„Modern Radiobiology“
State of the Art and Future Perspectives

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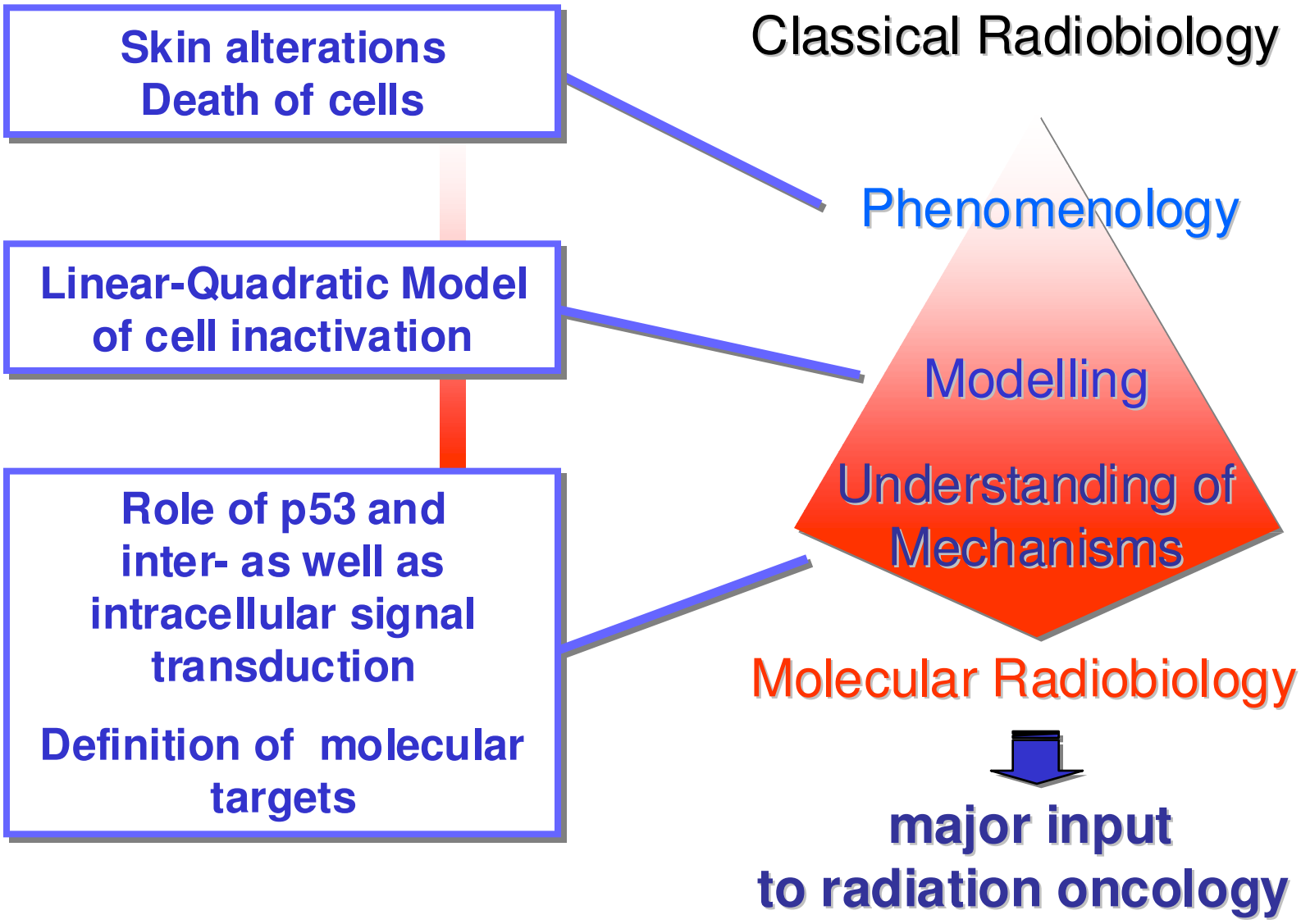
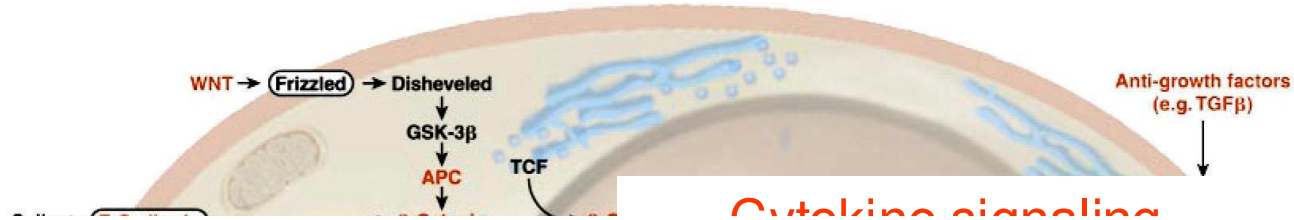
Radiobiology: A changing science



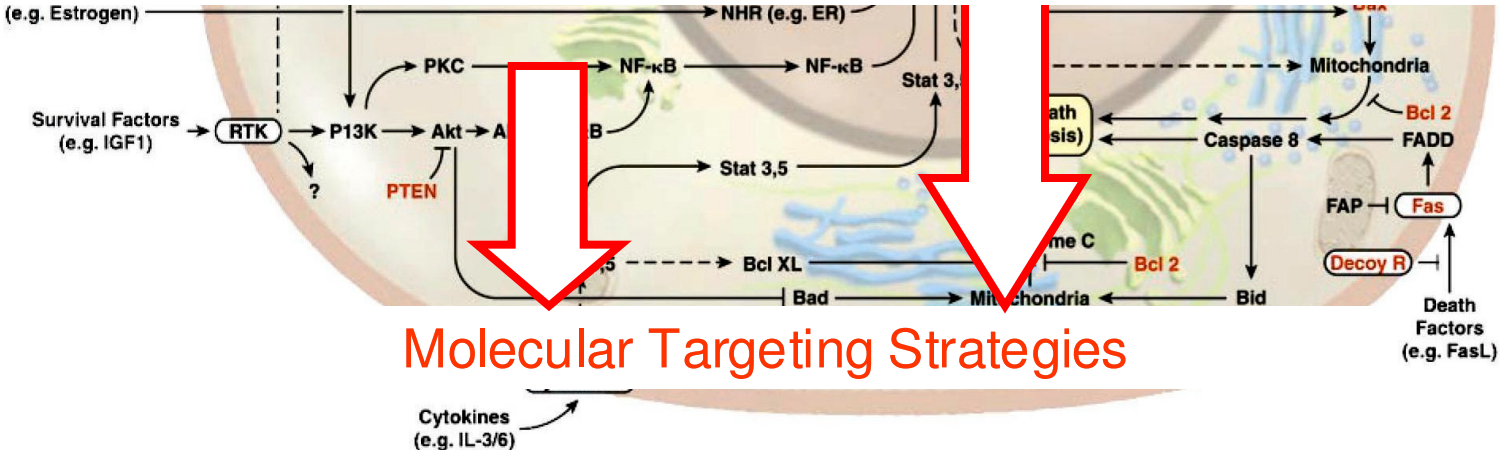
What are the future perspectives ?

- Identification of molecular targets for radiosensitization / radioprotection in the context of their normal and pathological mechanisms
- Identification of tissue specific target structures on the basis of biological / molecular imaging
- Identification of genetic markers of individual radiation sensitivity (*Genomics / Proteomics*)
- Development of molecular prediction for RT (*Theranostics*)
- Application of stem cells to rescue damaged normal tissue

Target identification

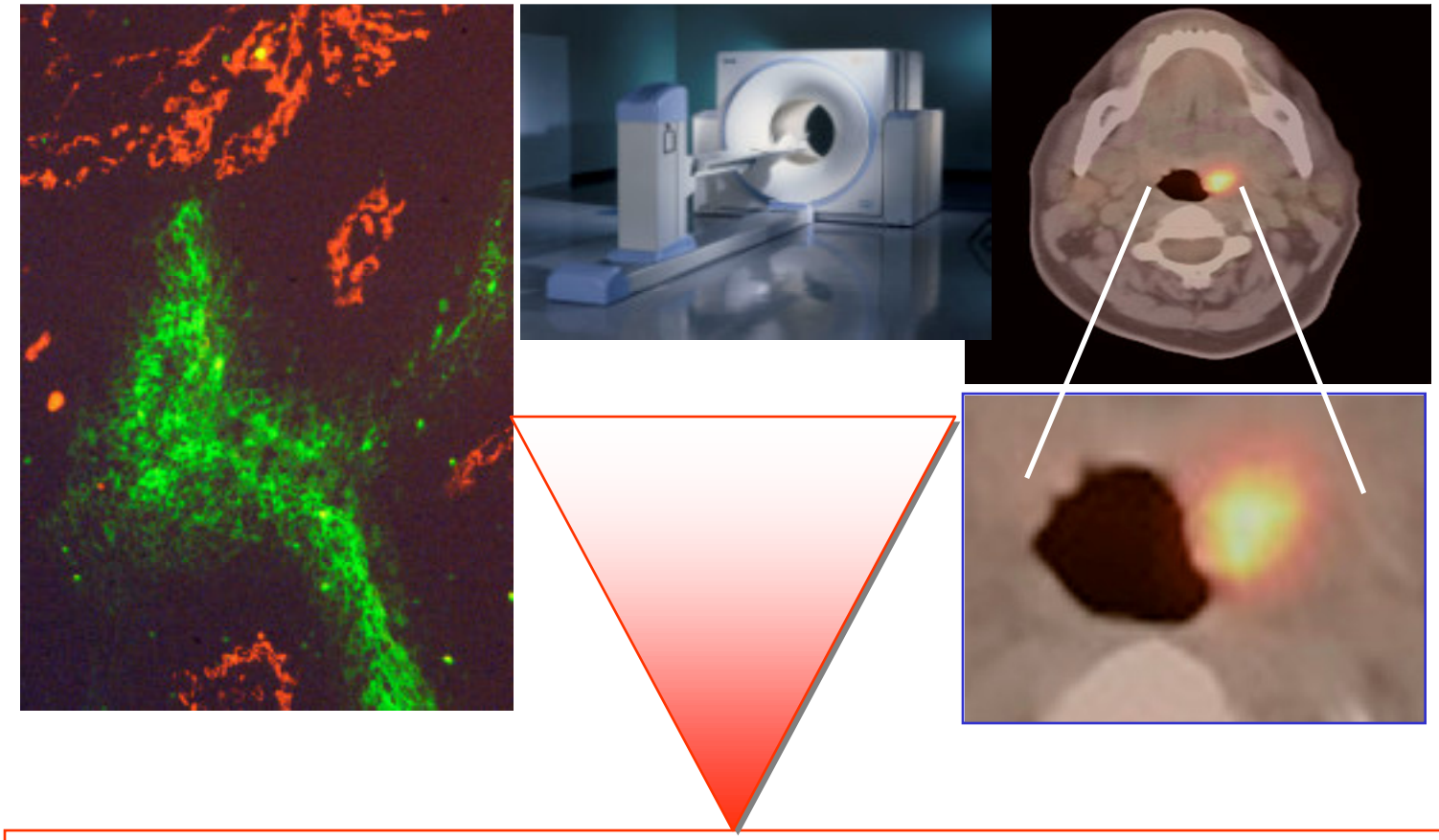


If we understand all the details, the most relevant molecular targets can be defined to develop the best possible therapeutic strategy in RO !



