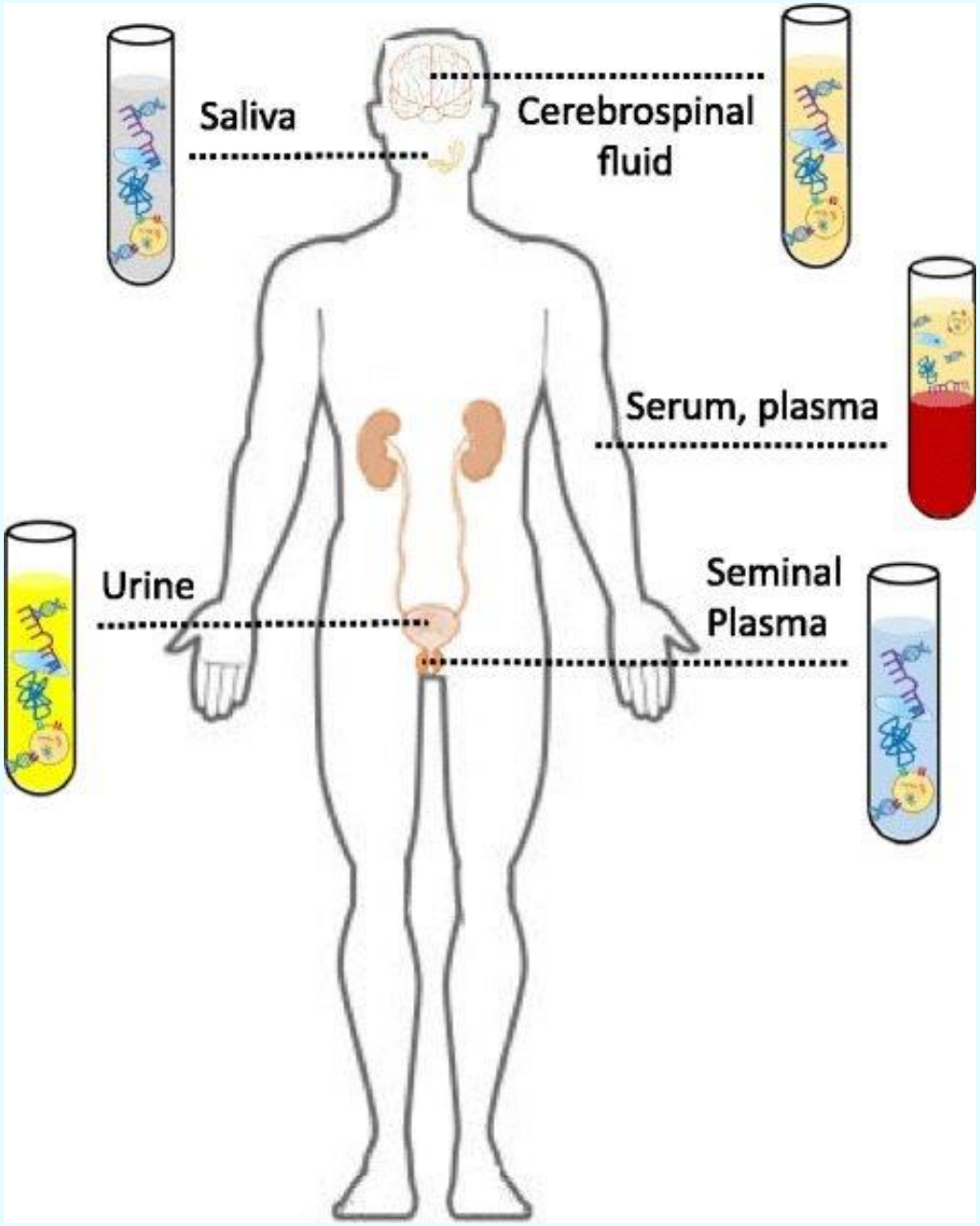
- Biomarkers can be used for multiple purposes:
  - Estimation or validation of received dose
  - Investigation of correlation between exposure and biological responses e.g., therapeutic effects of radiotherapy
  - Investigation of individual susceptibility
  - Early detection of a radiation induced health effect


Circulating Biomarkers
(Metabolic Diseases, Inflammation/ Immunology, Oncology, Neuroscience, Toxicity)
Blood Vessel
Extracellular
GPCR
Cytokine receptor
Ca2+
Intracellular proteins
(Cell Signaling Pathways)
Intracellular
Cell functions
Cell functions
Transcription factors
DNA
Nuclear

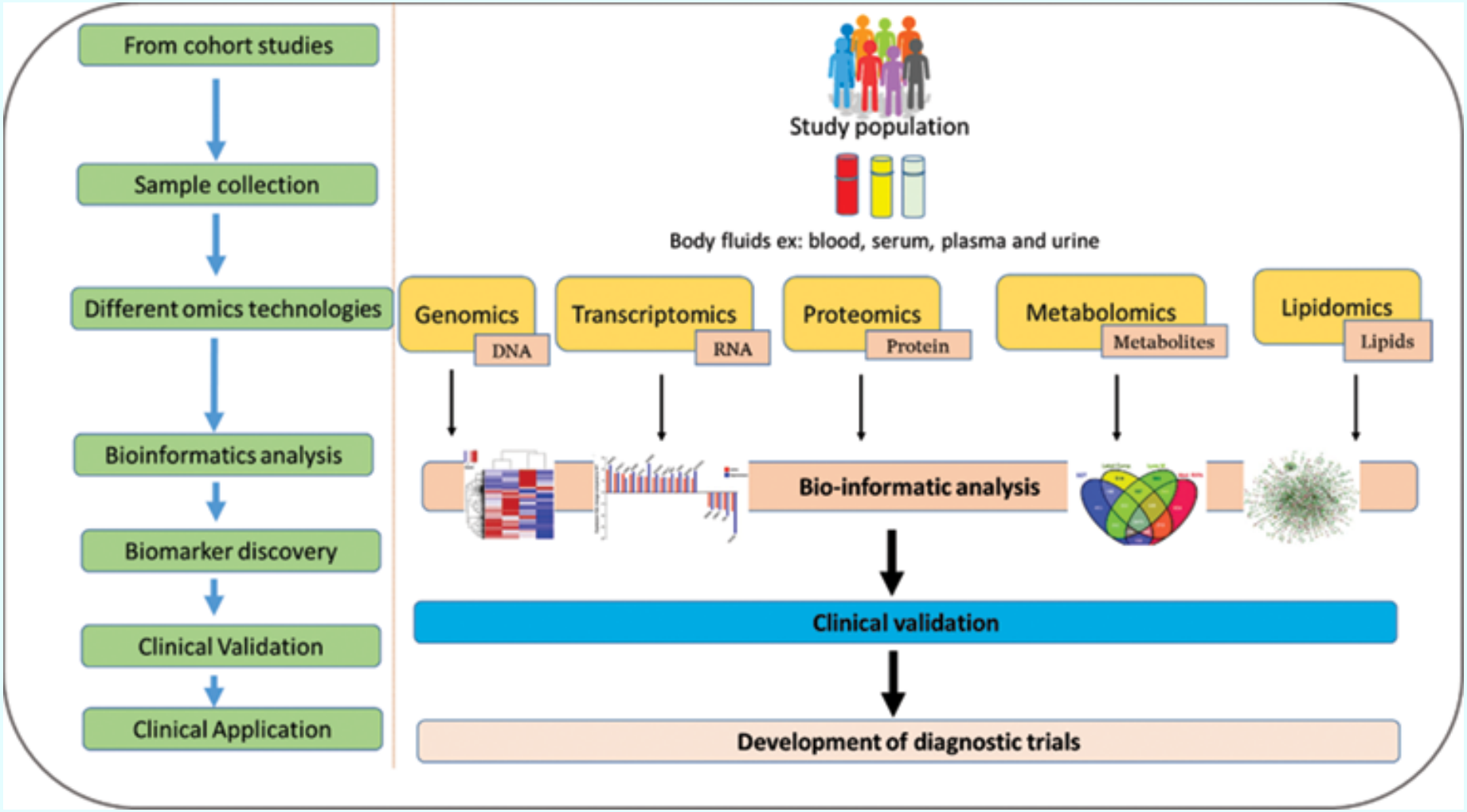


What is in plasma?

cell-free:
DNA
microRNA
exosome vesicles
proteins
metabolites


Saliva
Cerebrospinal fluid
Serum, plasma
Urine
Seminal Plasma

Retrospective or prospective cohorts



Example of biomarker for monitoring effect of treatment:

Serum level of thymidine kinase 1

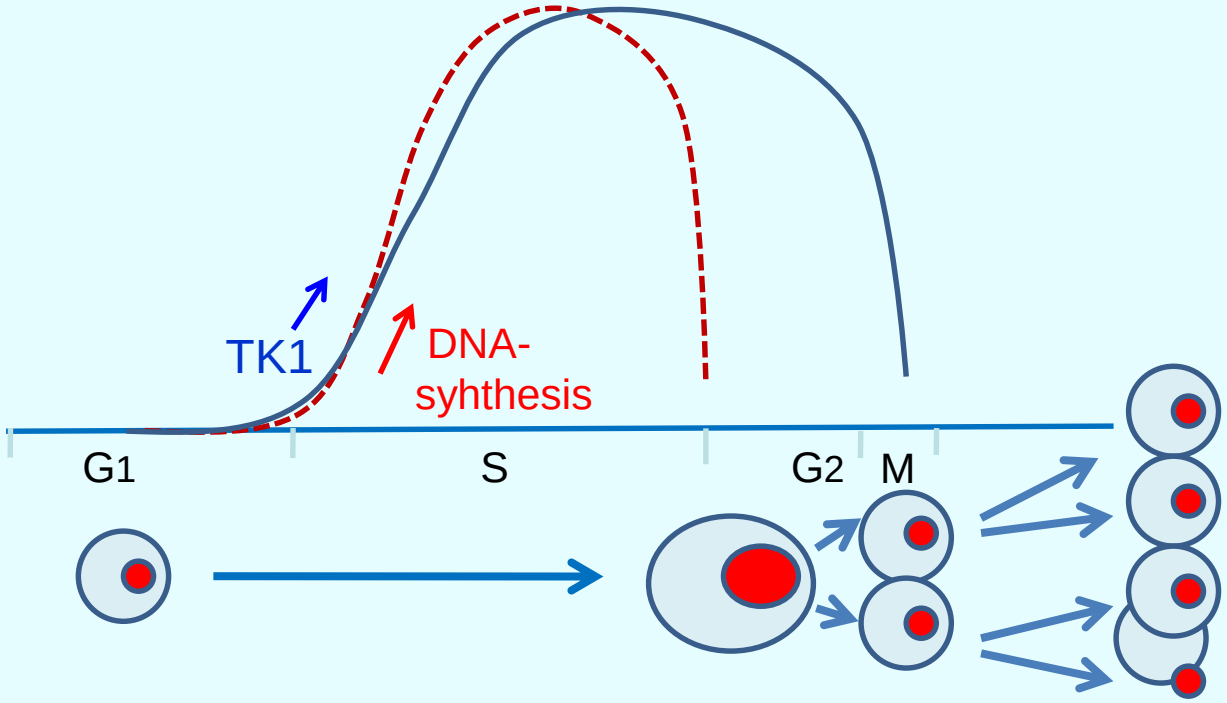
1951: TK1 was discovered by Prof Peter Richard and his research group

Late 1954: TK1 was suggested as a proliferation/growth marker in tumour biopsy

1986 – 2000: developed a sensitive TK1 test for detect of TK1 in human blood serum