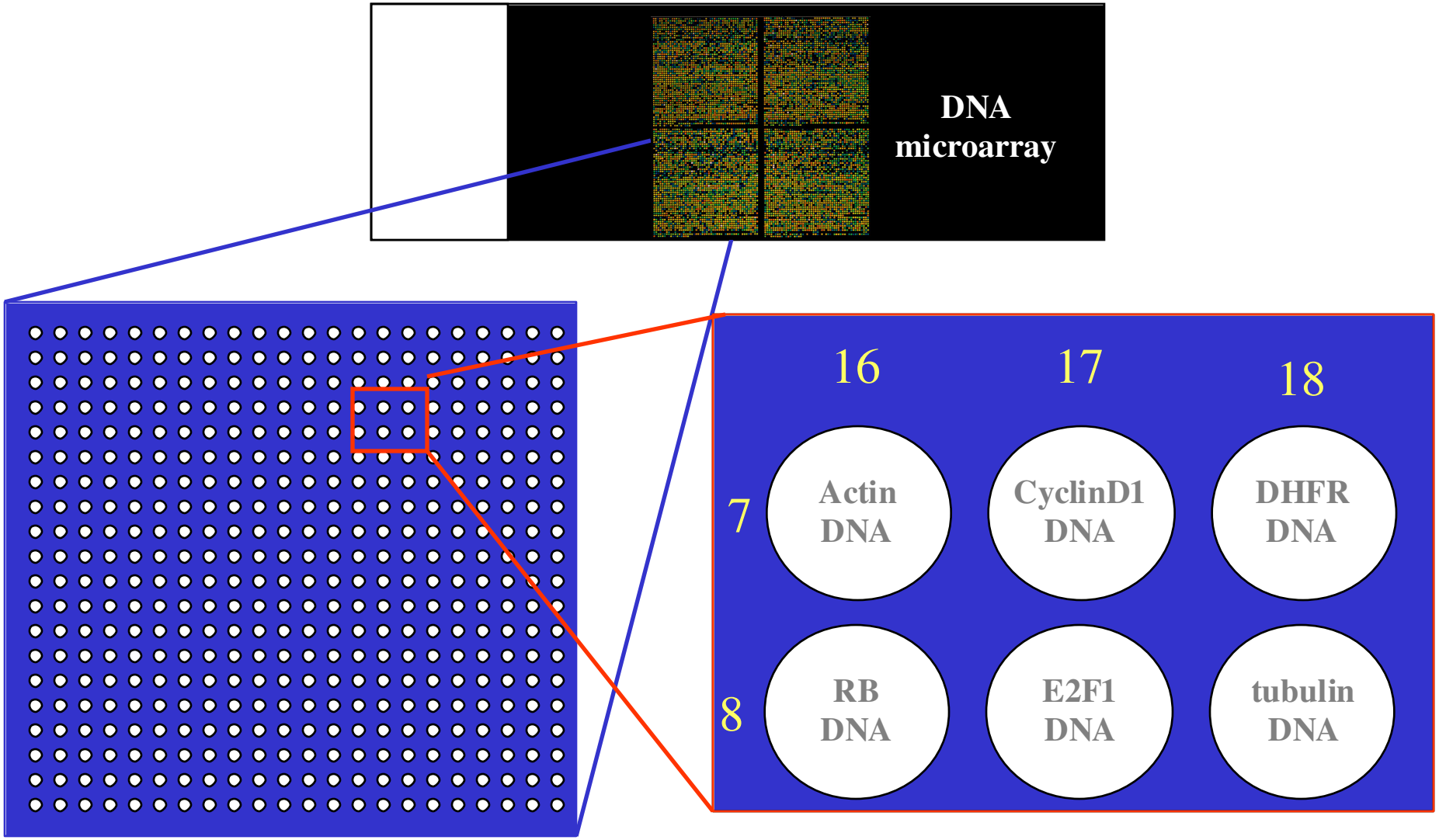
Molecular Targeting Strategies

Functional molecular imaging



high resolution PET-CT / PET-MRI

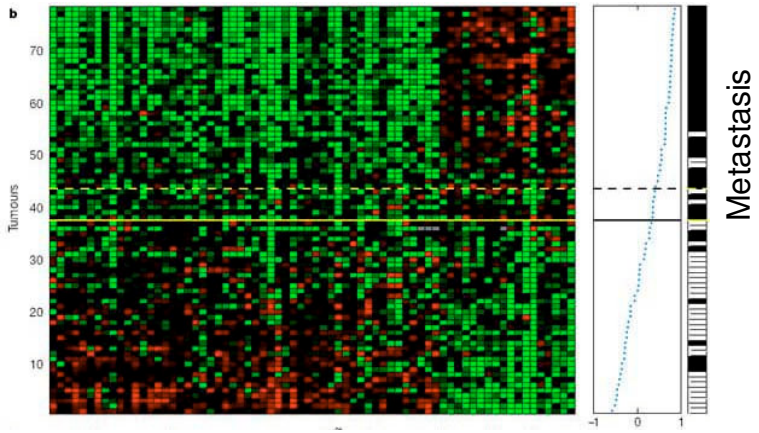
Genomics


DNA
microarray
16
17
18
7
Actin
DNA
CyclinD1
DNA
DHFR
DNA
8
RB
DNA
E2F1
DNA
tubulin
DNA

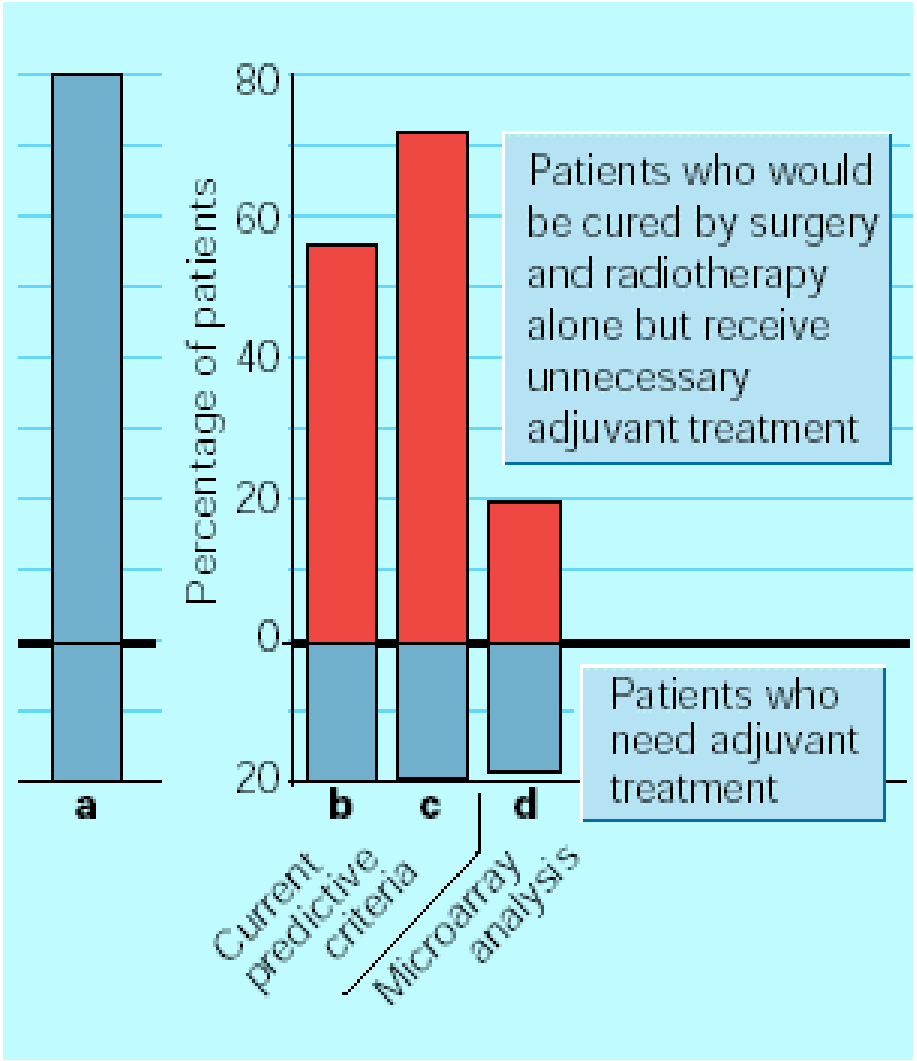
Prognostic profiling

Gene expression profiling predicts clinical outcome of breast cancer

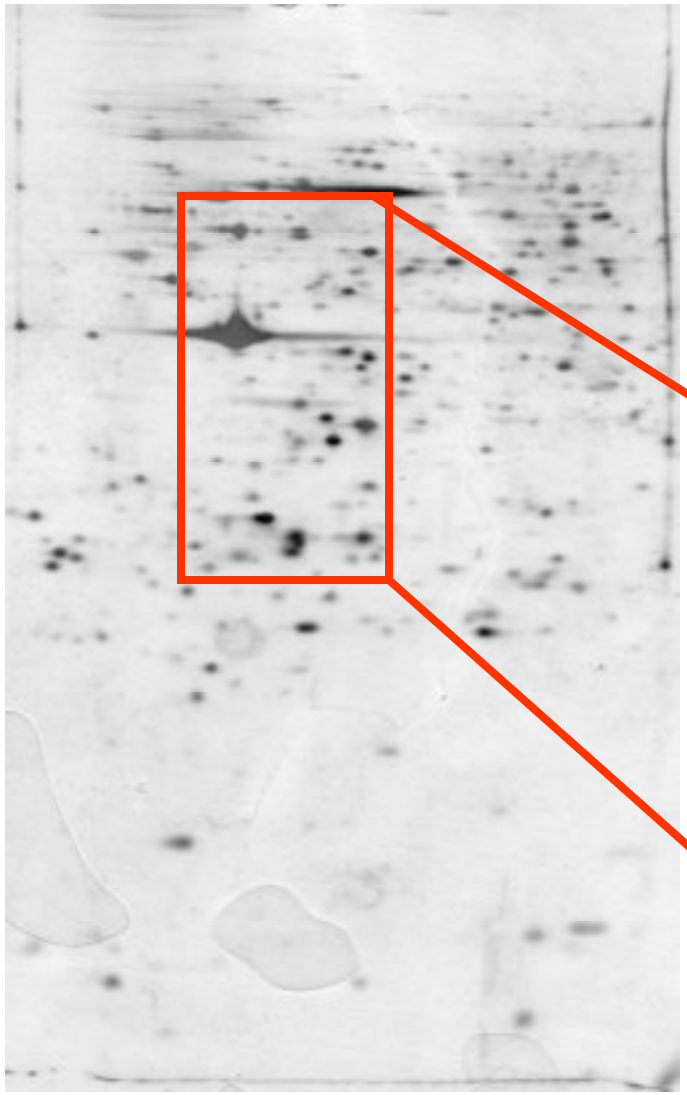
Laura J. van 't Veer*†, Hongyue Dai†‡, Marc J. van de Vijver*†, Yudong D. He‡, Augustinus A. M. Hart*, Mao Mao‡, Hans L. Peterse*, Karin van der Kooy*, Matthew J. Marton‡, Anke T. Witteveen*, George J. Schreiber‡, Ron M. Kerkhoven*, Chris Roberts‡, Peter S. Linsley‡, René Bernards* & Stephen H. Friend‡