2002: SSTK company was set up to produce TK1 kit

TK1 in relation to DNA-synthesis and the cell cycle



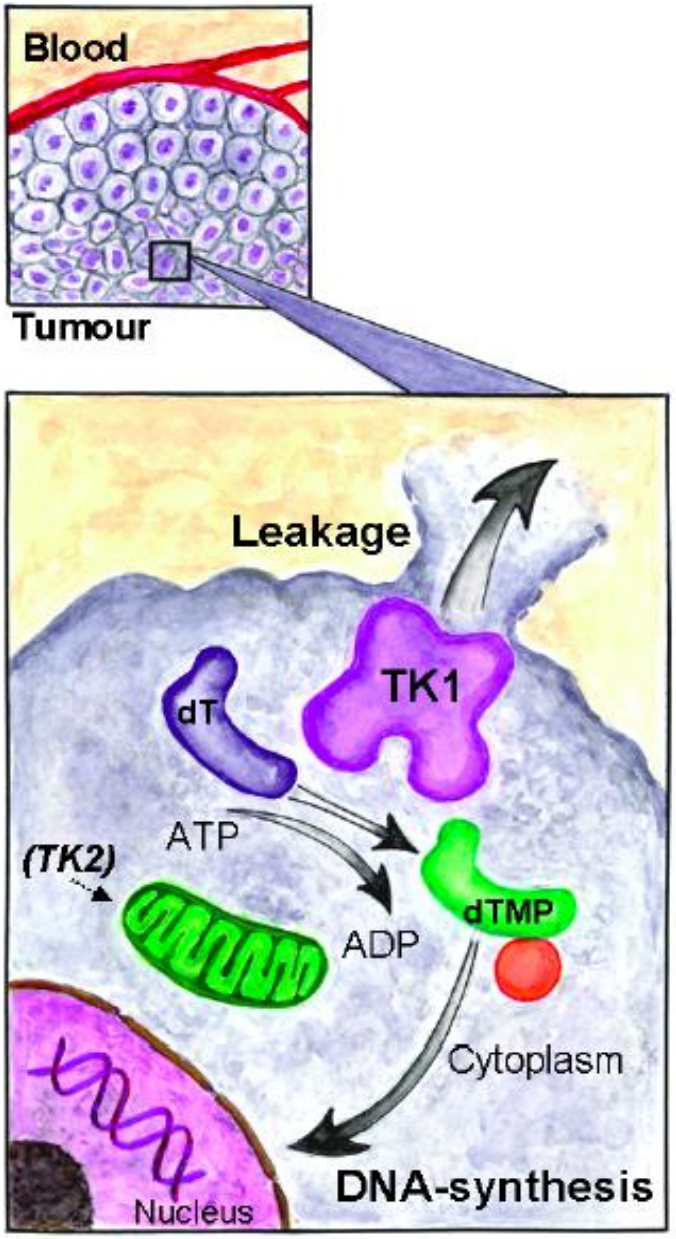
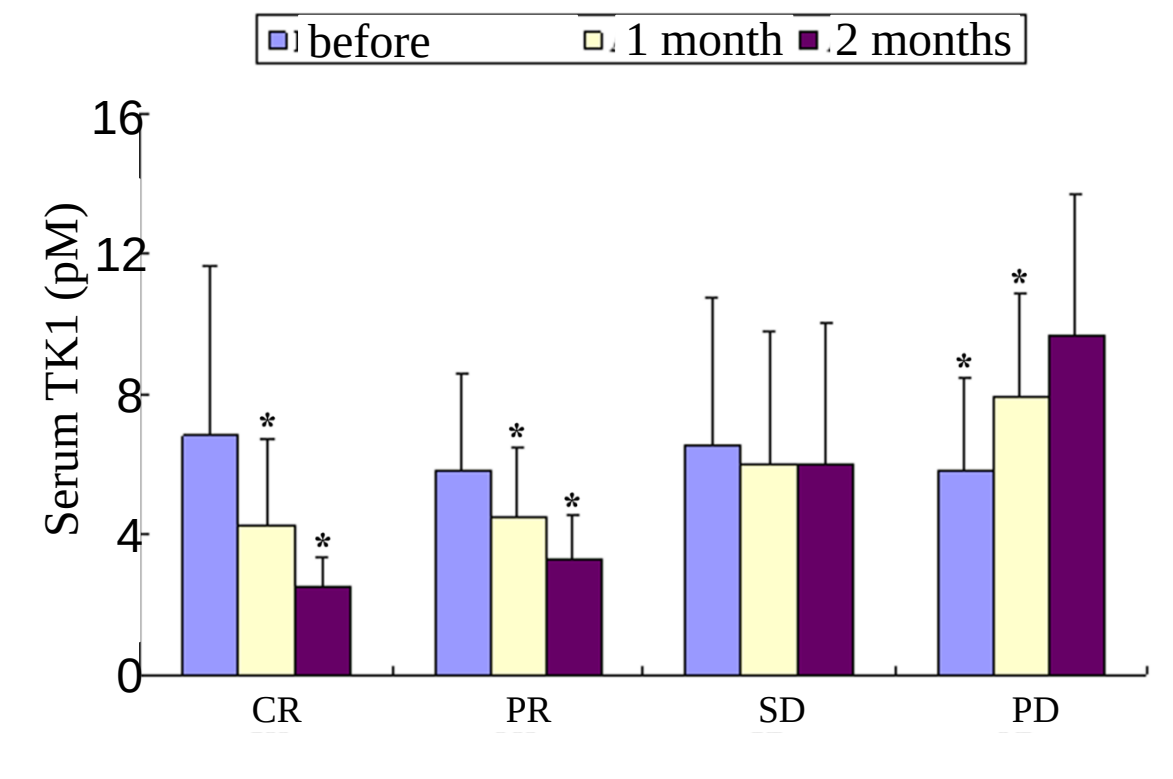

Blood
Tumour
Leakage
TK1
dT
ATP
(TK2)
dTMP
ADP
Cytoplasm
DNA-synthesis
Nucleus

Fig 1

Gastric, monitoring chemo-therapy



CR: complete remission,
PR: partial remission, 50% of tumor disappear.
SD: stable diseases, the tumor size did not change.
PD: progressive diseases, tumor size increase.

EXPERIMENTAL AND THERAPEUTIC MEDICINE
SPANDIDOS PUBLICATIONS

Exp Ther Med. 2011 Nov-Dec; 2(6): 1177–1181. PMCID: PMC3440839
Published online 2011 Aug 17. doi: 10.3892/etm.2011.338 PMID: 22977640

Changes in serum thymidine kinase 1 levels during chemotherapy correlate with objective response in patients with advanced gastric cancer

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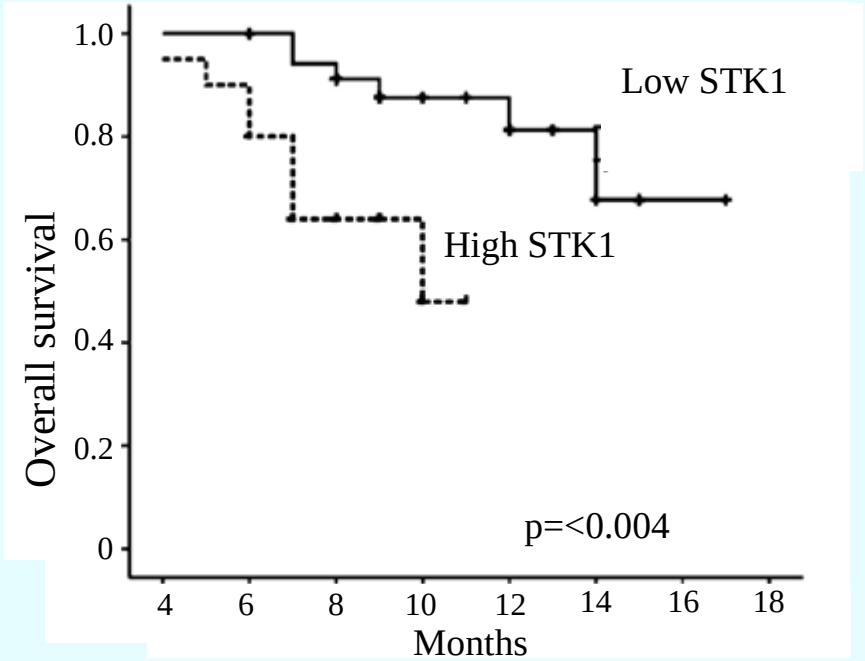
Gastric, survival



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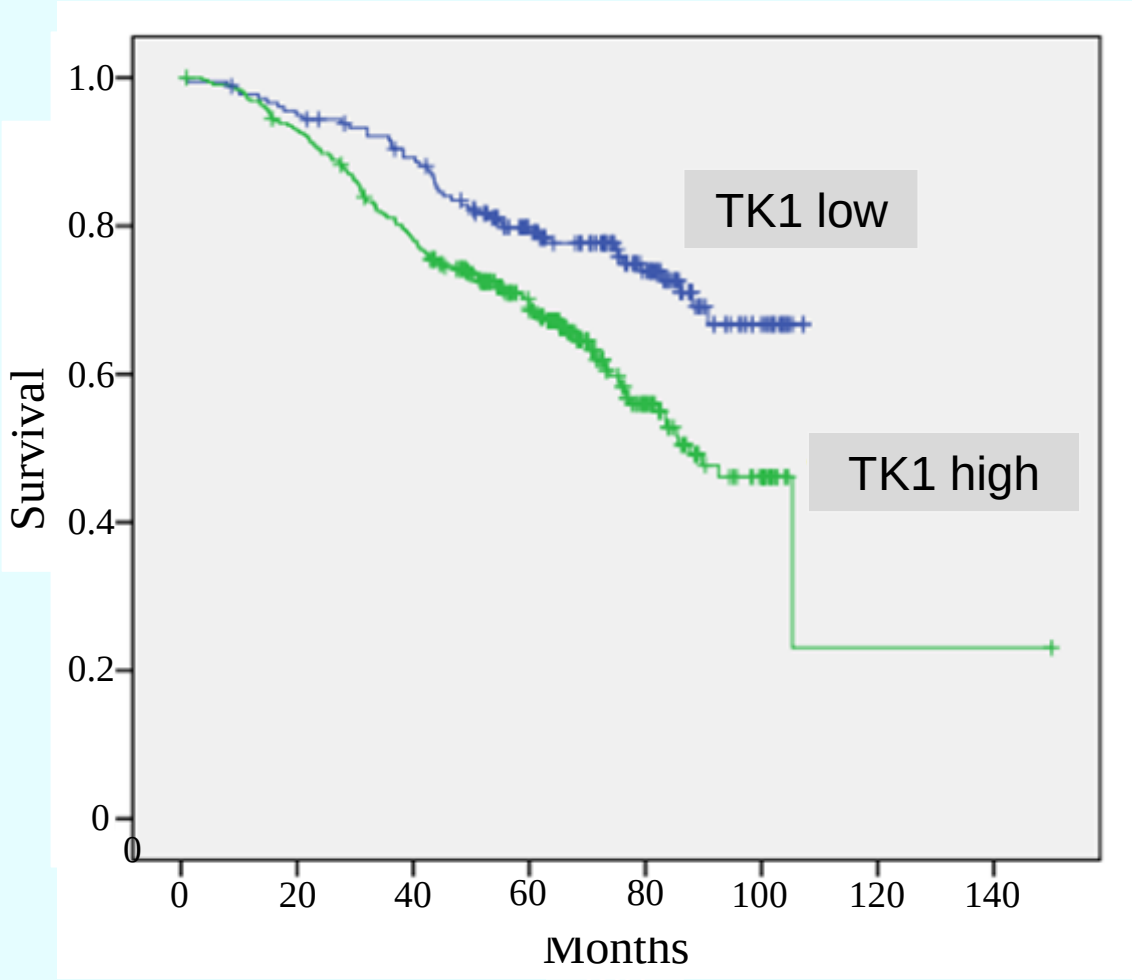
Similar results:
Breast cancer survival
Hodgkin disease
Prostate cancer

Colorectal, survival

504 patients



STK1

1. Prognosis of patient survival: High or low levels before treatment

2. Early diagnosis of Relapse: Increase level after treatment indicate relapse

3.Results of treatment: Decreasing level after treatment

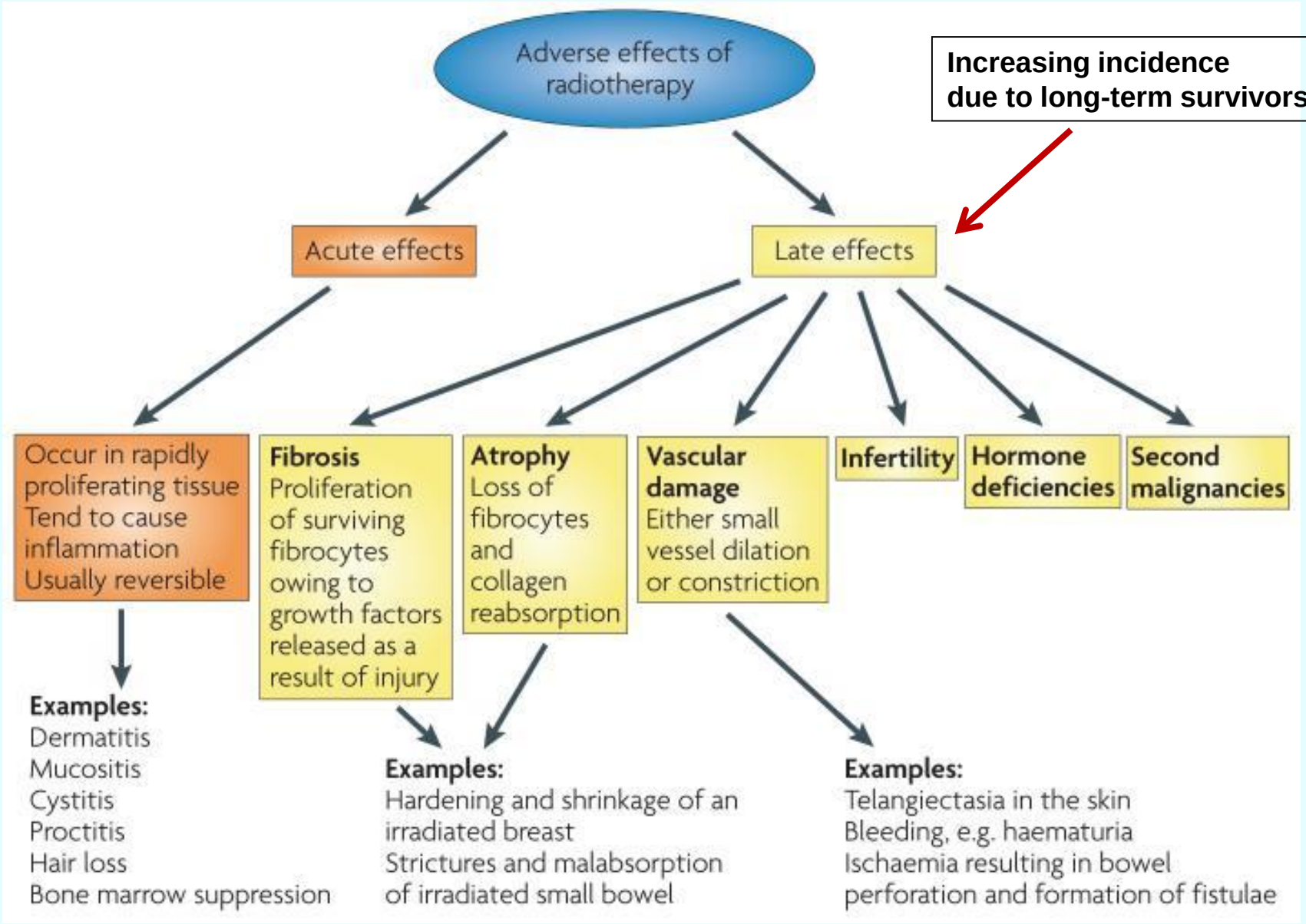
4.Health screening of pre-tumor and early small tumor

(low TK1 value, good prognosis)

Ongoing experiments to find biomarker of individual radiosensitivity: 1999-

7 PhD students
20 Master students
3 Post docs
35 Publications

Healthy tissue reaction in radiotherapy



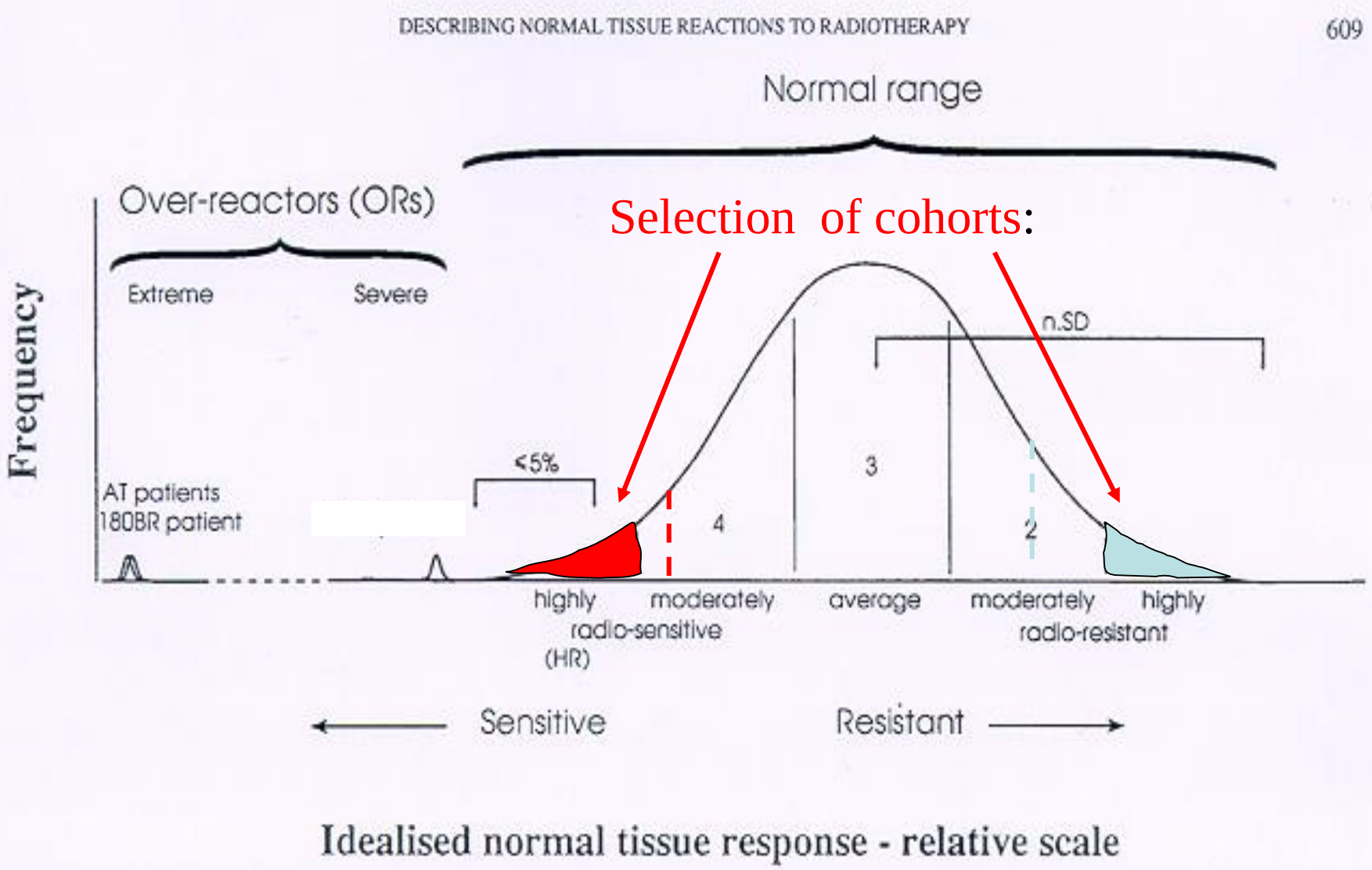
Bernett, G, et. al. 2009

Factors influenzing side effects

- Dose per fraction/dose rate
- Total dose
- Target volume and irradiated organ
- Radiation quality

- Life style …
- Health status
- Individual radiosensitivity: genetic background

Normal distribution of tissue reactions to radiotherapy



Burnet et al, Int. J. Cancer, 1998, 79, 606

Radiation sensitivity

- About 60 percent of all cancer patients receive radiotherapy
- Approximately 20% of RT patients experience **adverse effects** and 3-5% experience **severe adverse effects** are accepted
- The dose is adjusted to the most sensitive individuals

Longterm aim:

To have predictive assay to be able to distinguish between extreme sensitive and normal senstive patients perior radiotherapy

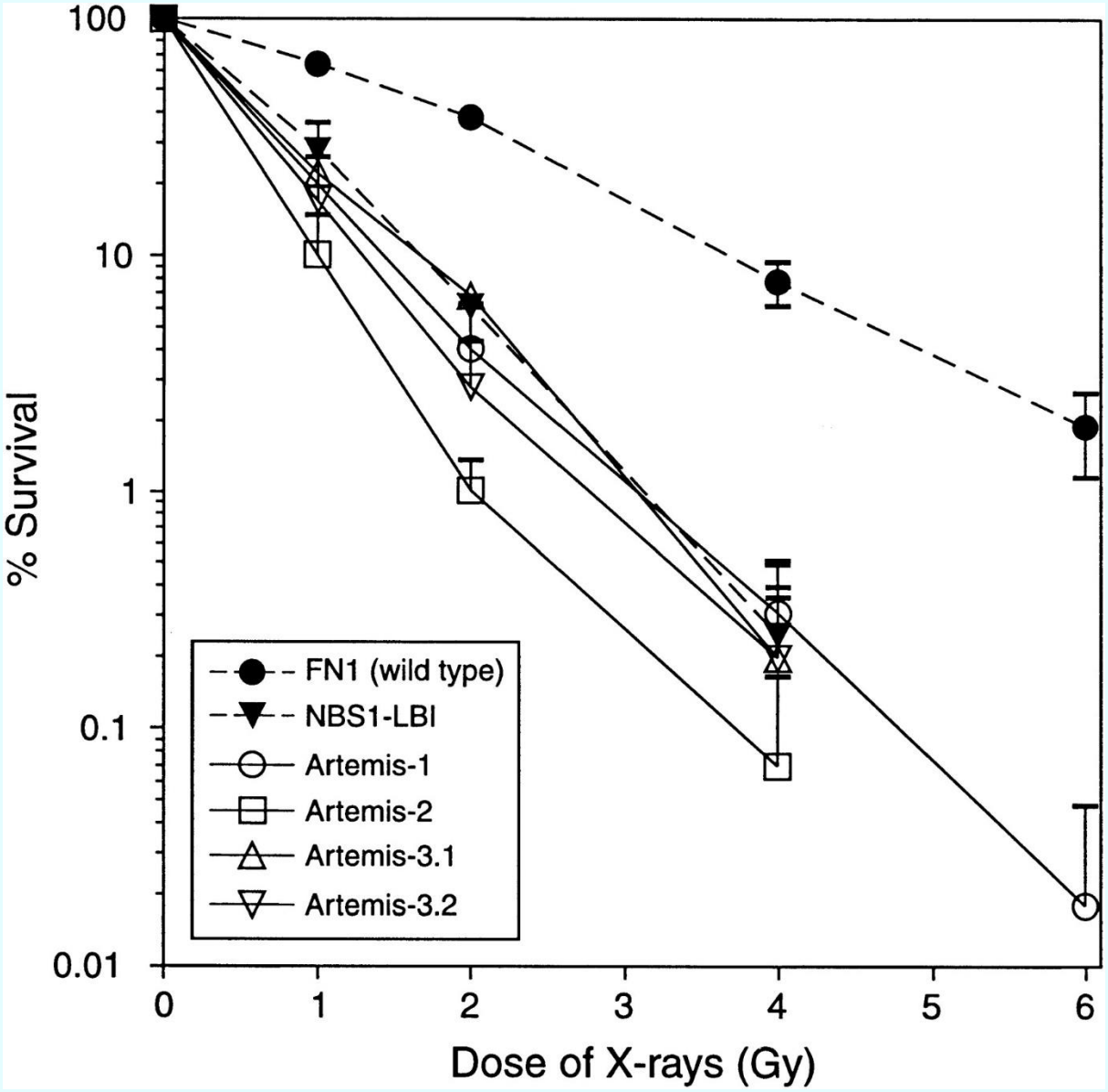
Focus is healthy tissue side effect as dose limiting factor

Nomber of DNA damade per Gy/cell

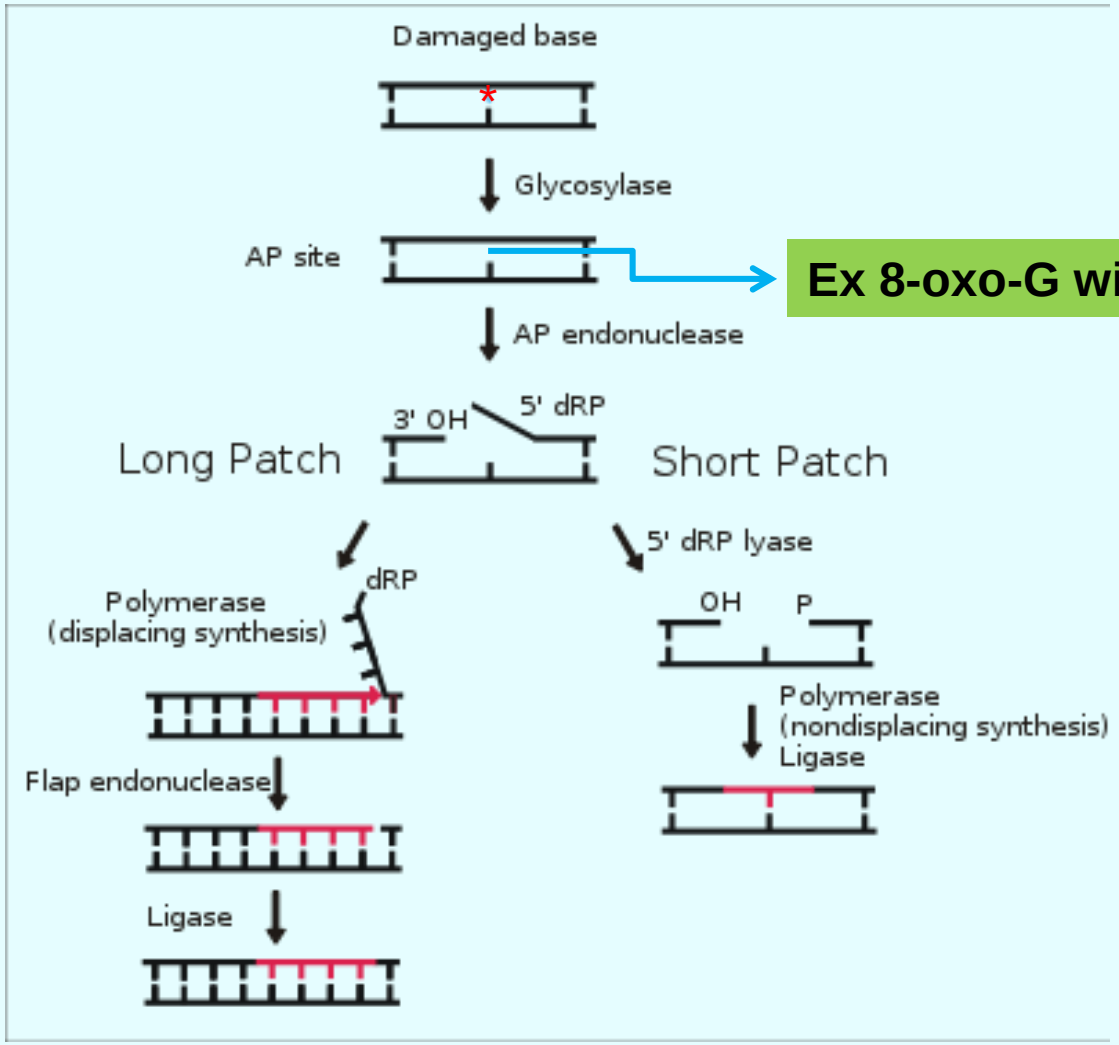
1 Gy photon

- ~ 20-40 DSB
- ~1000 SSB
- ~2000 Base damages

Radiosensitivty of DNA repair deficient cells



Base exicision repair pathway



Ex 8-oxo-G with hOGG1

Out from body via urine and saliva

* Damaged base

Hypothsis

Normo-sensensitive patients has better DNA repair, BER, capacity.

If 2 individual with different DNA repair capacities receive same dose:

Lower 8-oxo-dG should be detected in radiosensitive patients after irradiation as compared to normo-sensitive individual

Can urinary 8-oxo-dG be used as a predictor for individual radiosensitivity?