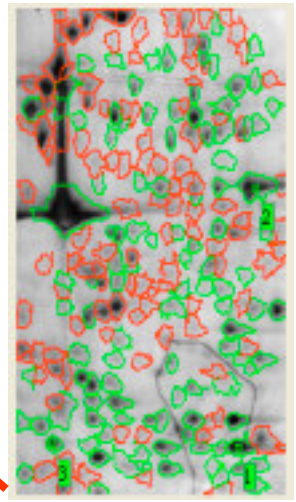
Nature, Vol 415, 31 January 2002



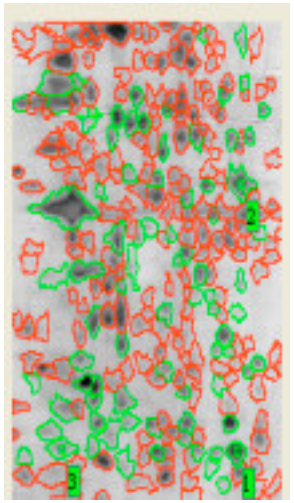
Proteomics



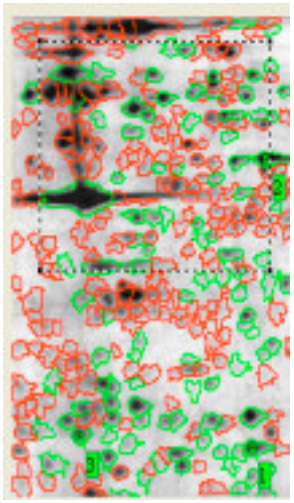
2D-PAGE (pH range 4-7) of non-irradiated lymphocytes from normal, radiation sensitive and radiation resistant identical twins !



normal
87 proteins



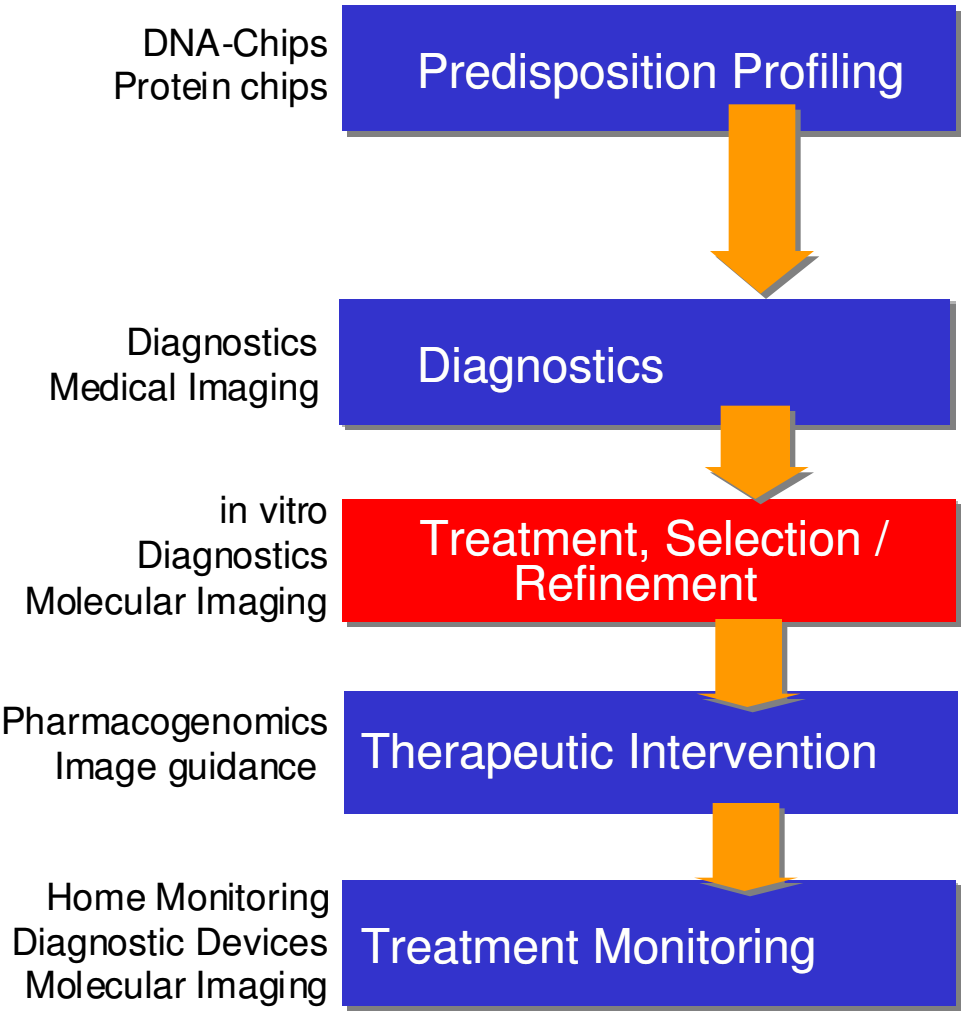
sensitive
–12 proteins



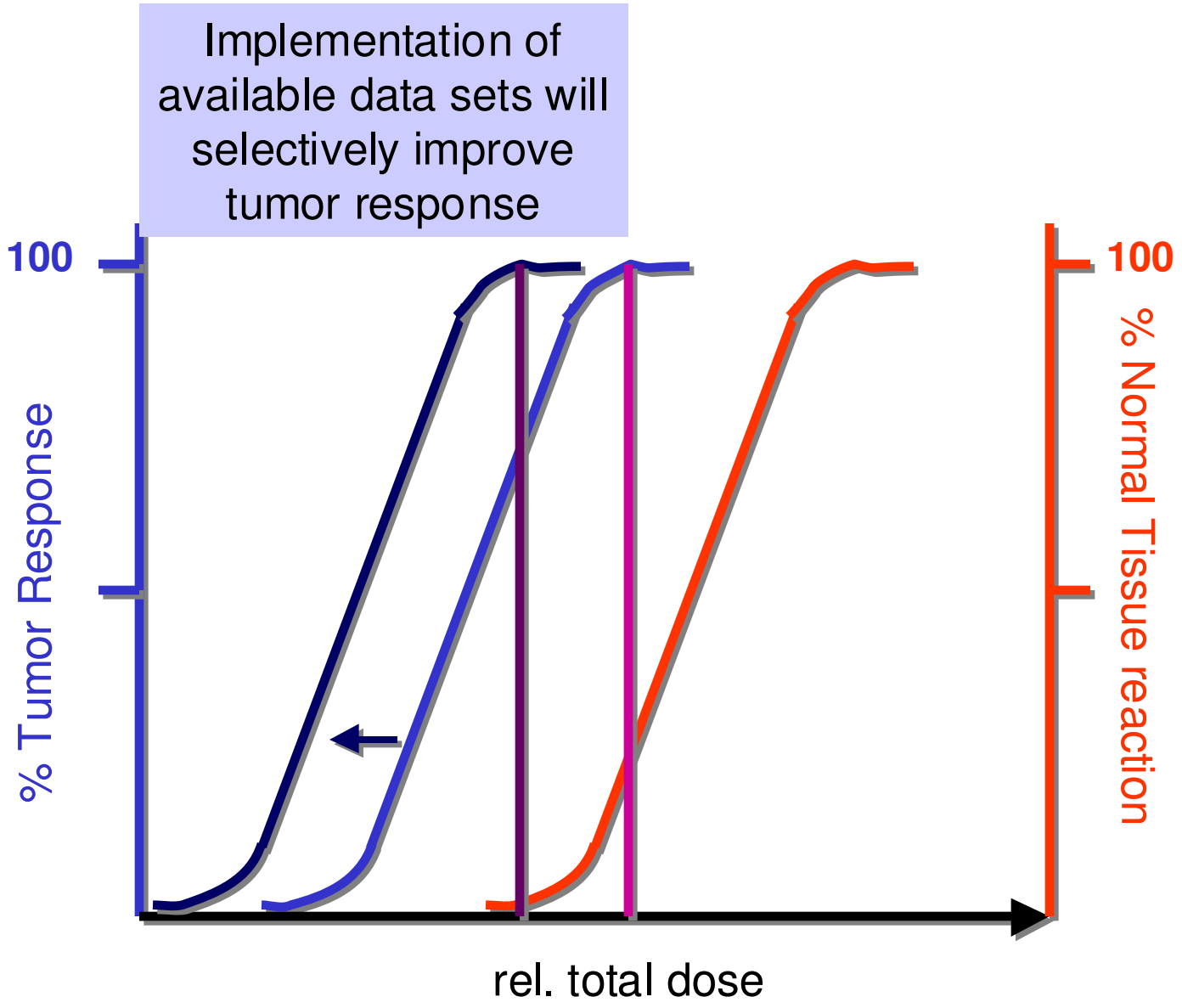
resistant
+15 proteins

Theranostics

Theranostics: link between diagnostics and therapy



The „Holthusen-Principle“ is still valid



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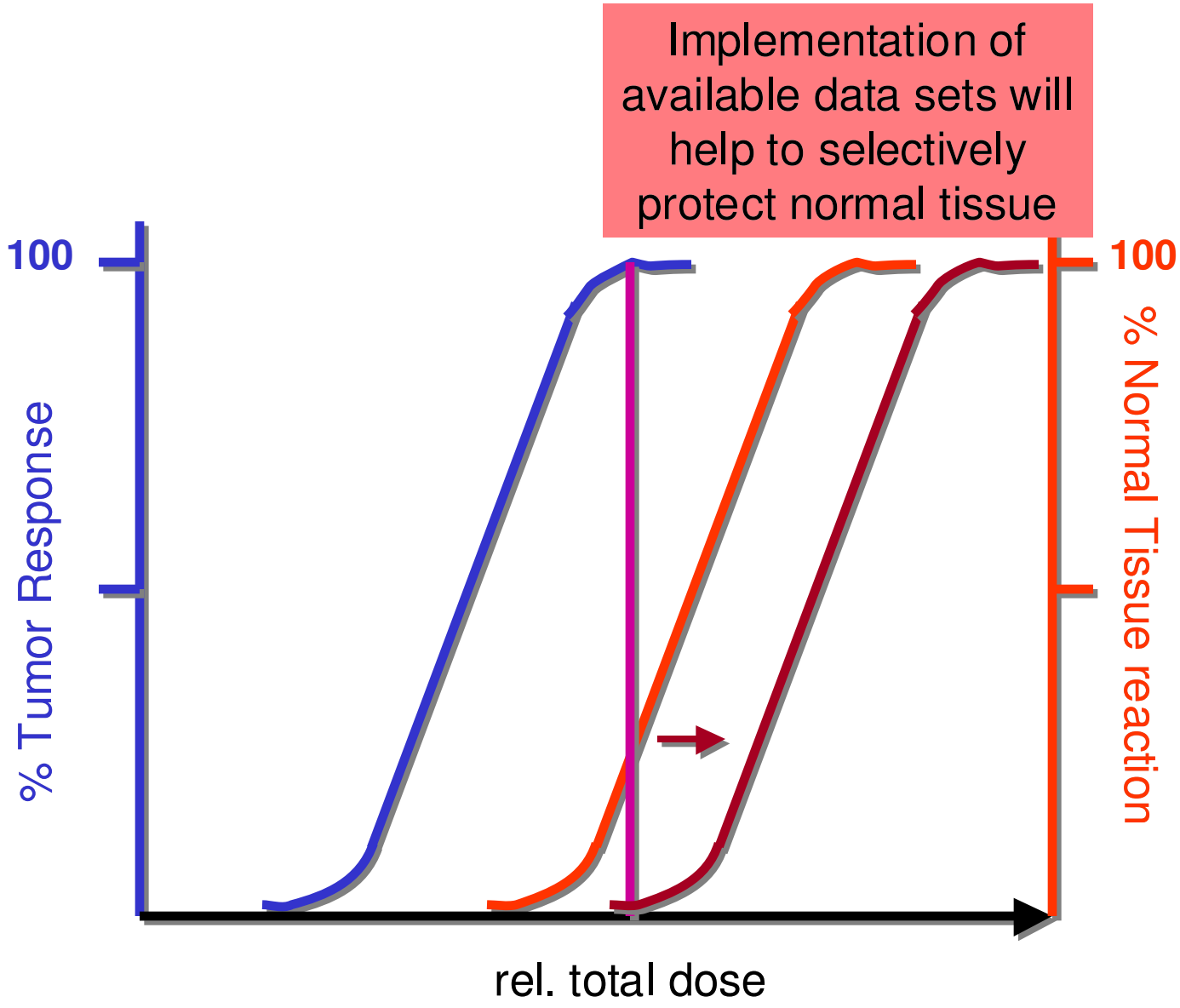


Molecular Targeting Strategies to improve tumor and prevent normal tissue reponses

EXAMPLE 1 : *EGF-Receptor antagonists as target to improve tumor response*

EXAMPLE 2 : *TGFß1-production/signaling as target to prevent radiation-induced fibrosis*

EXAMPLE 3 : *Bowman Birk Protease Inhibitor (BBI) and Phospho-Tyrosine as tools to selectively protect normal tissue*

EGFR

EGFR overexpression in human tumors

Solid tumors presenting overexpressed or mutated EGFR

• H&N	80-100%
• Renal	50-90%
• Breast	14-91%
• Esophageal	43-89%
• Prostate	40-80%
• NSCLC	40-80%
• Colorectal	25-77%
• Gastric	33-74%
• Ovarian	35-70%
• Glioma	40-63%
• Pancreatic	30-50%
• Bladder	31-48%

overexpression / mutation is associated with …

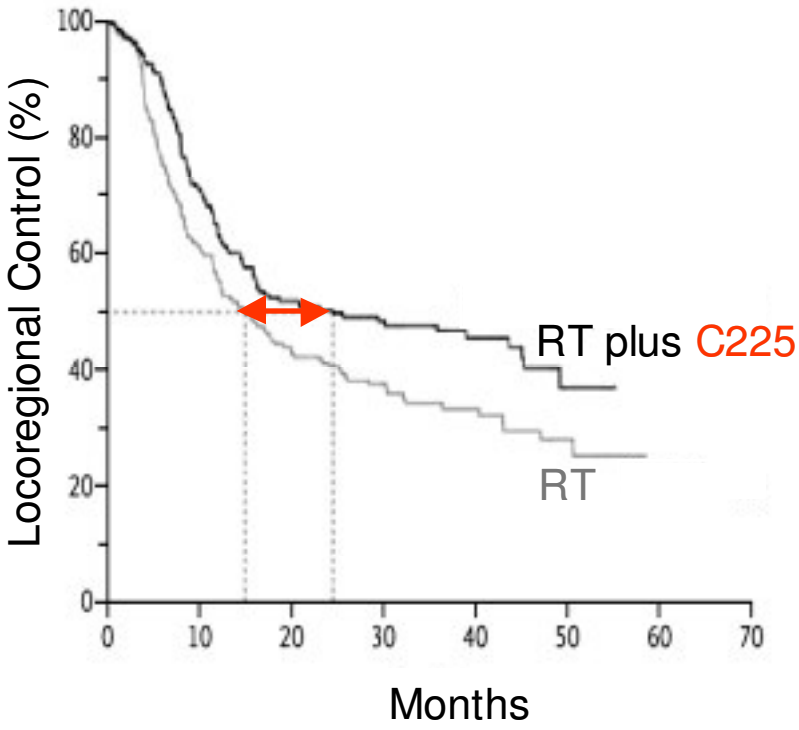
- enhanced receptor signaling
- massive tumor growth
- enhanced invasive and metastatic potential

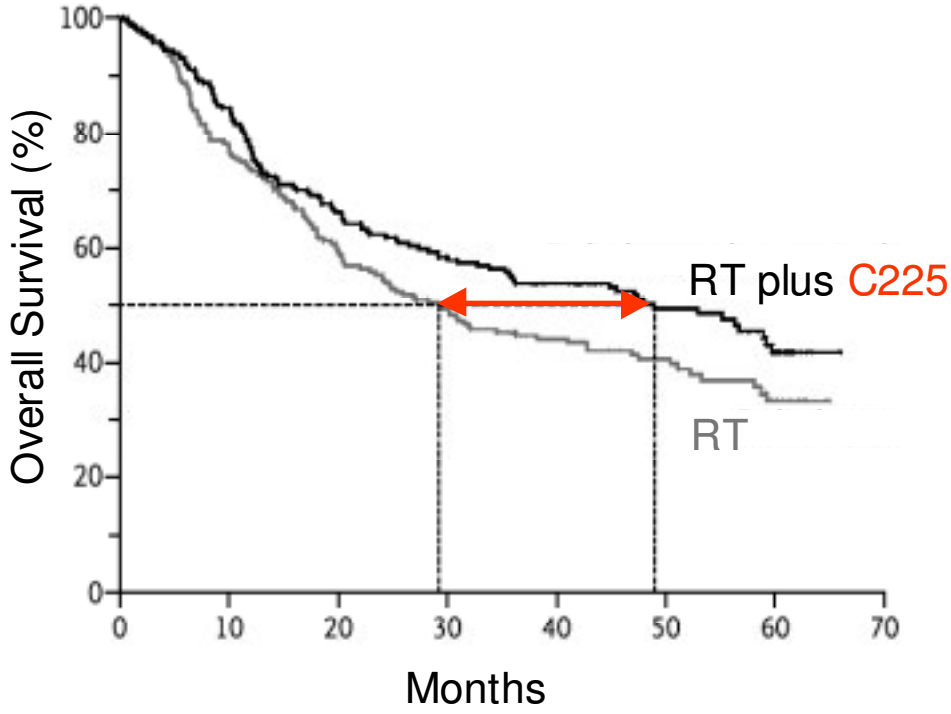
…and is generally correlated with resistance to chemo-/ radiotherapy