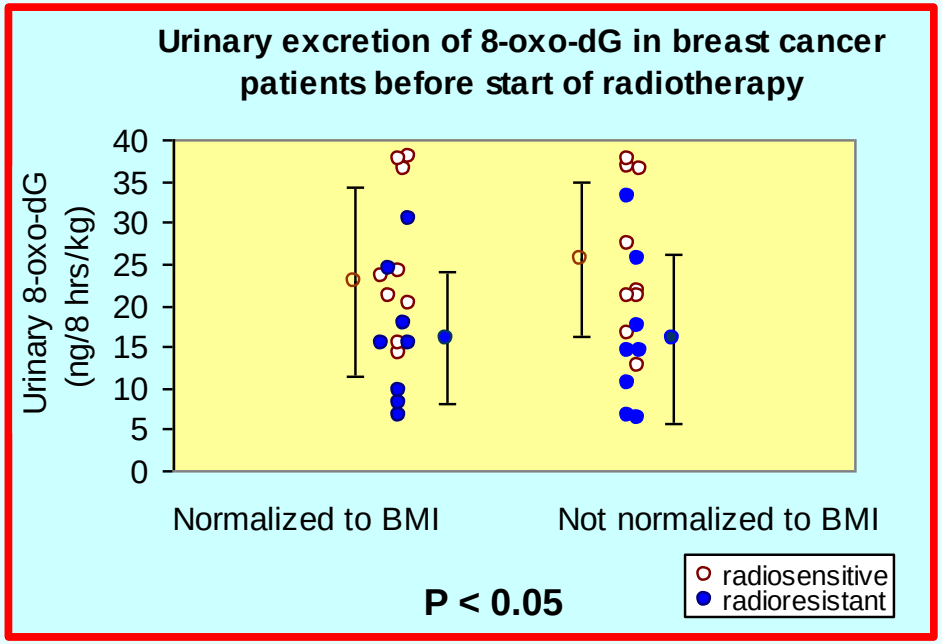
Haghdoost s. et al Intl. J. of Radiation Oncology, Biology, Physics,

- **Patients**

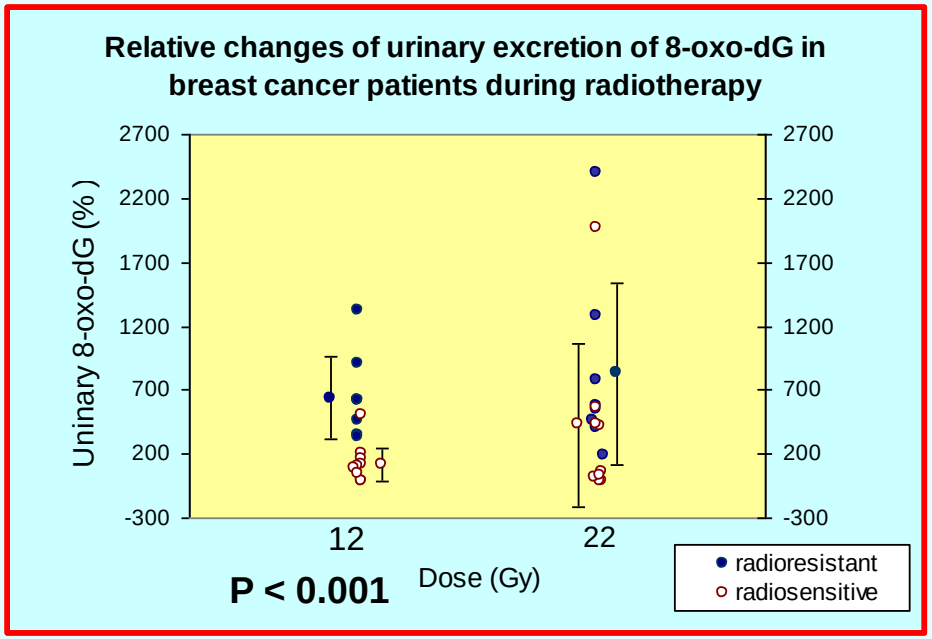
  17 breast cancer patients, radiotherapy after surgery (46 Gy with 2 Gy fractions) to breast and regional lymph nodes

Measured urinary 8-oxo-dG by HPLC-EC before and during radiotherapy

Radiosensitive group:
High background levels and low therapy related increase of urinary 8-oxo-dG



Non-sensitive group:
Low background levels and high therapy related increase of urinary 8-oxo-dG



Intl. J. of Radiation Oncology, Biology, Physics, 2001

Next step: in vitro test

Gamma Irradiation

1 hour incubation for repair

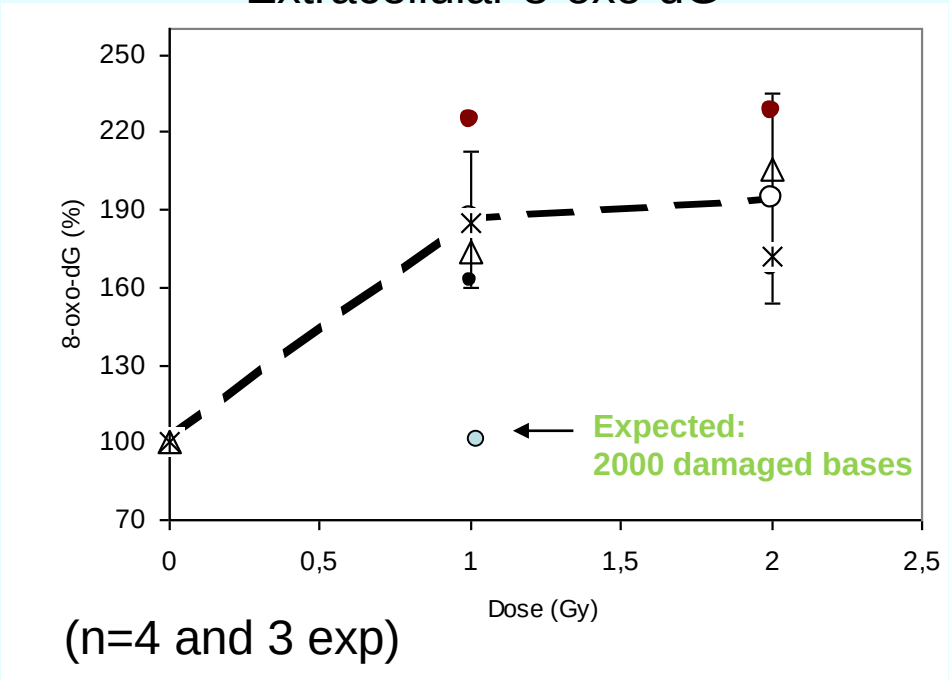
Detection of oxidized DNA base in serum

Extracellular 8-oxo-dG as a sensitive marker for oxidative stress in vivo and in vitro

Haghdoost S. et al. Free Radic. Res. 2005

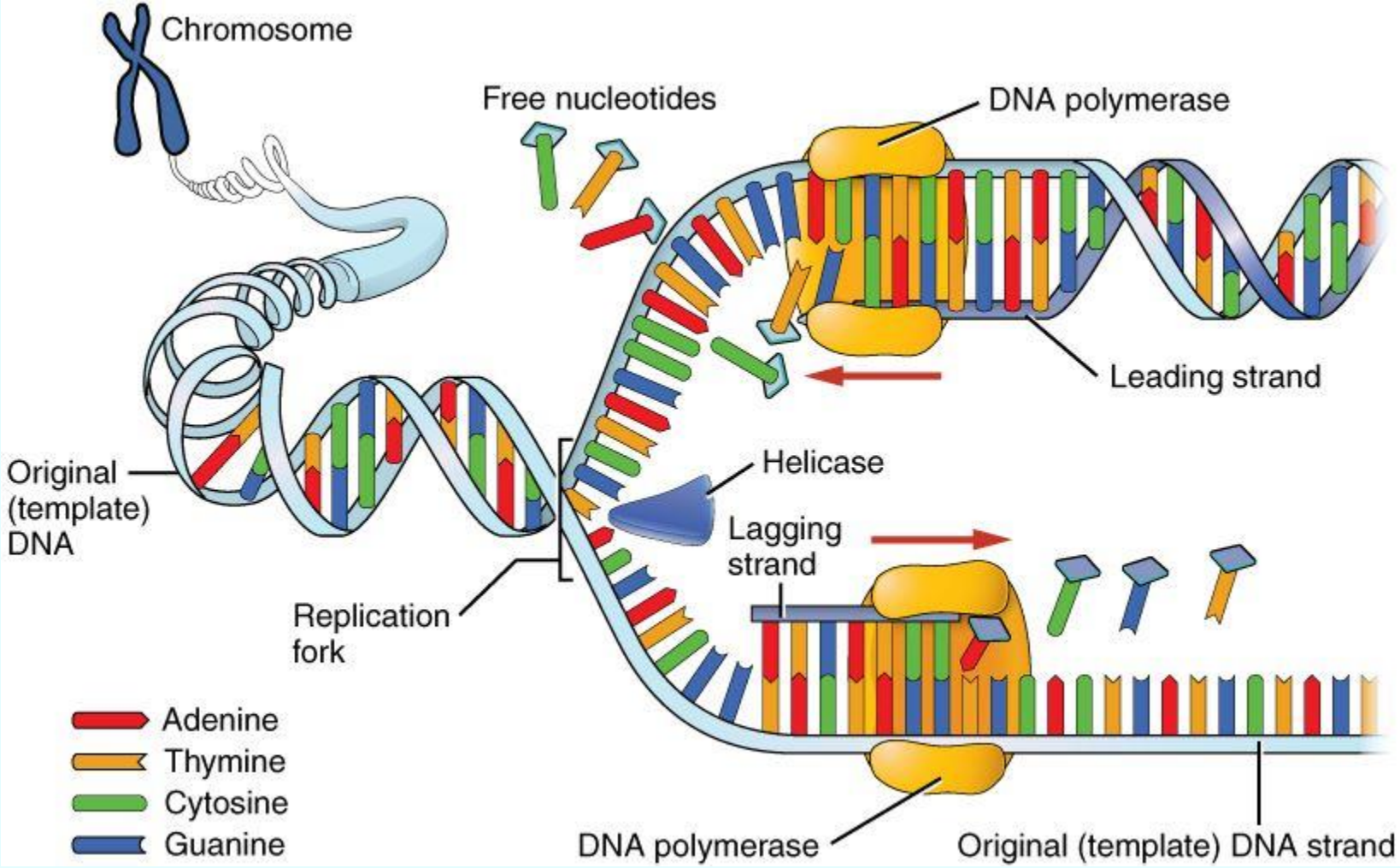
- Amount of 8-oxo-dG excreted by leukocytes, exposed to 1 Gy, is 35 times higher than what is expected to be formed in DNA.
- DNA is not the main source for extracellular 8-oxo-dG

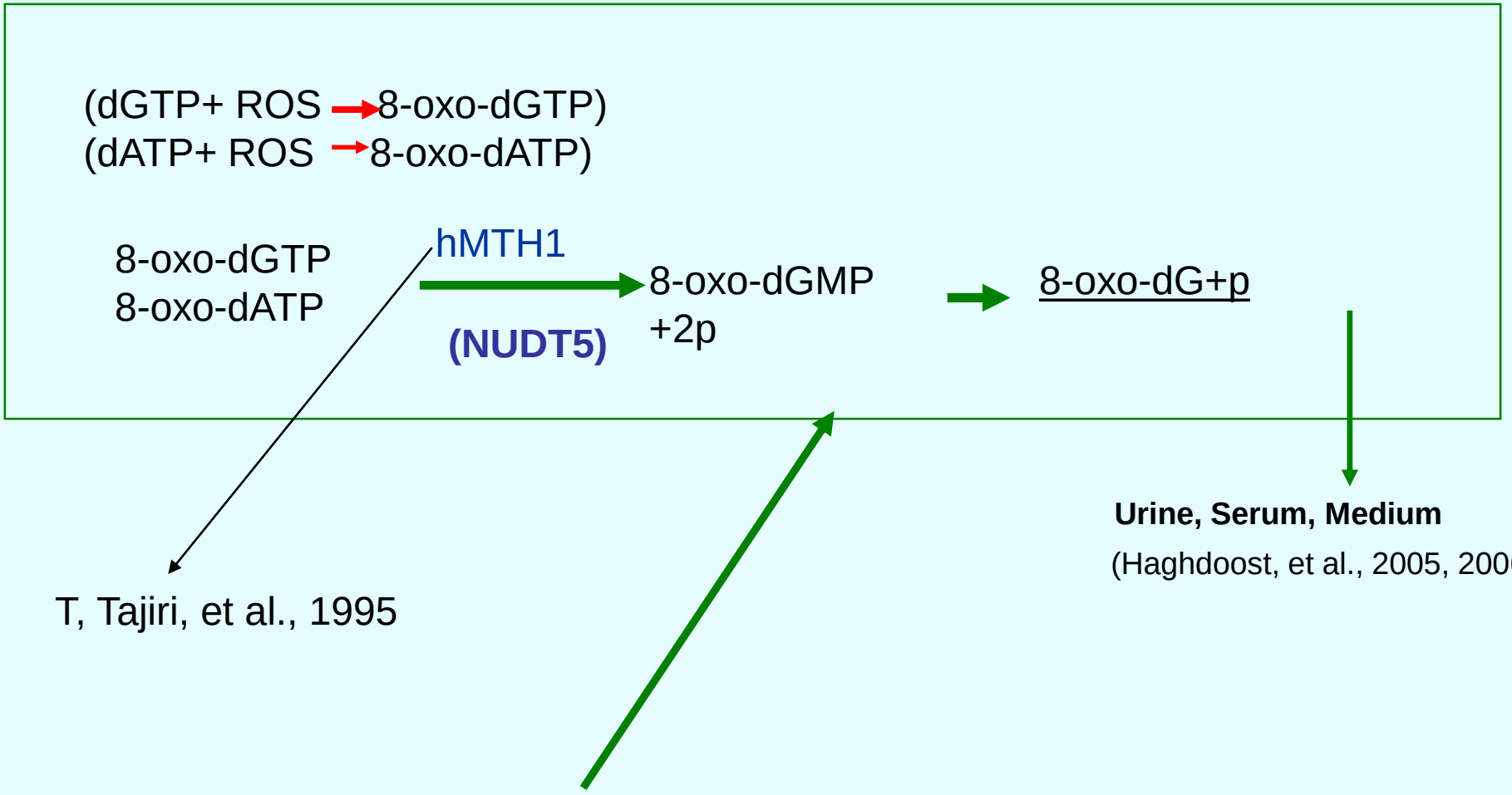
Extracellular 8-oxo-dG



(n=4 and 3 exp)

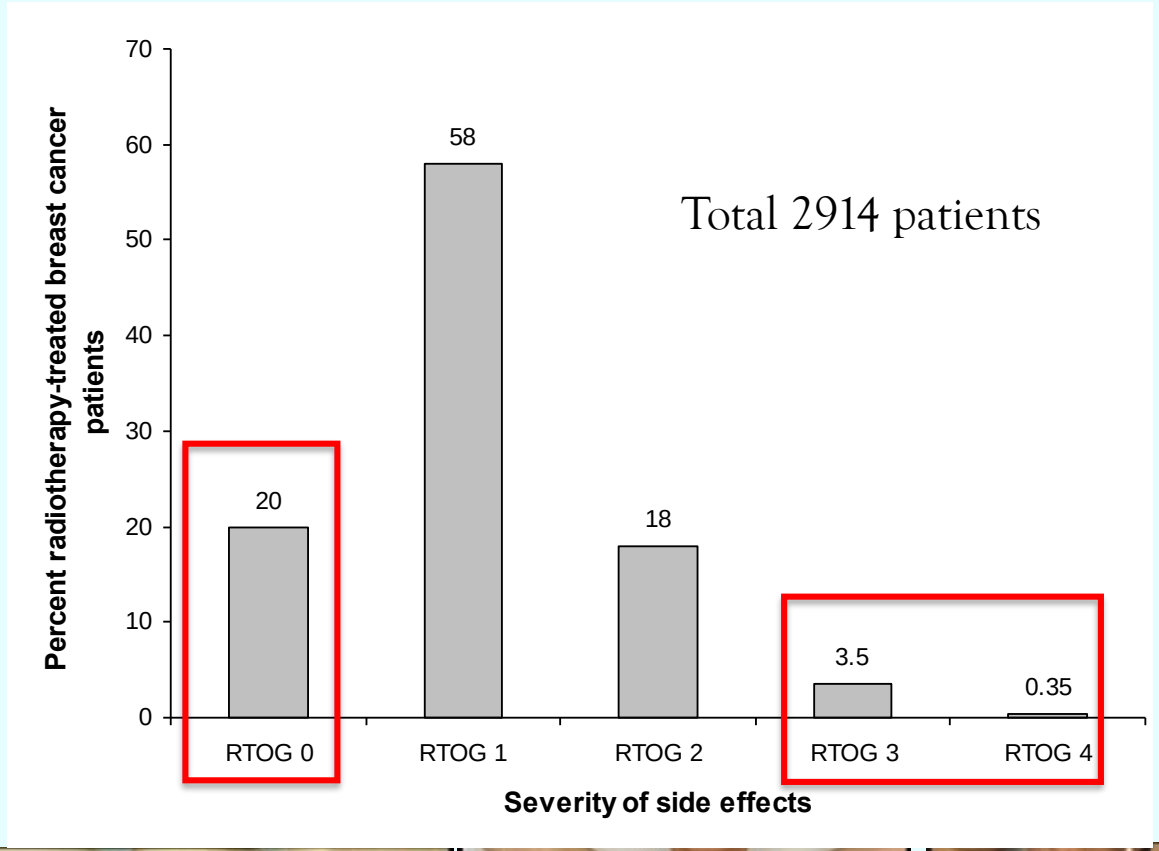
(***In vitro* study, HPLC**)