EGFR overexpression in human tumors

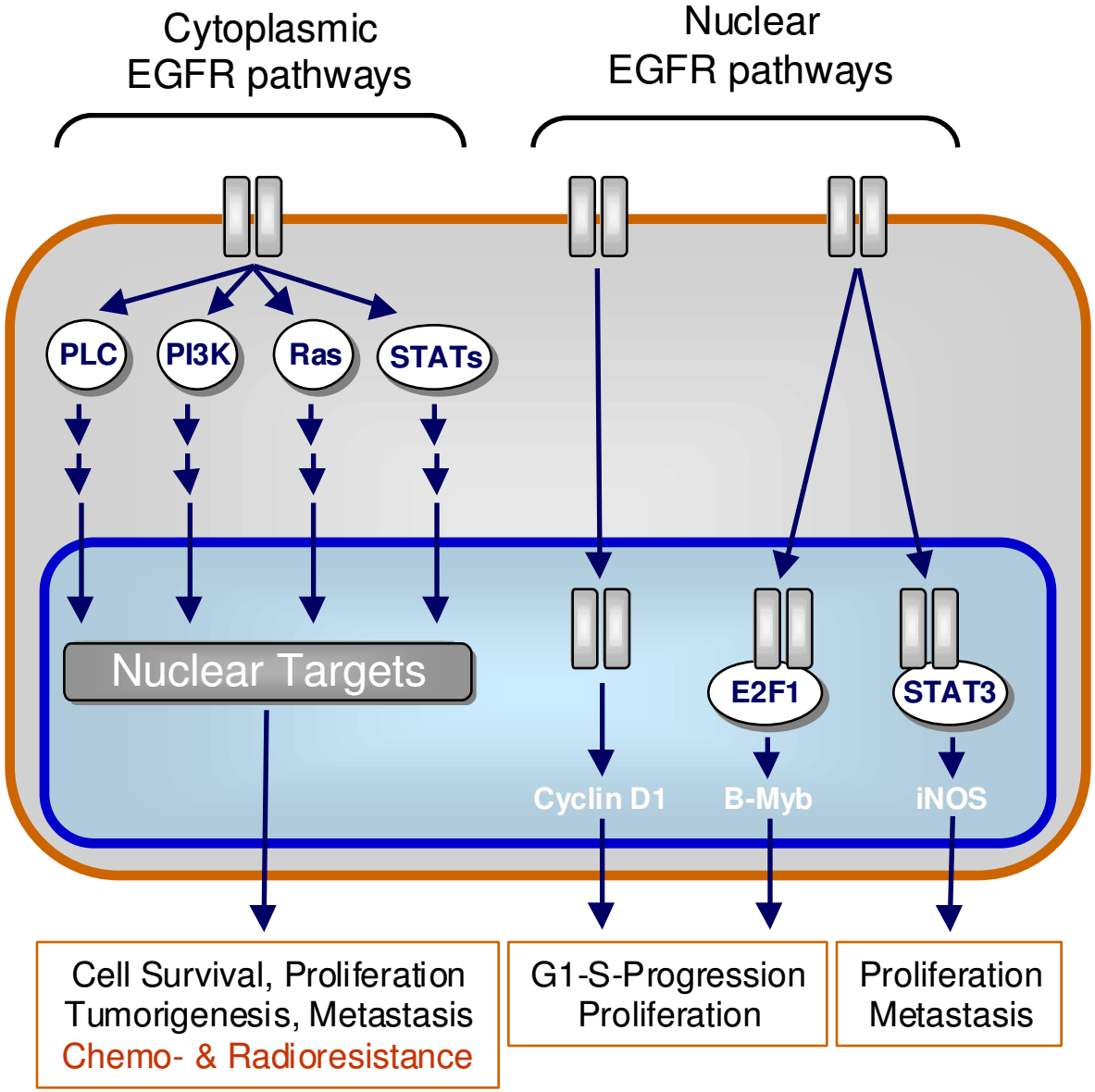
Bonner et al. New Engl J Med 2006

424 H&N tumor patients : 213 RT+Cetuximab (C225) / 211 RT alone

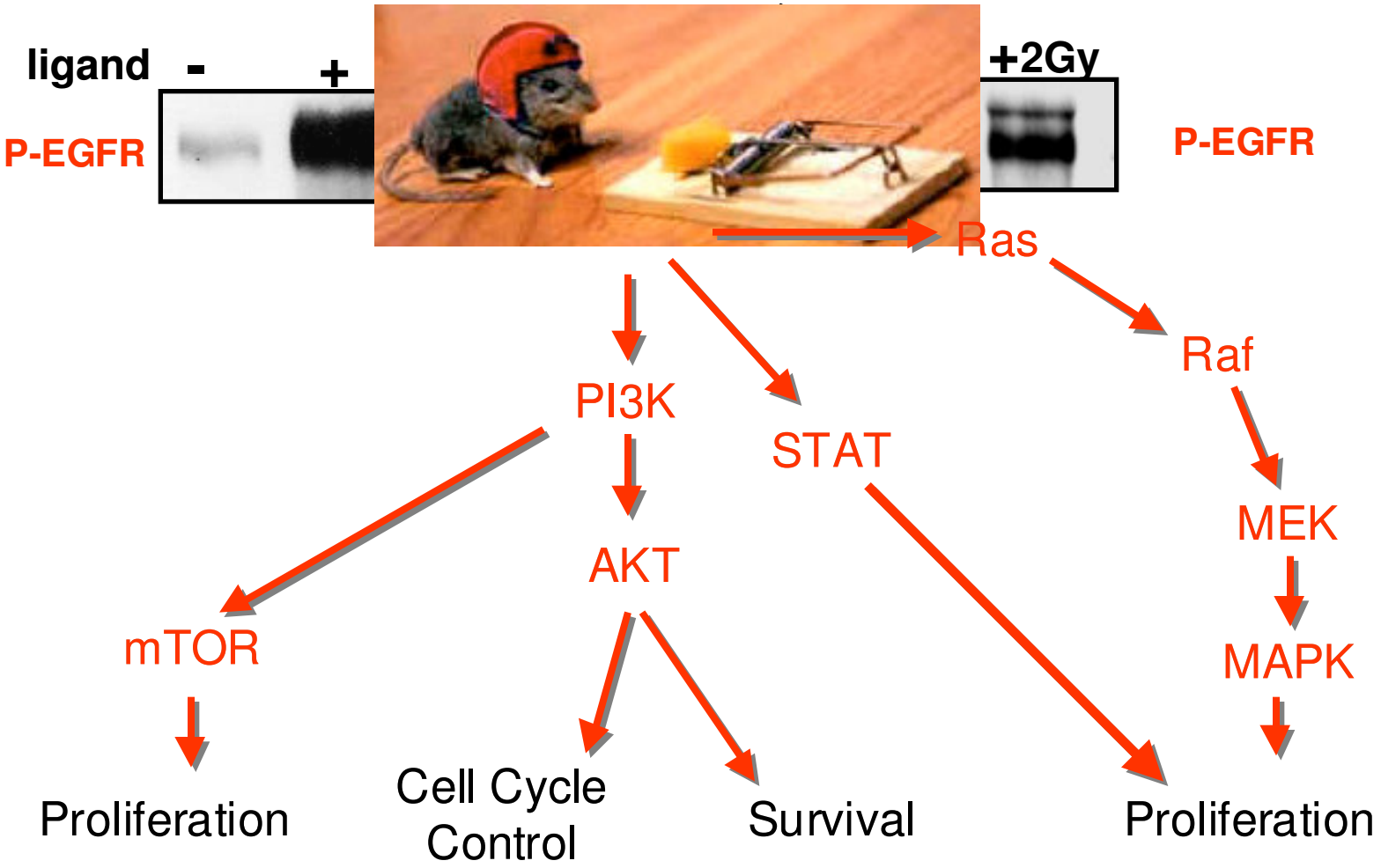




EGFR signaling

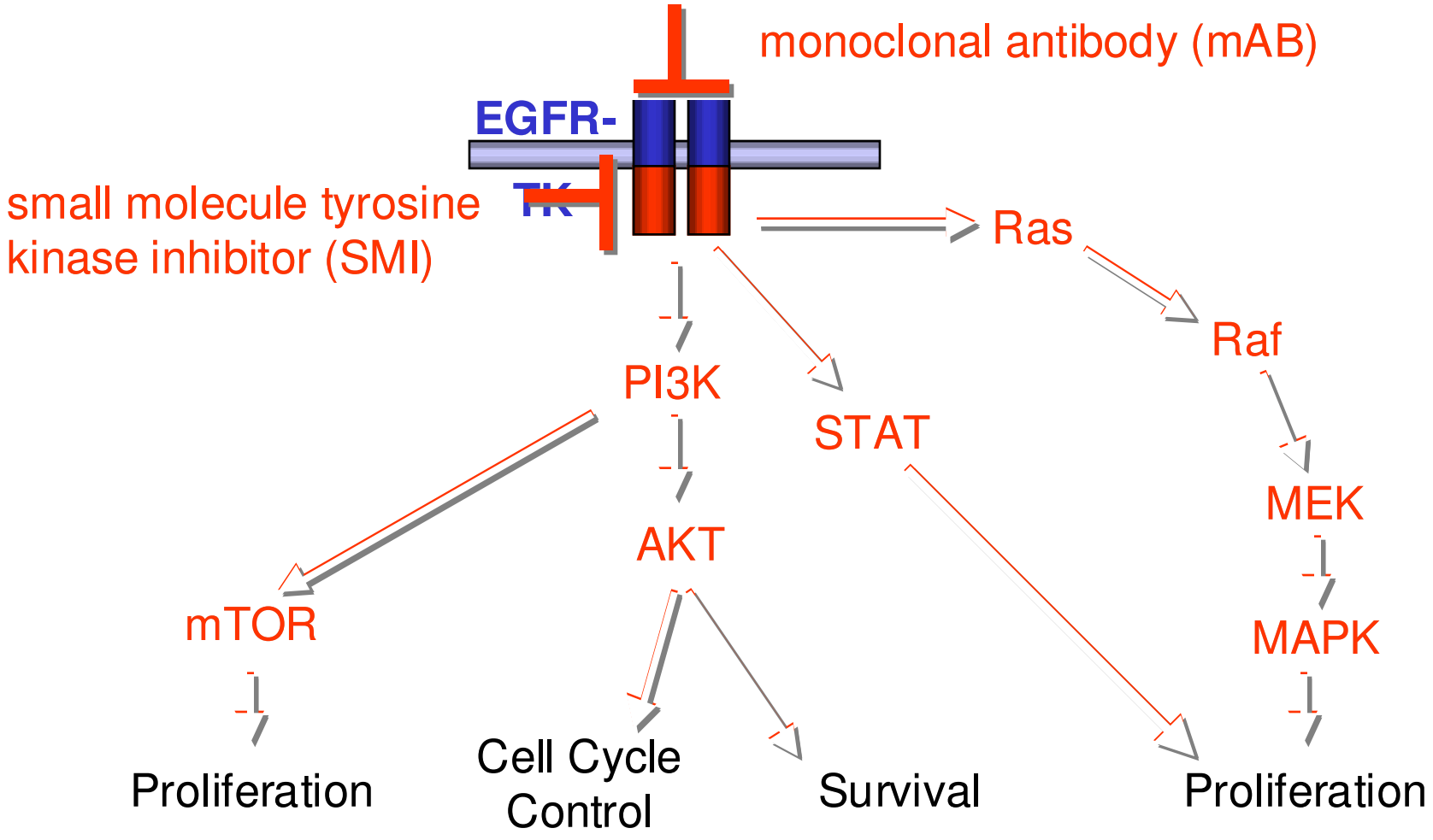


Activated EGF receptor and its cellular consequences



➢ **Radioresistance**

Targeting EGFR signaling



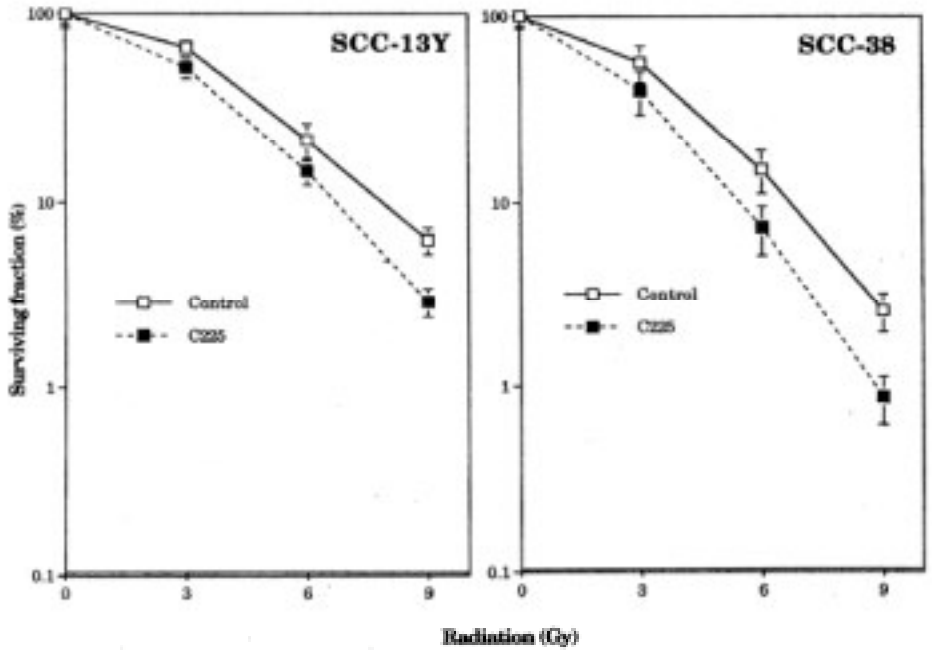
➢ **Radiosensitization**

C225 leads to radiosensitization in vitro and in vivo

Harari et al. 2001 IJROBP 49:427-433

Clonogenic assay in vitro

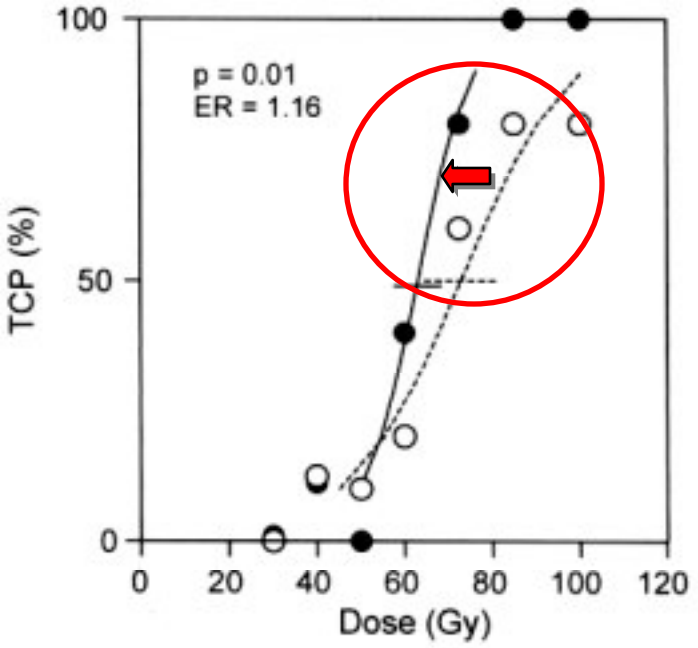
H&N SCC cell lines



Krause et al. 2005 Radiother. Oncol. 74:109-115

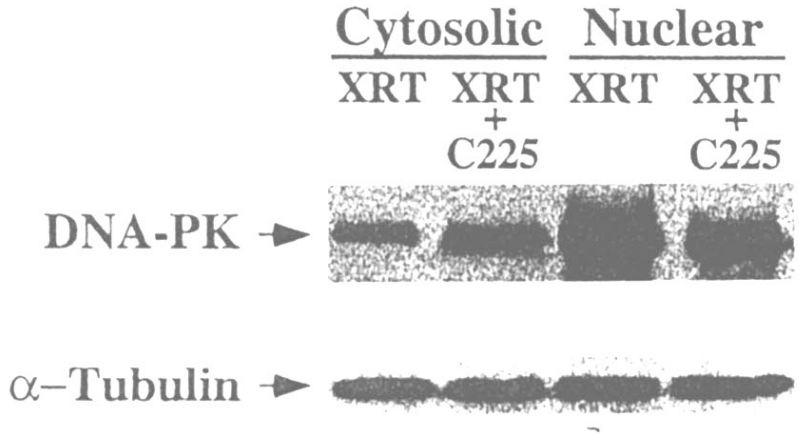
Tumor Control Study in vivo

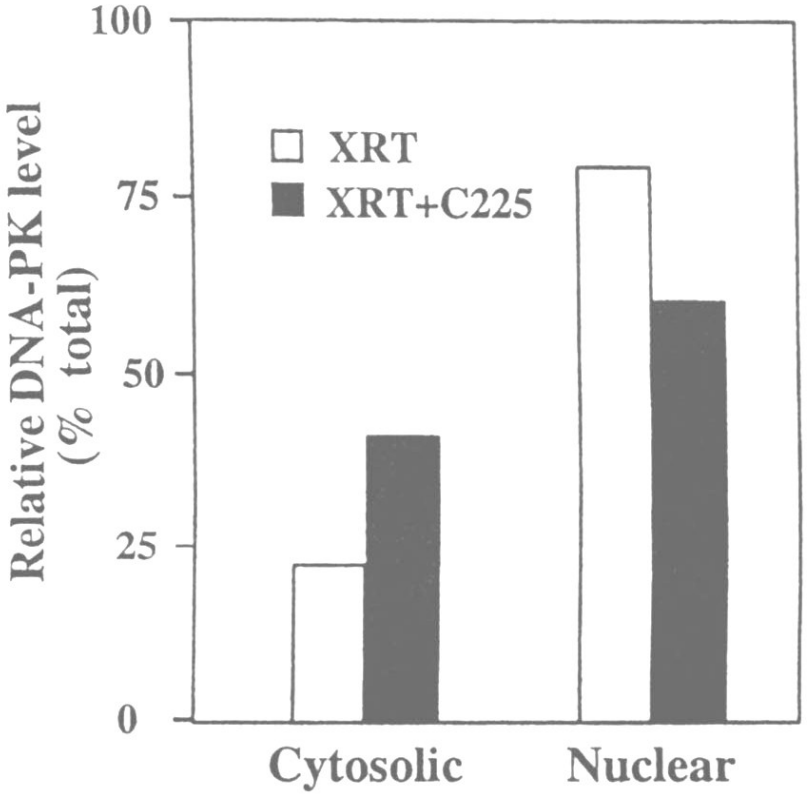
FaDu tumor xenograft model



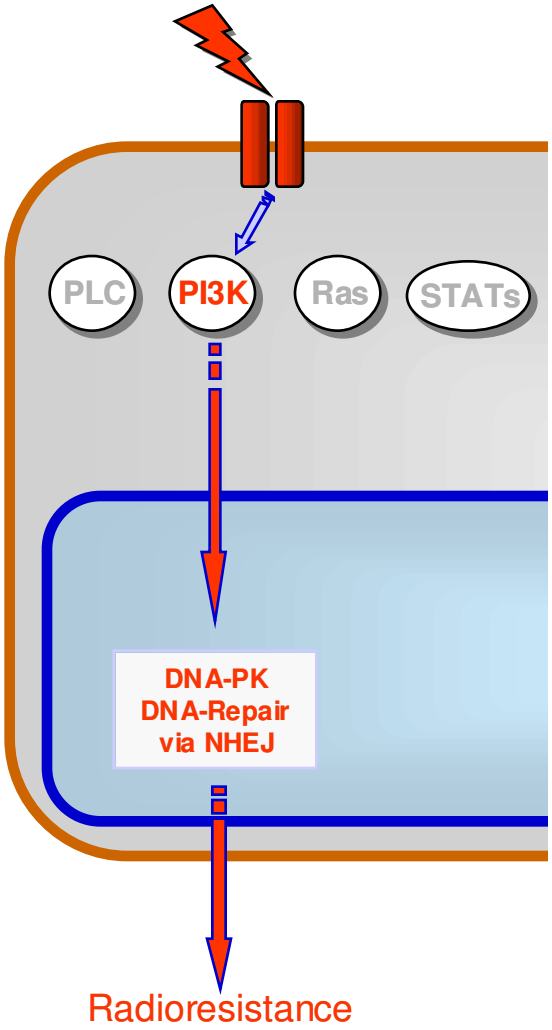
Effect of C225 on localisation of DNA-PK

Huang et al. 2000 Clin. Cancer Res. 6:2166-2174





Radiation-induced EGFR signaling

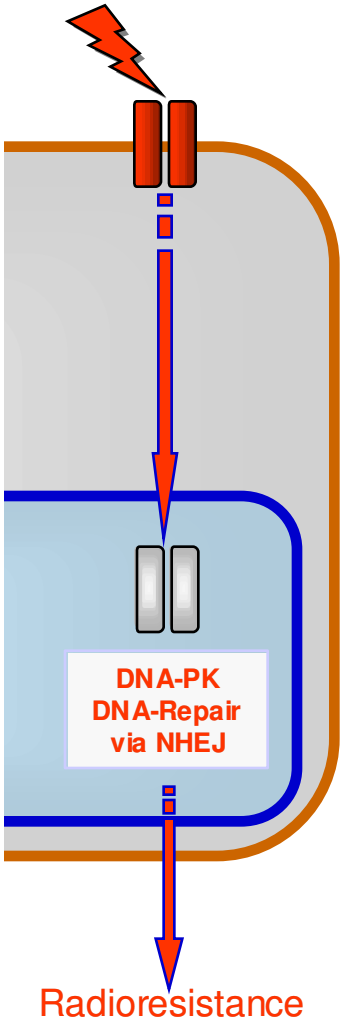


Güven et al.
J Biol Chem *2001*
Genes Chromos Cancer *2003*

Dittmann et al.
J Biol Chem *2005*
Radiother Oncol *2005*
Radiother Oncol *2007*
Int J Rad Oncol Biol Phys *2008*
Radiother Oncol *2008*

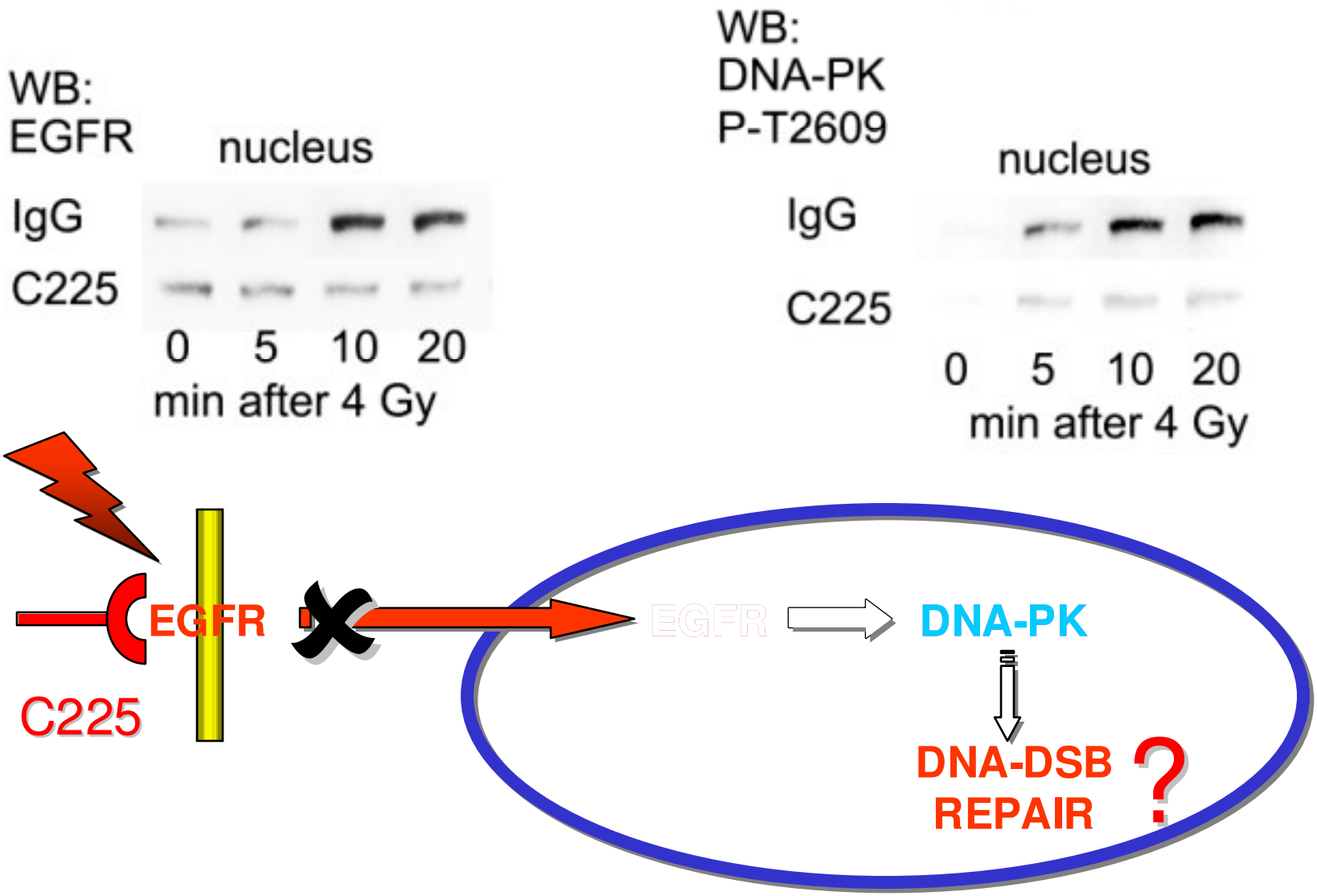
Toulany et al.
Radiother Oncol *2005a*
Radiother Oncol *2005b*
Clin Cancer Res *2006*
Mol Cancer Res *2007*

Rodemann et al.
Sem Rad Oncol *2007*
Int J Rad Biol *2007*



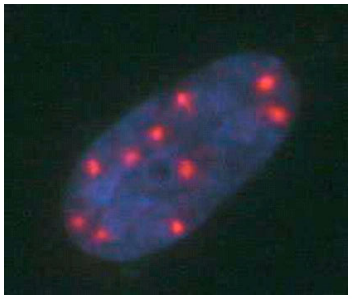
C225 prevents radiation-induced nuclear translocation of EGFR and activation of DNA-PK