Chromosome
Free nucleotides
DNA polymerase
Leading strand
Original (template) DNA
Helicase
Lagging strand
Replication fork
Adenine
Thymine
Cytosine
Guanine
DNA polymerase
Original (template) DNA strand


(dGTP+ ROS → 8-oxo-dGTP)
(dATP+ ROS → 8-oxo-dATP)
8-oxo-dGTP
8-oxo-dATP
hMTH1
(NUDT5)
8-oxo-dGMP
+2p
8-oxo-dG+p
Urine, Serum, Medium
(Haghdoost, et al., 2005, 2006)
T, Tajiri, et al., 1995
Nucleotide pool cleaning up system

- Clinical relevans?

Retrospective breast cancer cohort



S Skiöld et.al. 2013 Mut. Res. 30; 756 (1-2): 152-7

0-1

2

3

4

Next step: In vitro test

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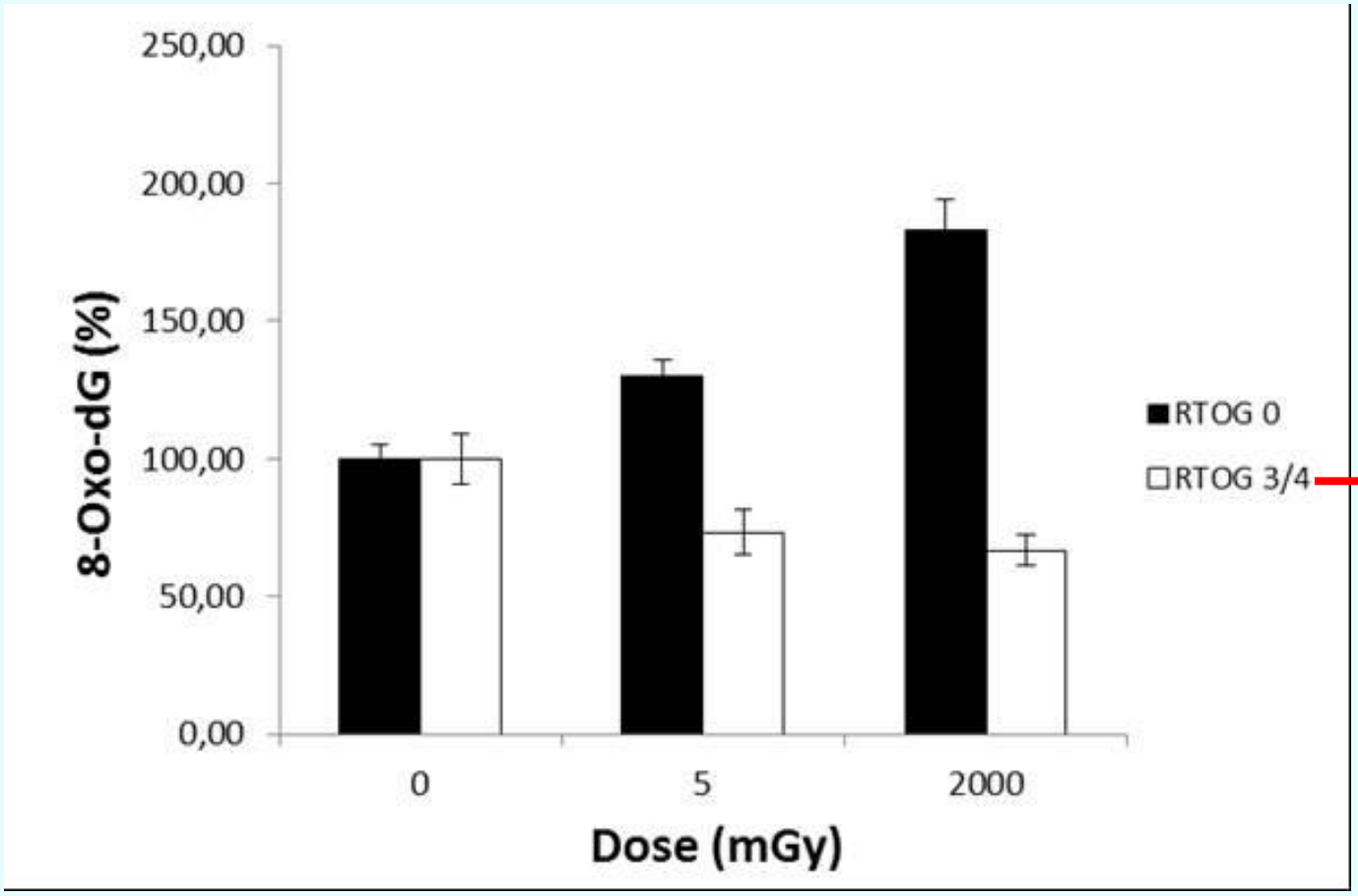
1 hour incubation for repair

Detection of oxidized DNA base in serum



Radiosensitive

Skiöld, S. et. al. Mut. Res. 2013.

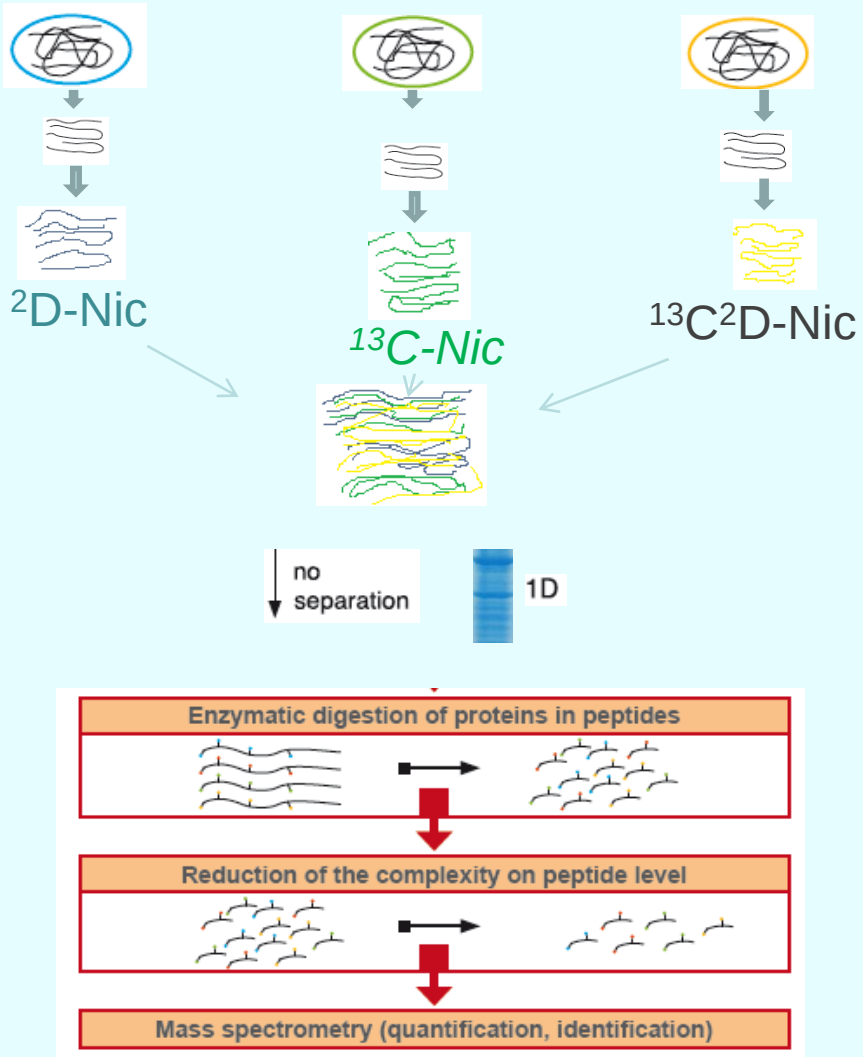
Discovery of mechanims: stable isotope labeling in combination with mass spectrometry