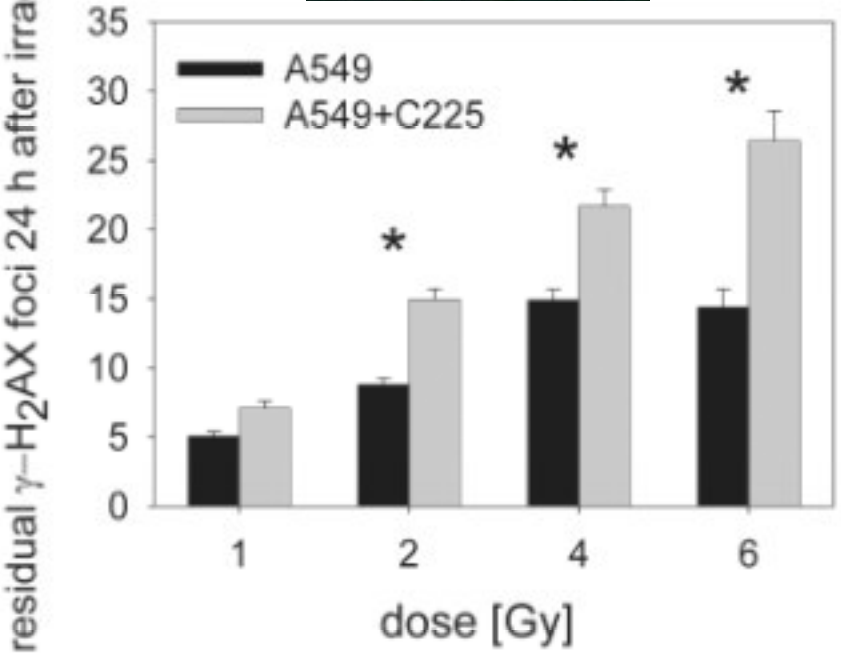
Dittmann et al. J. Biol. Chem. 2005

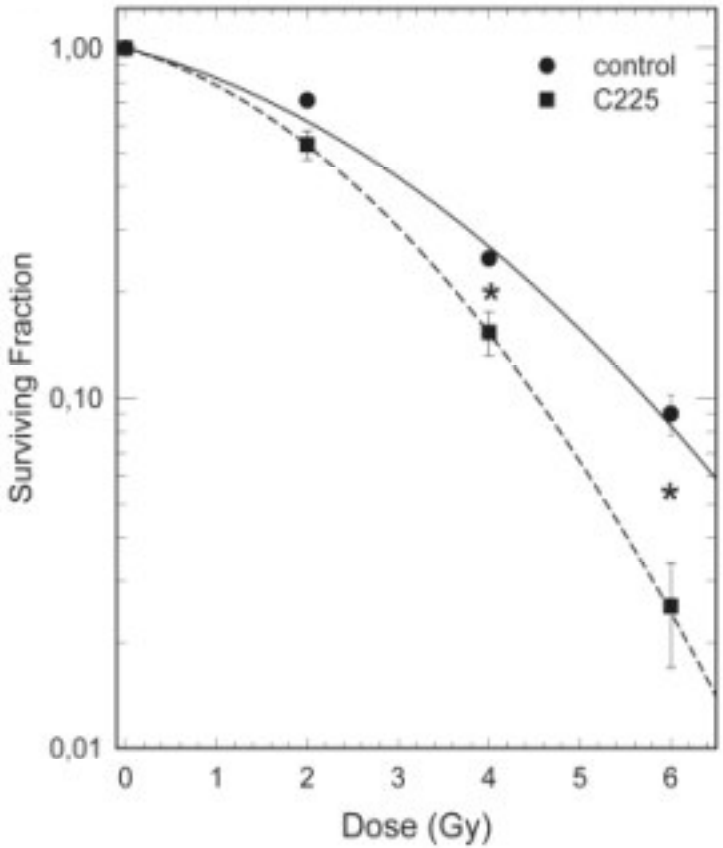


C225 mediates impaired DNA-DSB repair and enhances radiation sensitivity

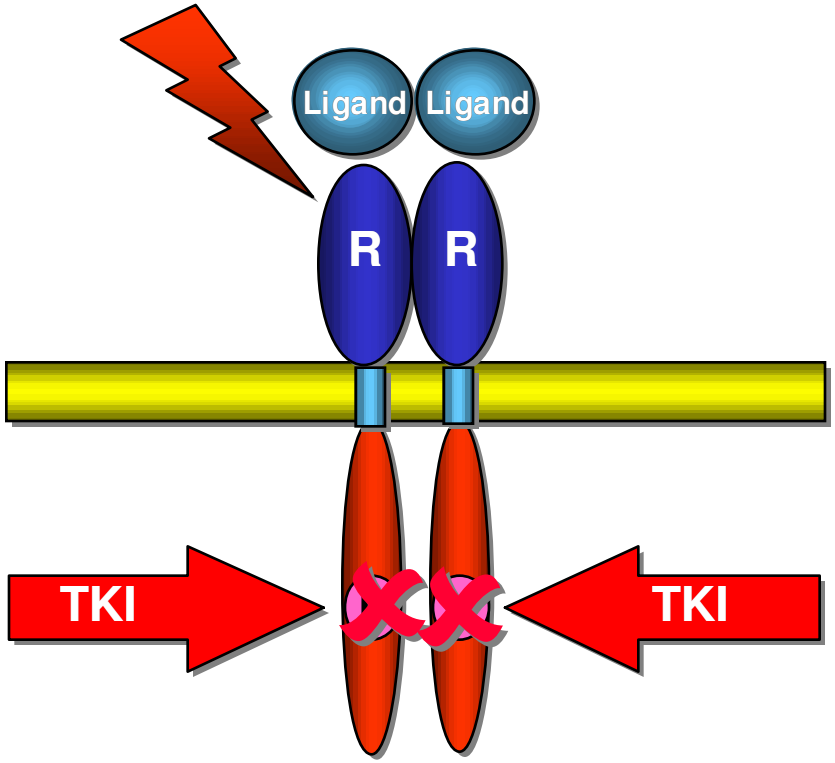
Dittmann et al. J. Biol. Chem. 2005







Specific inhibition of EGFR-tyrosine kinase activity



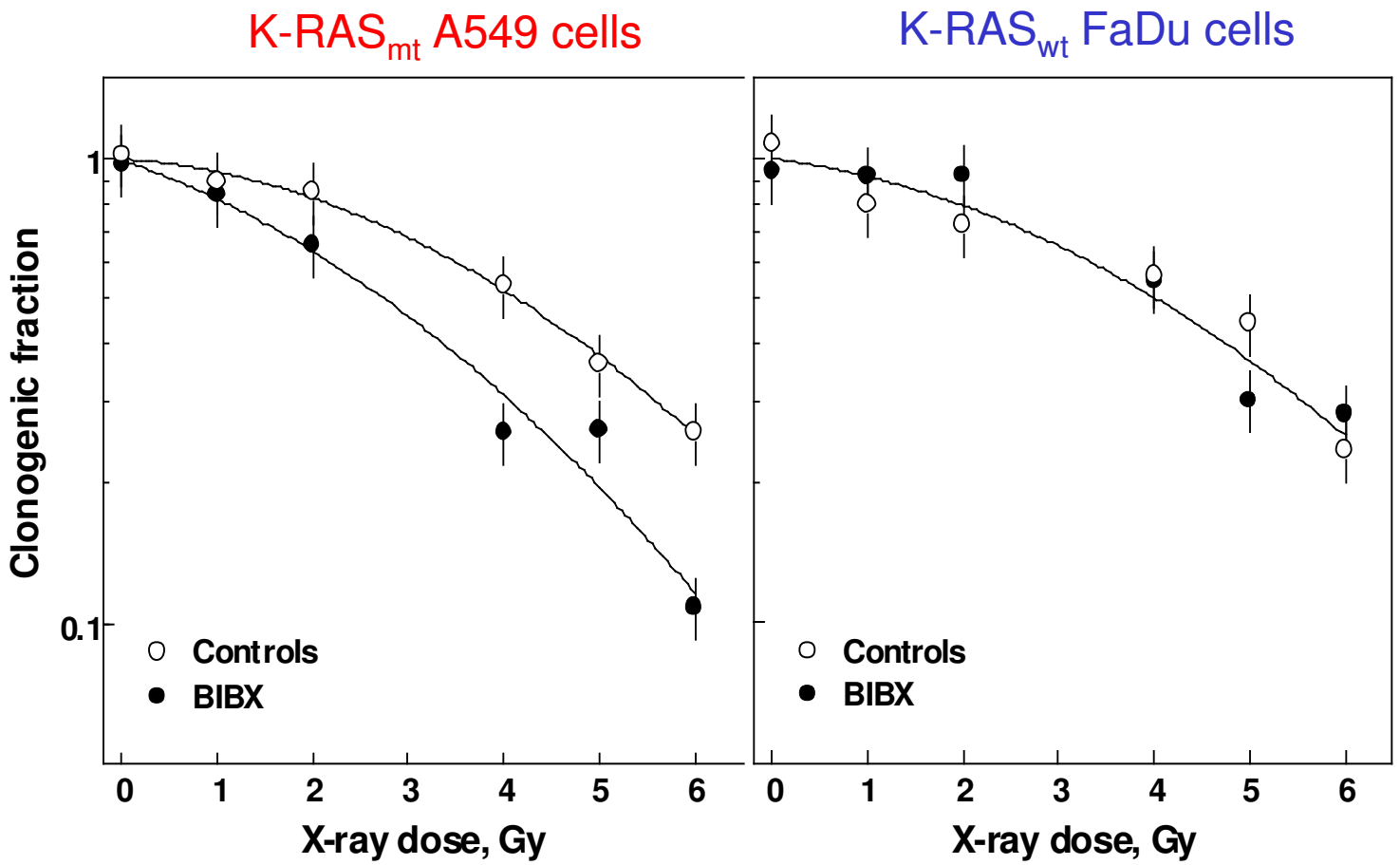
BIBX1382BS

- chemical class: pyrimido-[5,4-D]-pyrimidine-2,8-diamine,N8-(3-chloro-4-fluorophenyl)
- highly selective inhibitor of EGFR tyrosine kinase
- binds directly to intracellular tyrosine-kinase domain
- inhibits ligand-induced cell growth
  - erbB1-cells IC_{50} = 0.01 µM
  - erbB2-cells IC_{50} = 1.0 µM

Differential radiation response of Ras_{wt} or Ras_{mt} tumor cells to BIBX1382BS