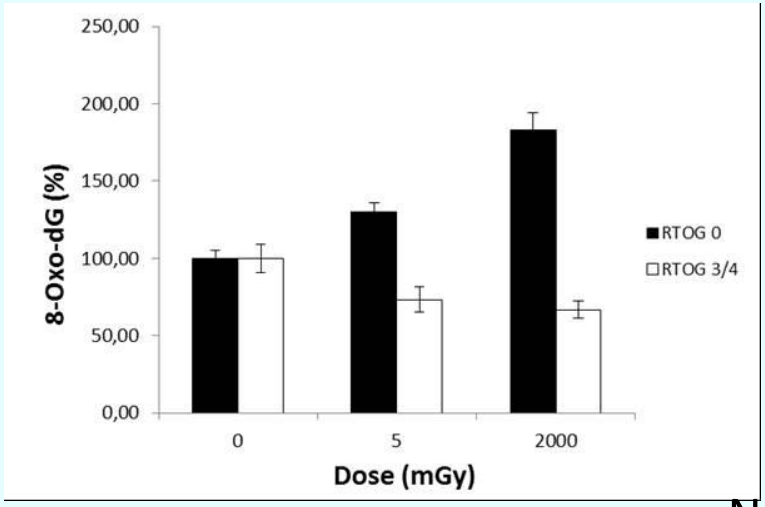
Denaturation

ICPL labelling

Combining

Reduction of complexity/ protein level





Normosensitive patients

Radiosensitive patients

Chages of
8-oxo-dG level

Steady-state levels

SOD1
PARK7
PRDX2

BLVRB
PRDX2

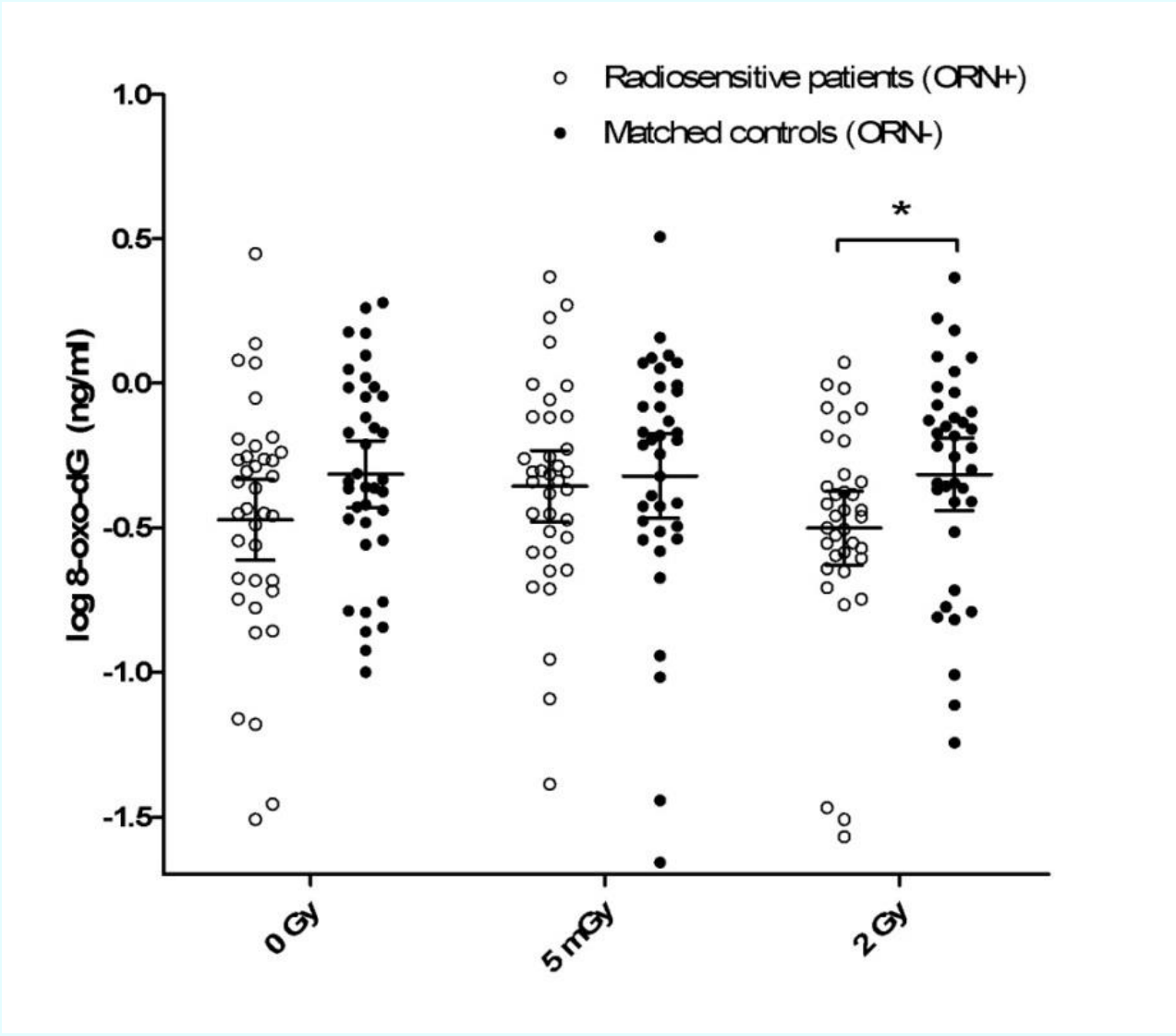
Head and neck cancer cohort

The incidence for ORN is ~5-8%

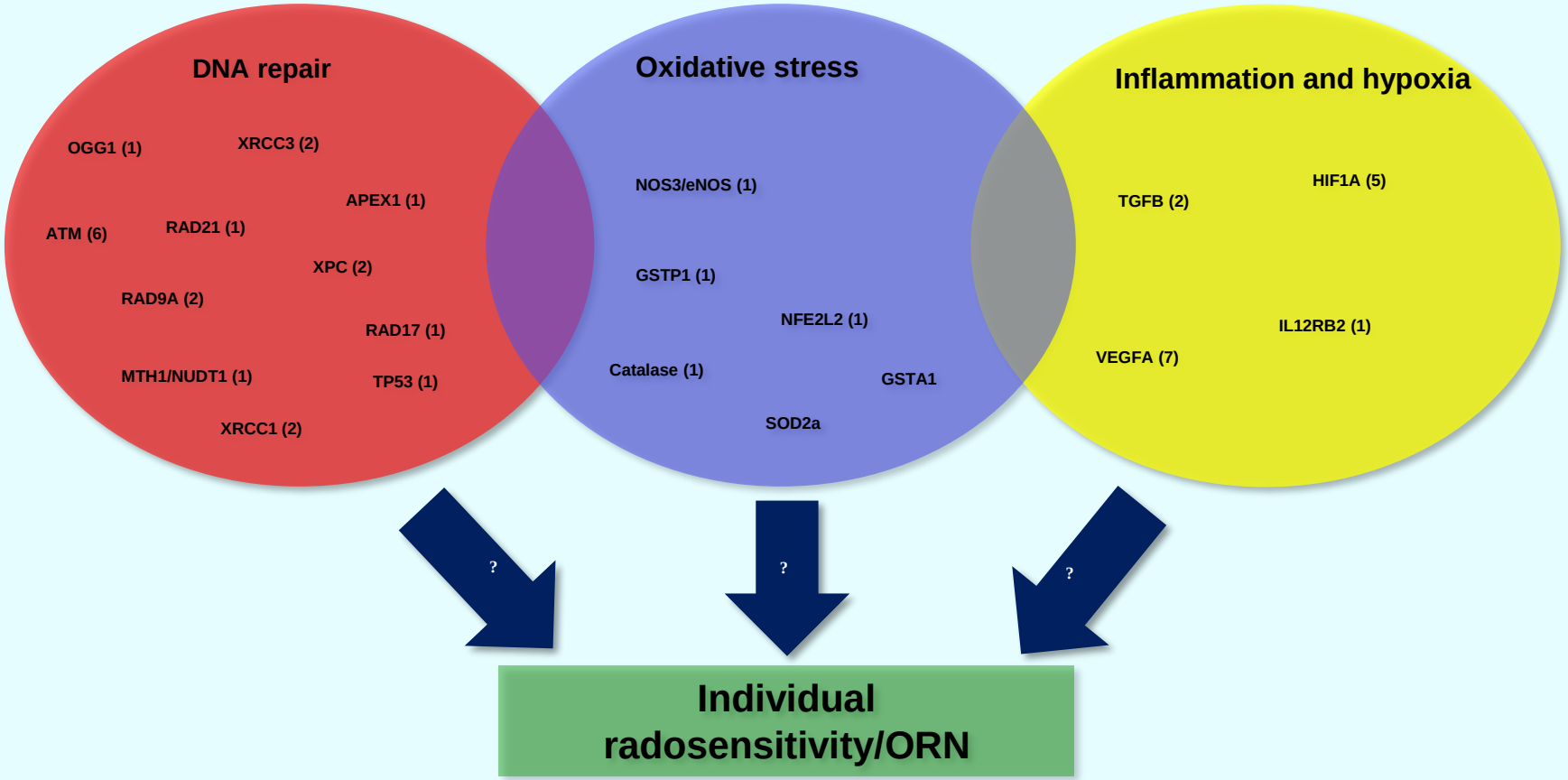
It is a late adverse effect to radiotherapy occurring 1-10 years after the end of the treatment.

Available:

37 patients with osteoradionecrosis (ORN) and 37 matched controls.

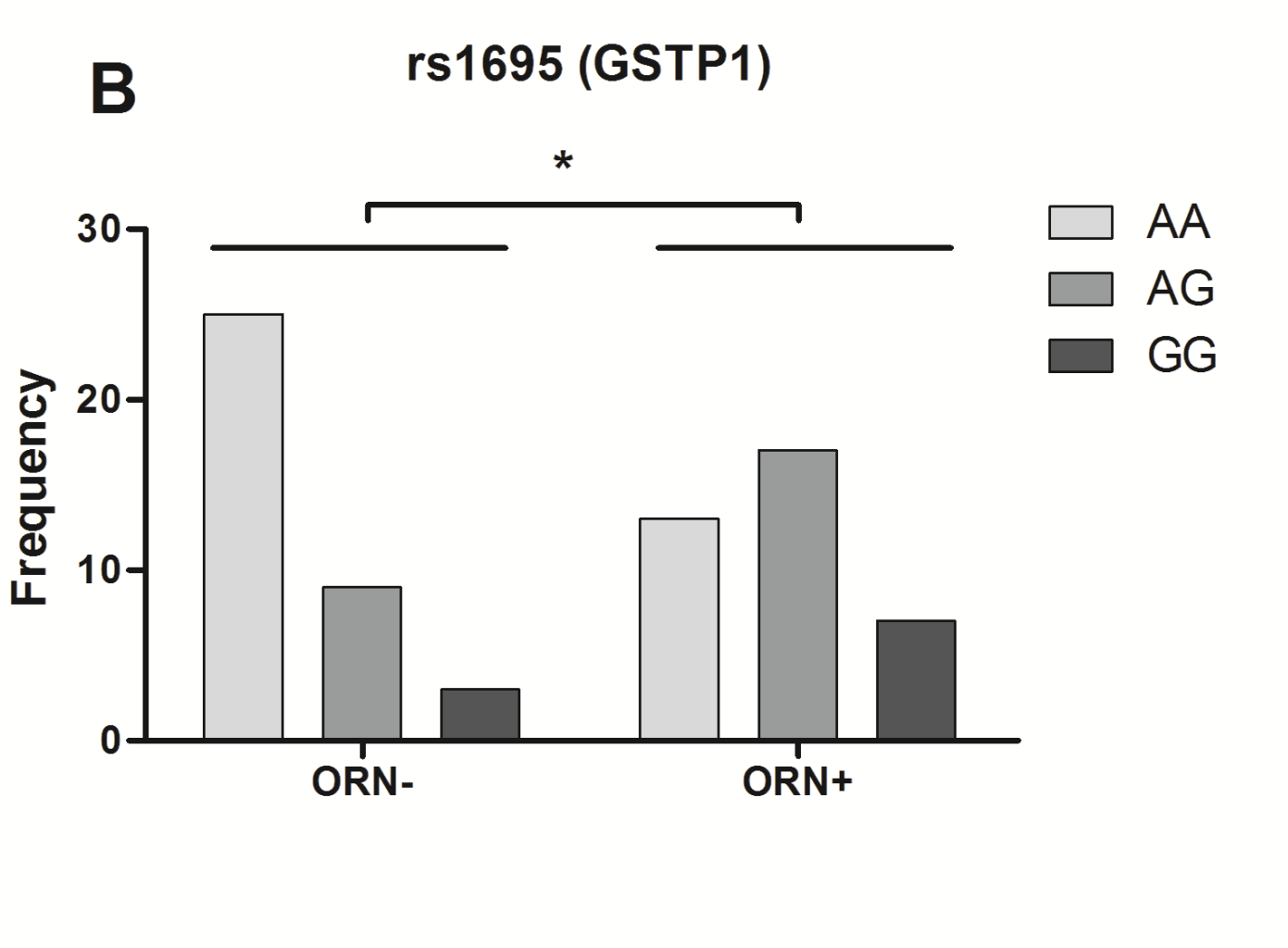


Danielsson, D. et. al. 2014, head and neck



Investigating 58 point mutations (SNPs) previously implicated in side effects to RT

Raw data just now sent from core facility, analysis soon to be initiated



Danielsson, D. et. al. 2014, head and neck

Model for predicting ORN

Predicted logit of (ORN) = 0.14 + (1.21*"brachytherapy") + (-1.90*"8-oxo-dG 2 Gy") + (1.31* "rs1695").

"brachytherapy" takes value 1 if the patient received/is planned to receive that treatment.

"8-Oxo-dG 2 Gy" is the level of 8-oxo-dG (ng/ml) in the blood serum 60 min after a 2 Gy *in vitro* radiation exposure of whole blood.

Variable "rs1695" takes value 1 if the patient is heterozygous/homozygous for the SNP in GSTP1.

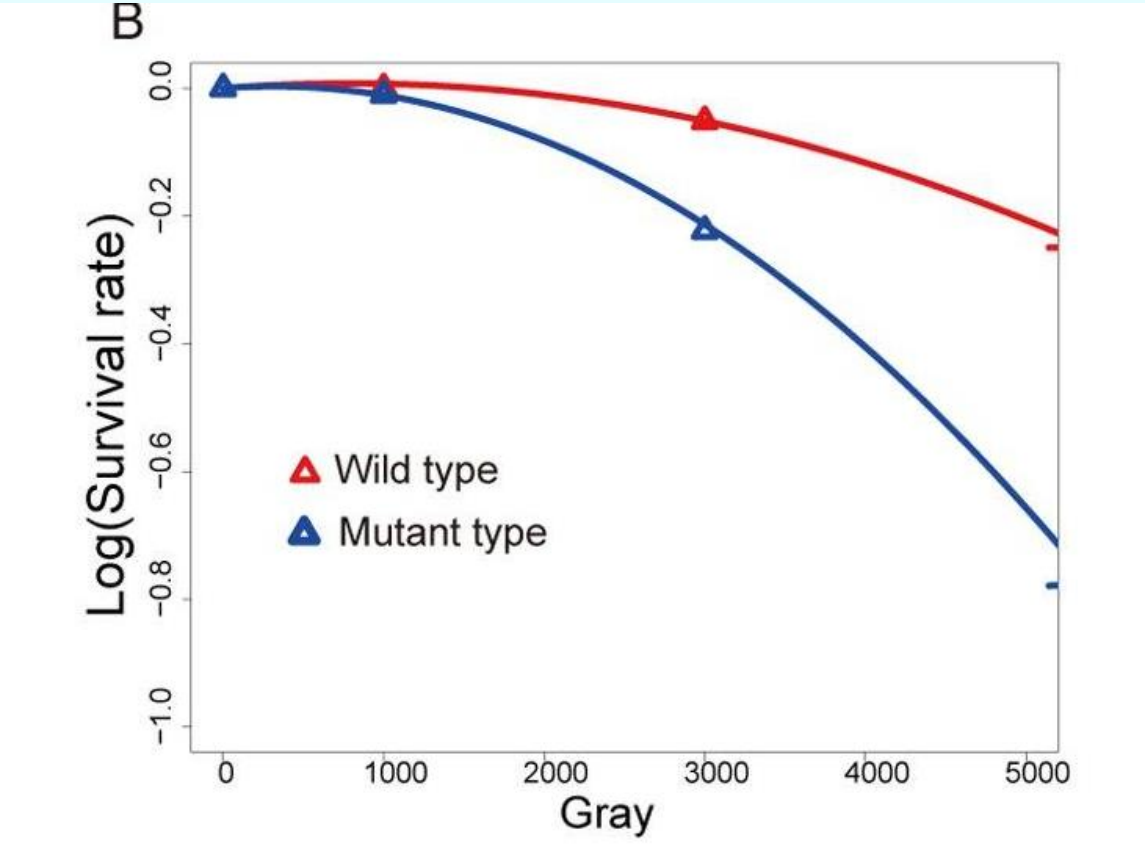
Conclussion

Oxidative stress response is related to clinical radiosensitivity:

- 8-oxo-dG levels
- protemic approache
- SNP in GSTP1

Indication that oxidative stress is an general factor influencing clinical radiosensitivity

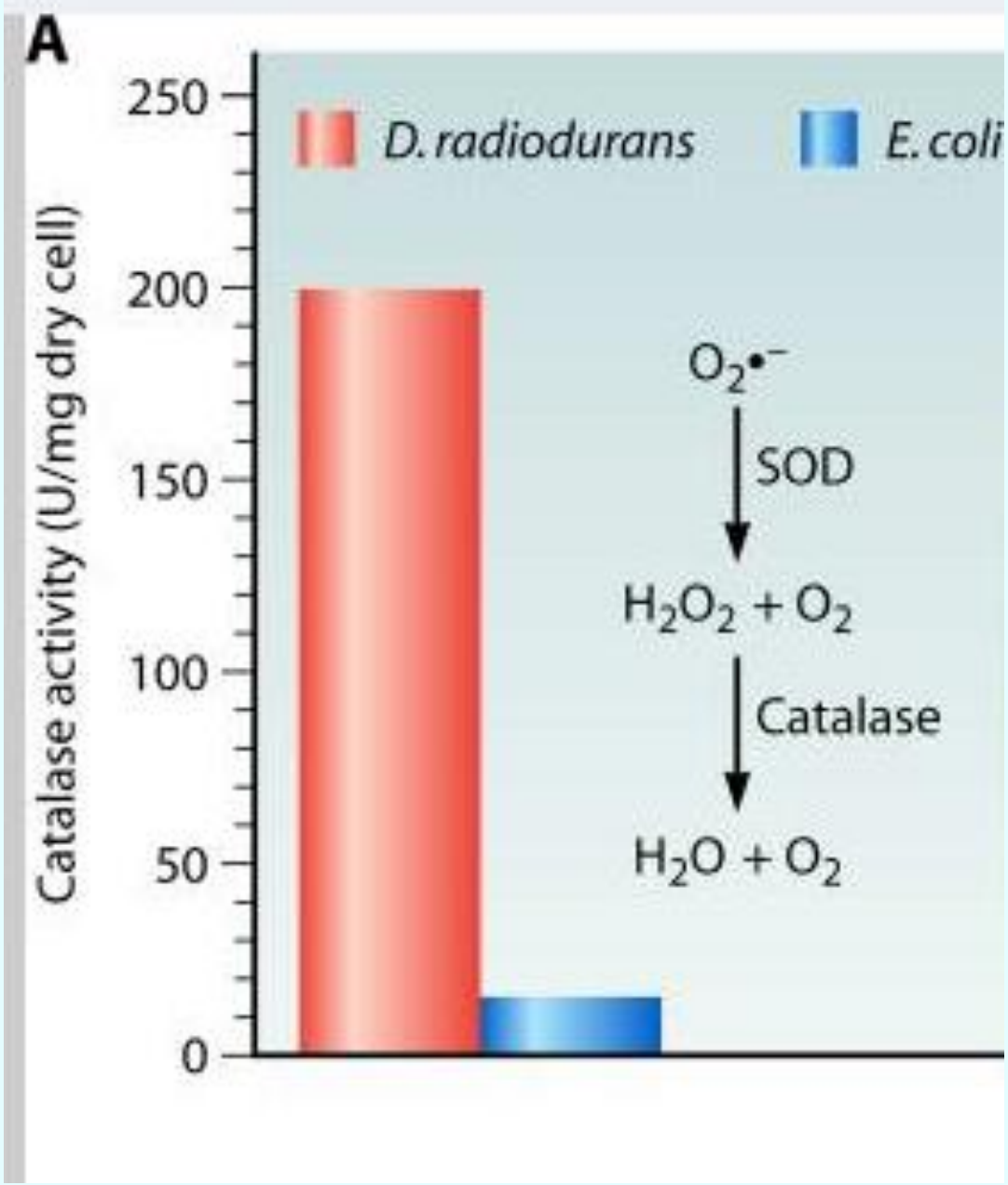
Effects of antioxidant in radiosensitivity



*crt*B mutant: *crt*B gene is involved in carotenoid biosynthesis

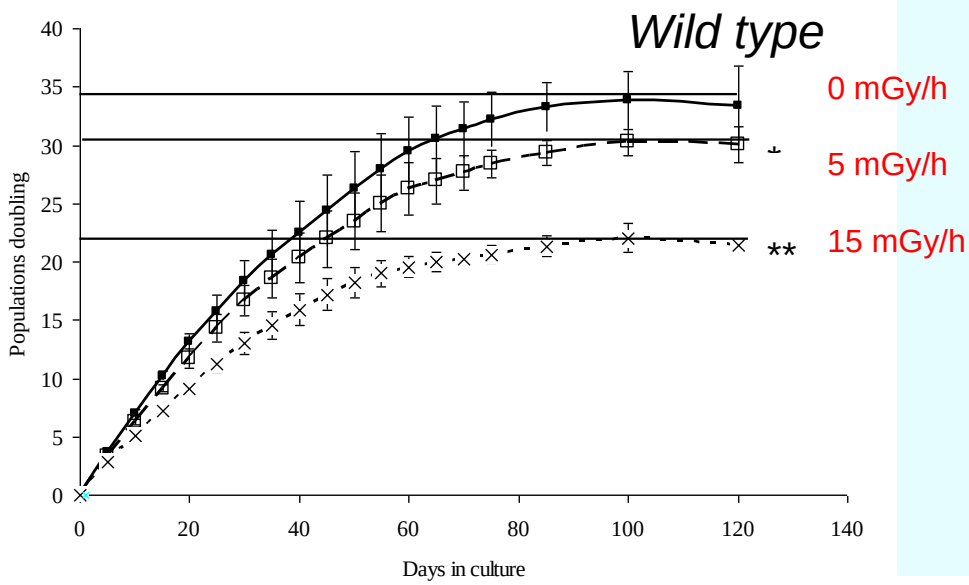
The *crt*B mutant

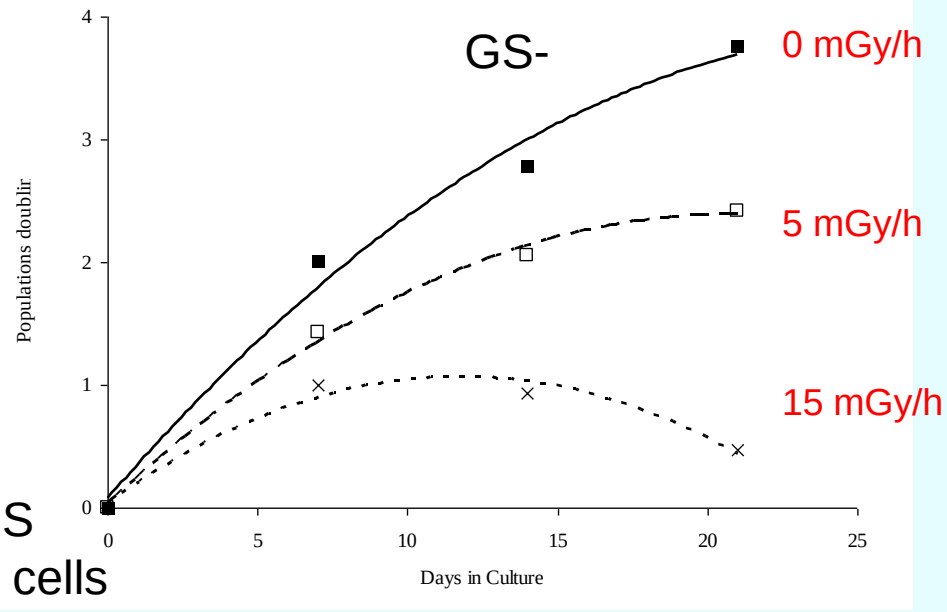
Dea Slade, et al., 2011



Dea Slade, et al., 2011

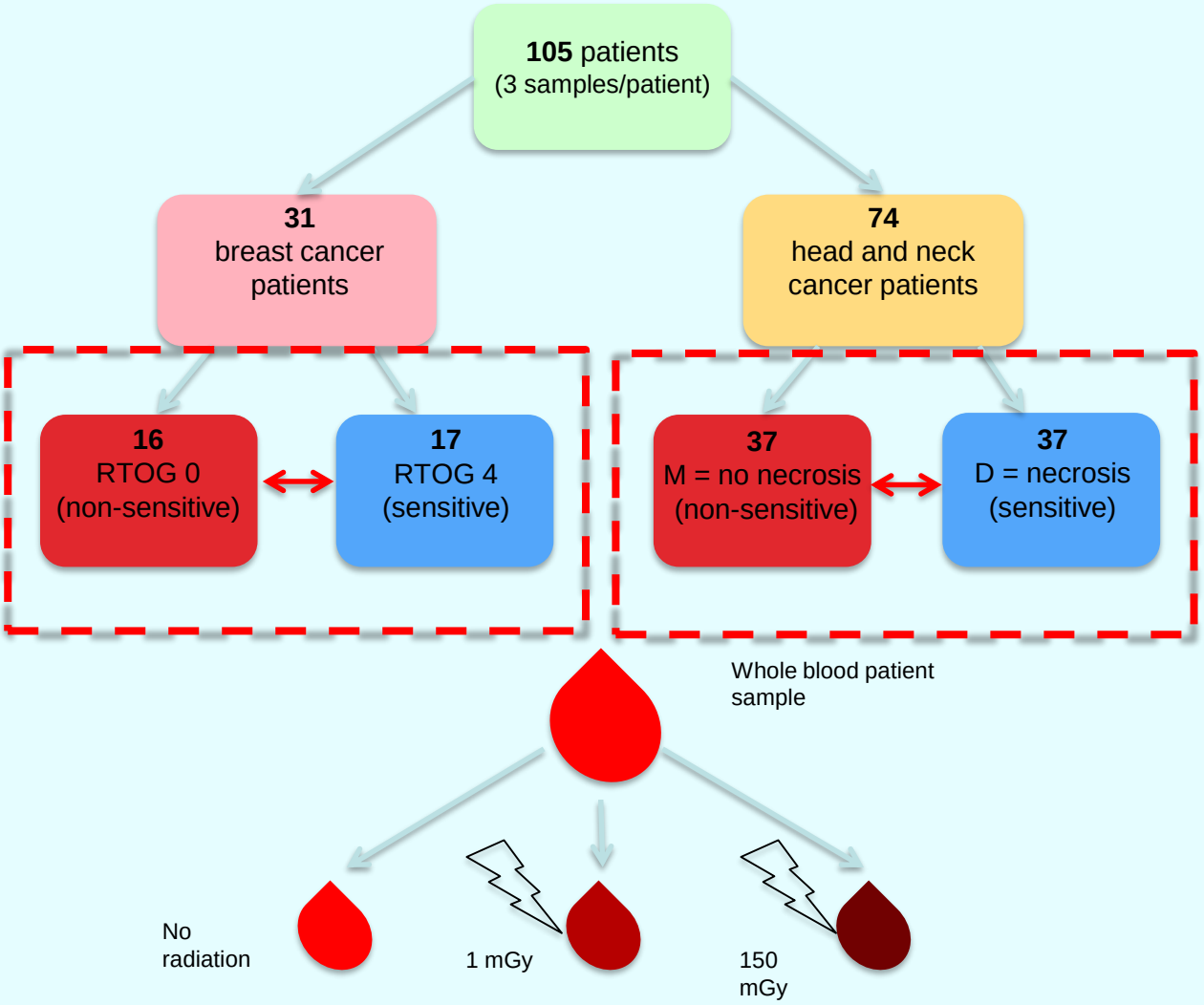
Growth rate of fibroblast with low levels of glutathione synthetase activity under chronic irradiation





The GS fibroblasts have 15% of the GS activity left as compared to the control cells

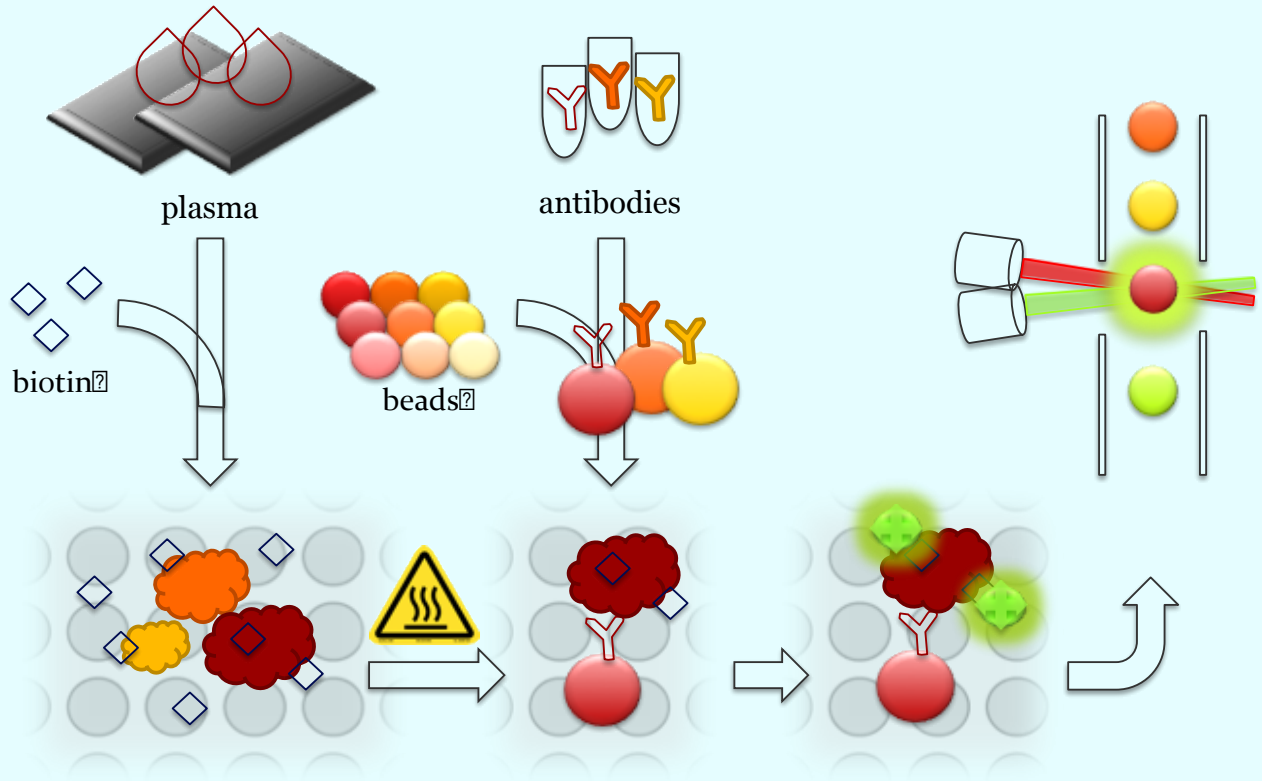
Plasma protein profiling to find common mechanisms



Experimental workflow – Discovery
Antibody suspension bead array

384 proteins can be analysed in 50 ul plasma

Beads are colour coded and connected to particular antibody



Study design: 315 samples

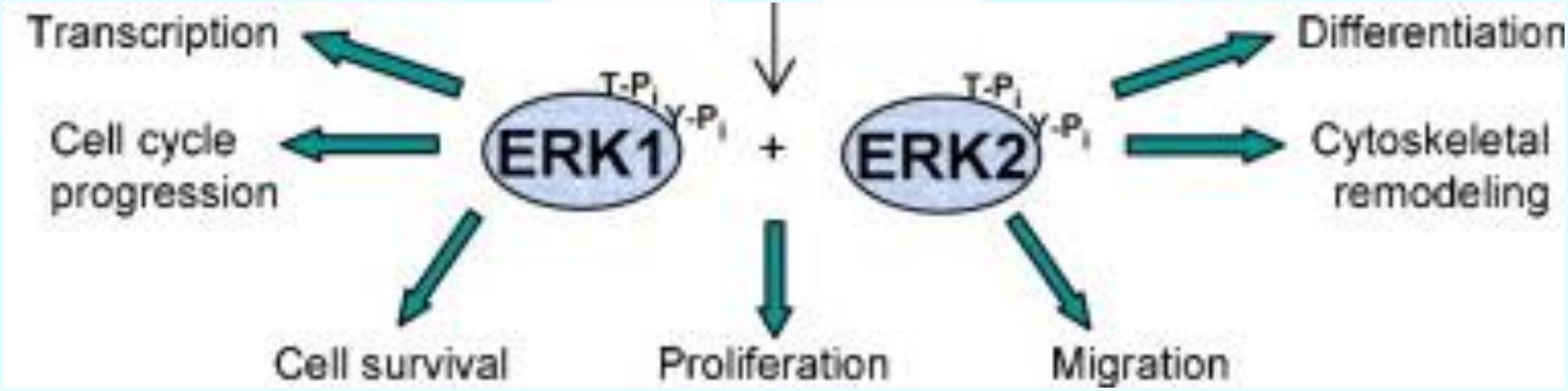
Protein targets

- 202 proteins (chosen from previous studies and from literature)
- Covered by 259 antibodies

Found almost 40 top candidate proteins

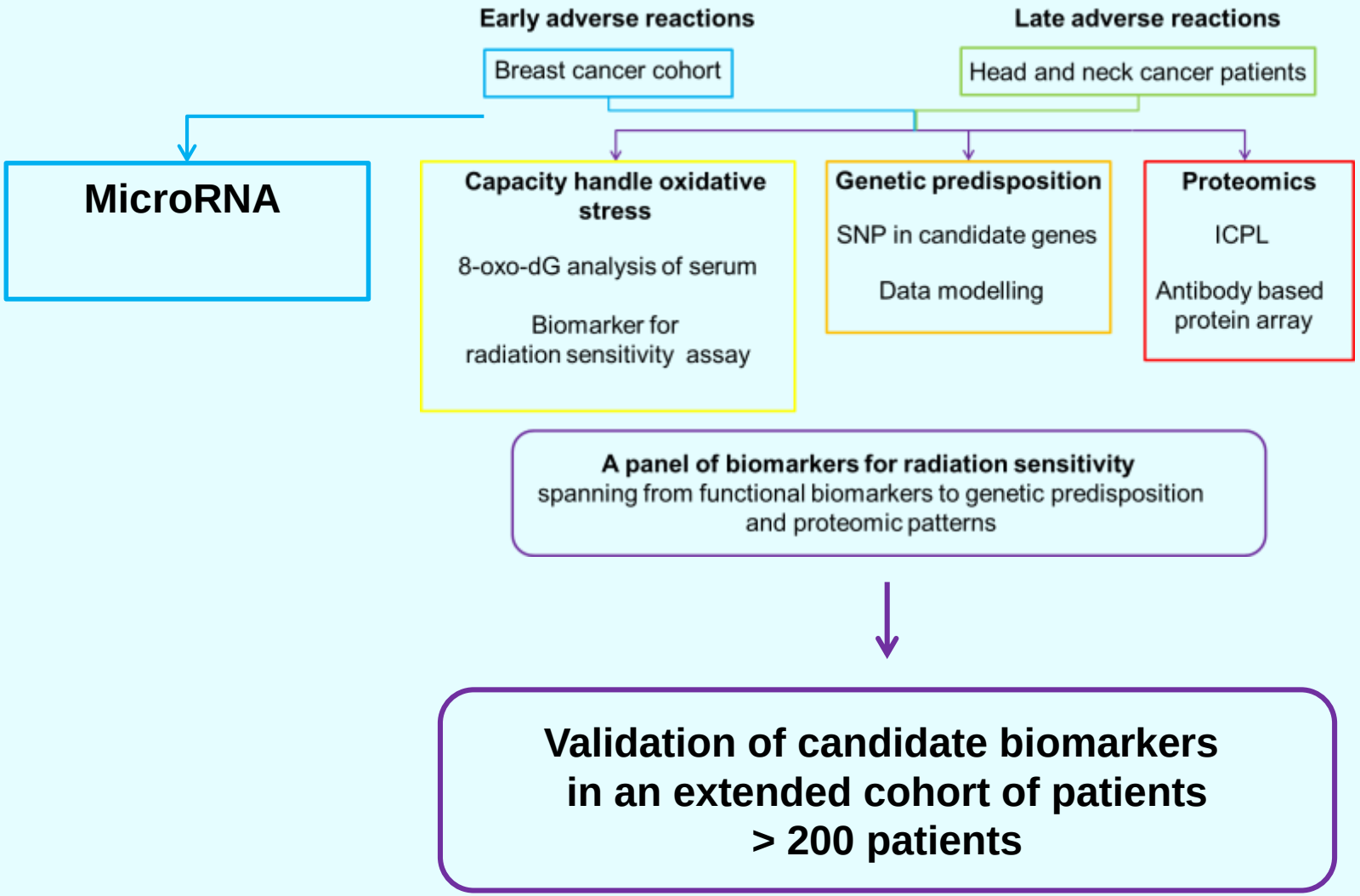
Ongoing evaluation:

Biological Process (GO)
pathway description
blood coagulation
wound healing
positive regulation of ERK1 and ERK2 cascade


Transcription
Cell cycle progression
Cell survival
T-P
Y-P
ERK1
+
Proliferation
ERK2
Differentiation
Cytoskeletal remodeling
Migration

Retrospective cohorts

Example of experimental design: Saliva, Plasma proteins

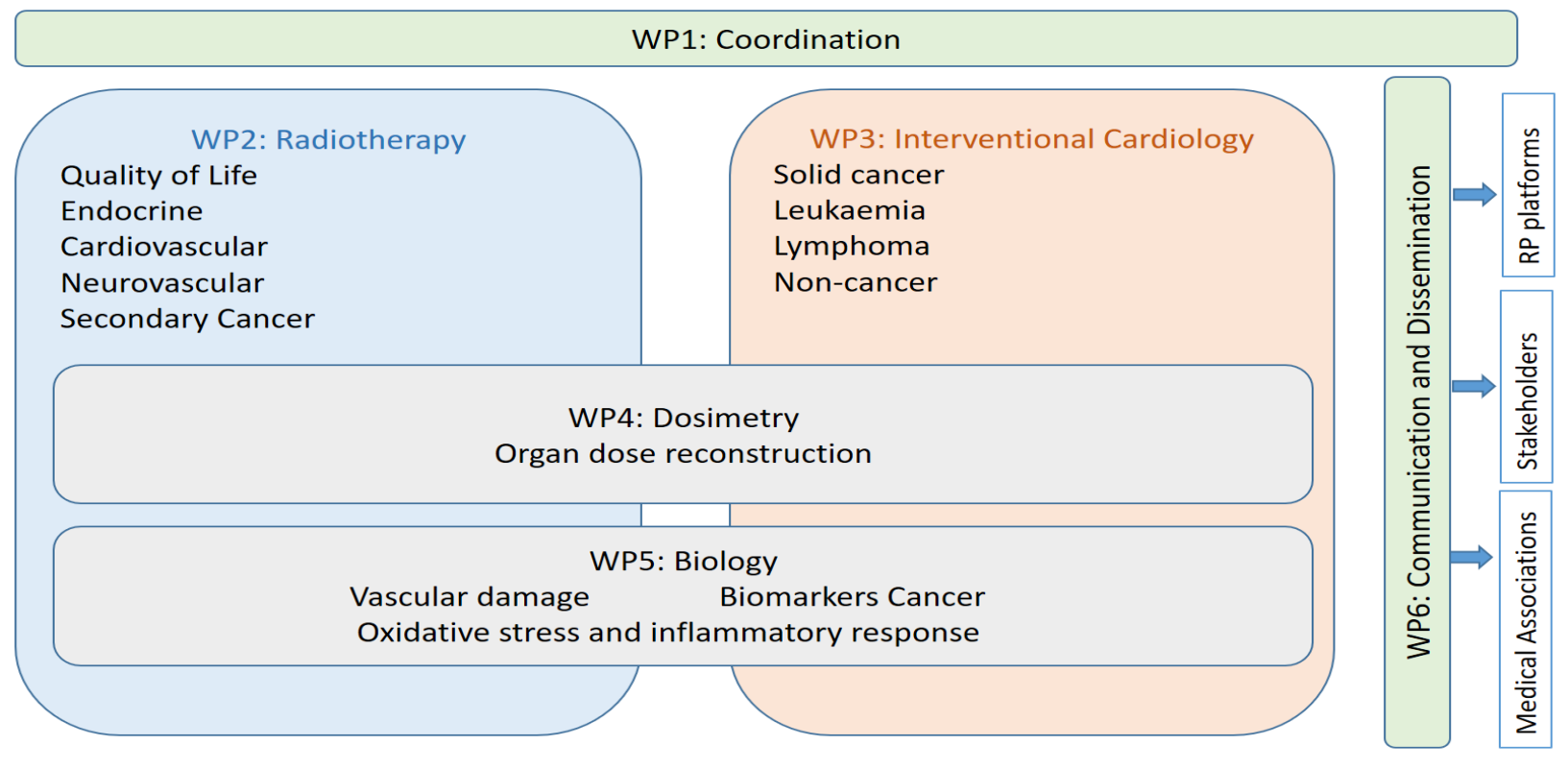


Health effects of cArdiac fluoRoscopy and **MOderN radIotherapy in paediatrics**

Coordinator: IsoGlobal, total budget 7 million Euro

Objectives

The HARMONIC project aims at improving the understanding of the biological and health effects of medical ionising radiation exposure of **paediatric patients**.

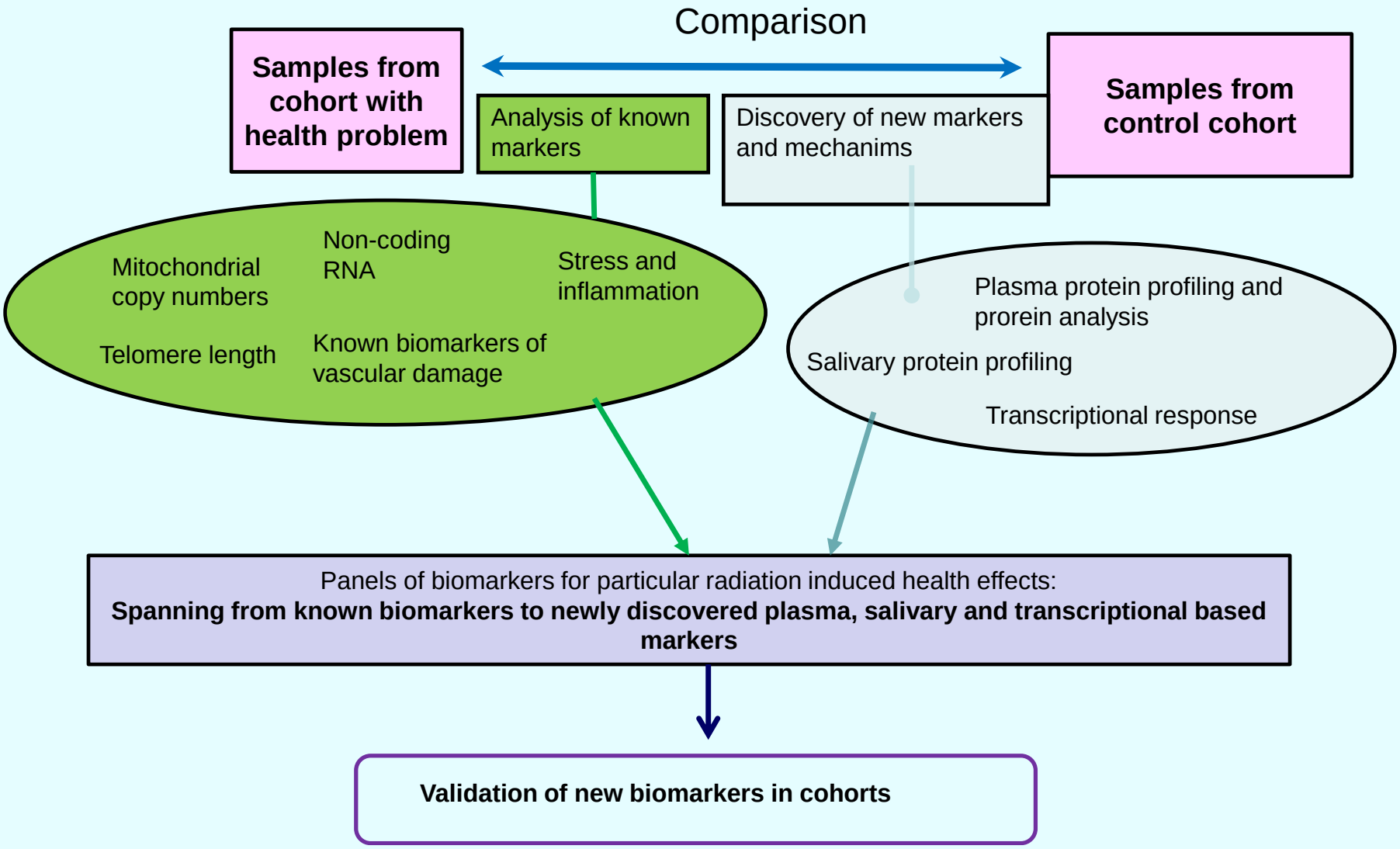


WP5-Biology: Unicaen/CIMAP (France), SU (Sweden), IFC-CNR (Italy) and GR (France)

Objective:

To investigate mechanisms and identify potential biomarkers that can be used

- For individualized therapy or providing rationale for selection of optimal diagnostic/therapeutic methods.
- Focus will be on oncogenic processes and vascular diseases. For molecular epidemiology to refine risk estimates for adverse health effects/disorders


Comparison
Samples from cohort with health problem
Samples from control cohort
Analysis of known markers
Discovery of new markers and mechanims
Mitochondrial copy numbers
Non-coding RNA
Stress and inflammation
Telomere length
Known biomarkers of vascular damage
Plasma protein profiling and prorein analysis
Salivary protein profiling
Transcriptional response
Panels of biomarkers for particular radiation induced health effects:
Spanning from known biomarkers to newly discovered plasma, salivary and transcriptional based markers
Validation of new biomarkers in cohorts

- Thank you for attention