Toulany et al. Clin. Cancer Res. 2006

Single dose irradiation



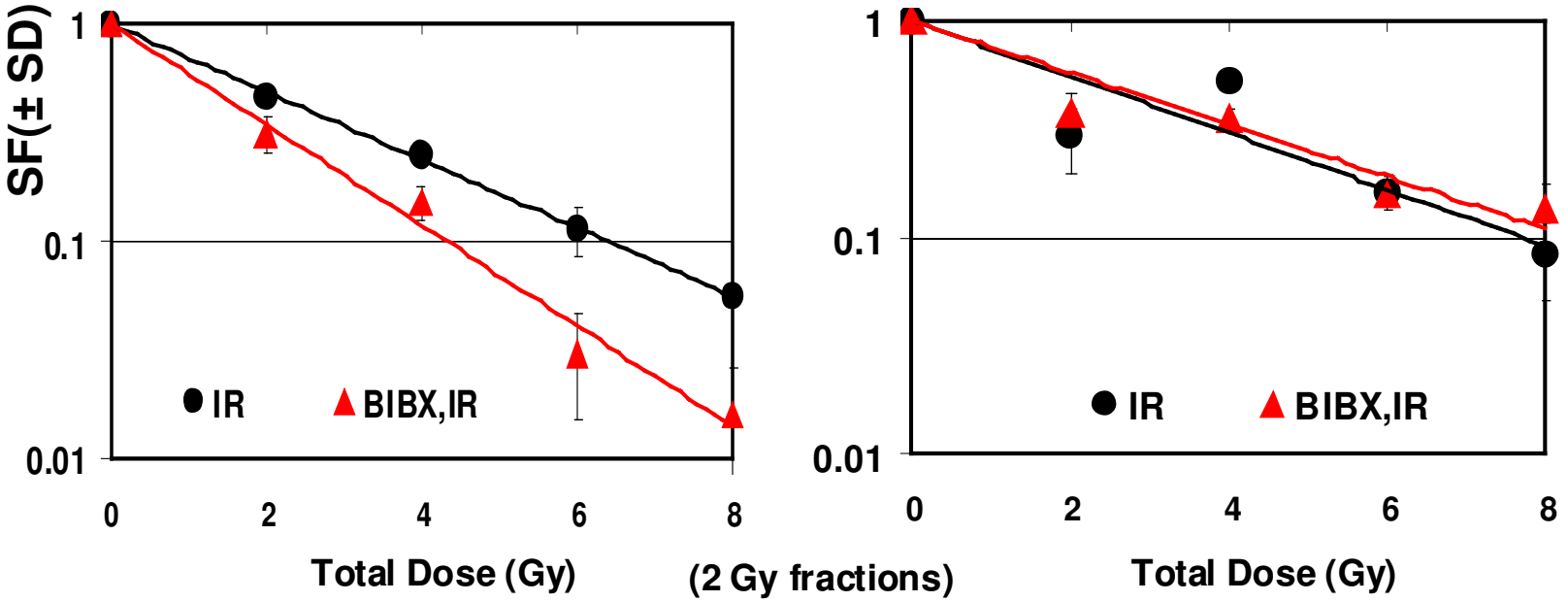
Differential radiation response of Ras_{wt} or Ras_{mt} tumor cells to BIBX1382BS

Toulany et al. Radiother. Oncol. 2005

Fractionated dose irradiation (4 x 2 Gy)

K-Ras mutated cell lines

K-Ras wildtype cell lines

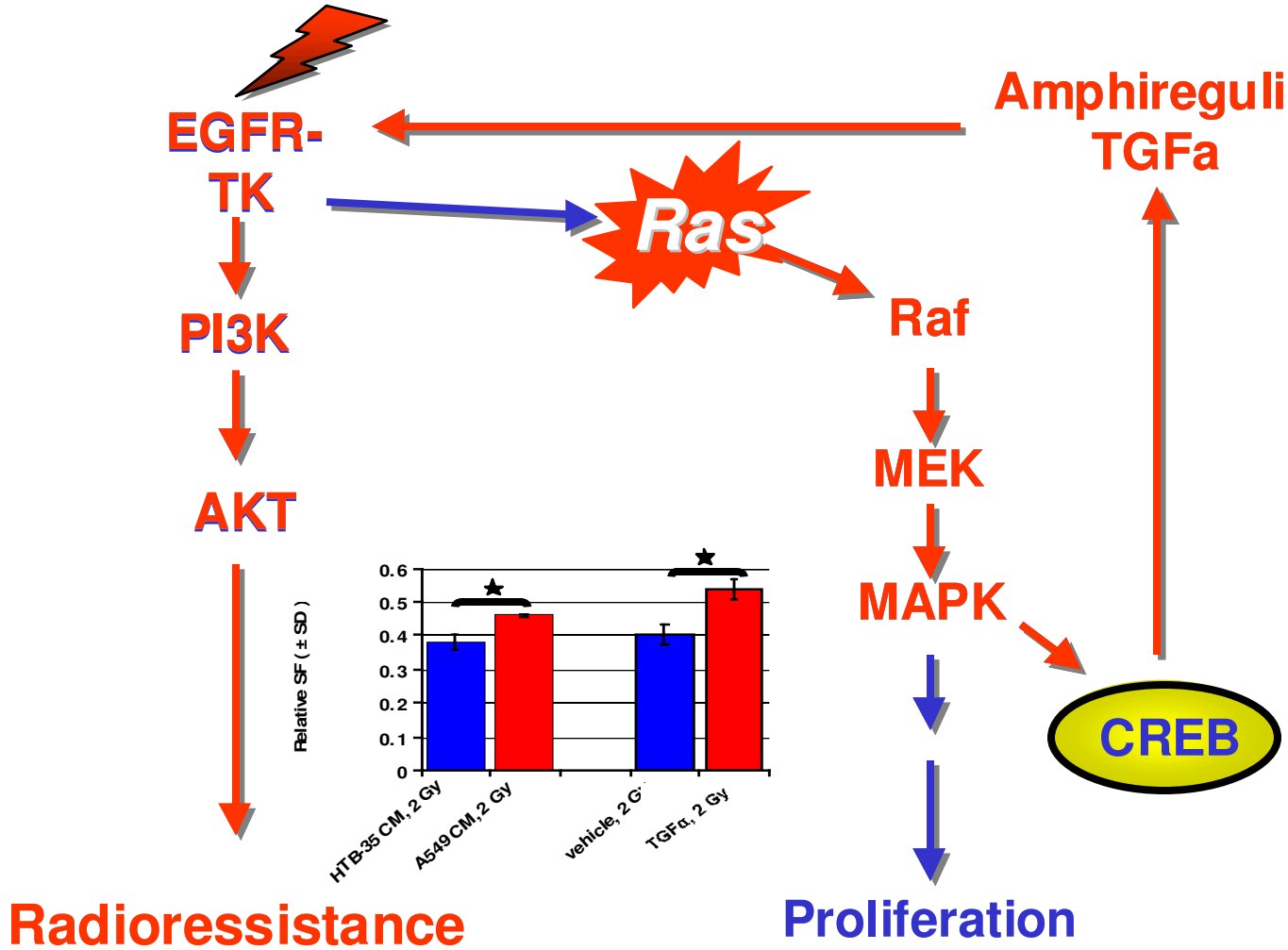


DMF 1.3-1.7 for Ras_{mt} cell lines
A549, MDA-MB231, PC3

No effect in normal Ras cell lines
FaDu, HTB35/SiHa, HH4DD

Autocrine activation of EGFR-PI3K-AKT signaling in K-RAS$_{mt}$ cells

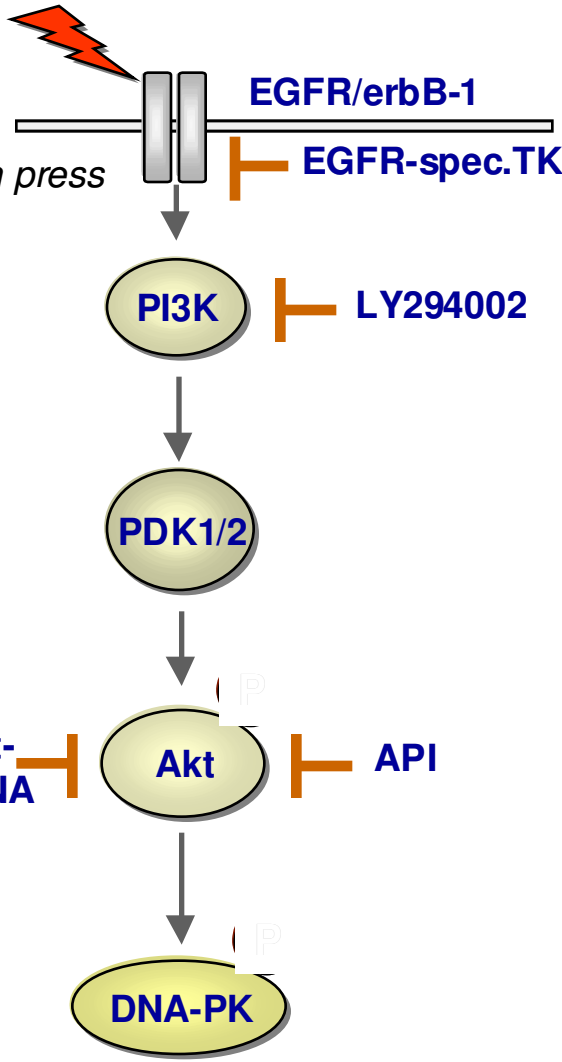
Toulany et al. 2005 Radiother. Oncol. 76:143-150

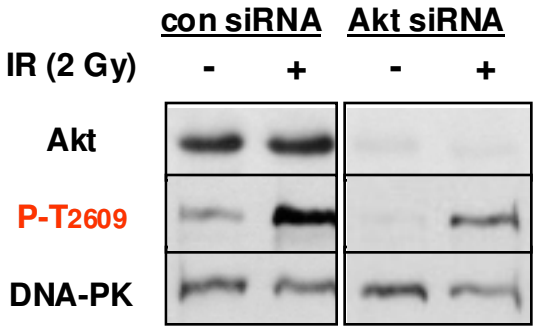


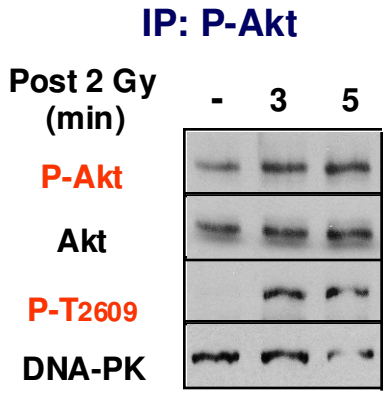
Role of AKT in regulating DNA-PK

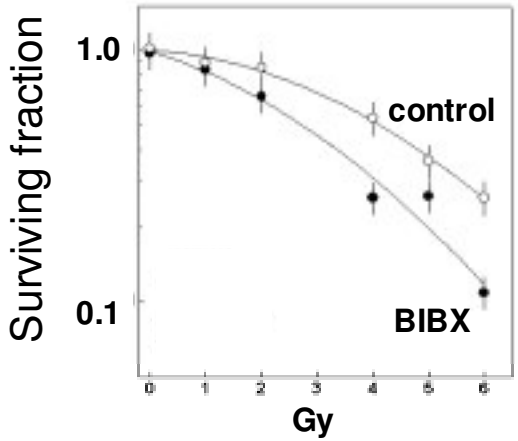
Toulany et al., Clin Cancer Res 2006

Toulany et al., Mol Cancer Ther 2008 in press









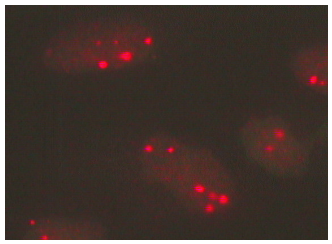
DNA-DSB repair is markedly inhibited !
RADIOSENSITIZATION

EGFR / PI3K inhibitors mediate enhanced residual DNA-damage

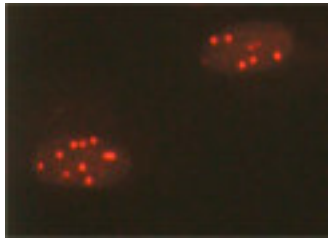
Toulany et al. Clin. Cancer Res. 2006

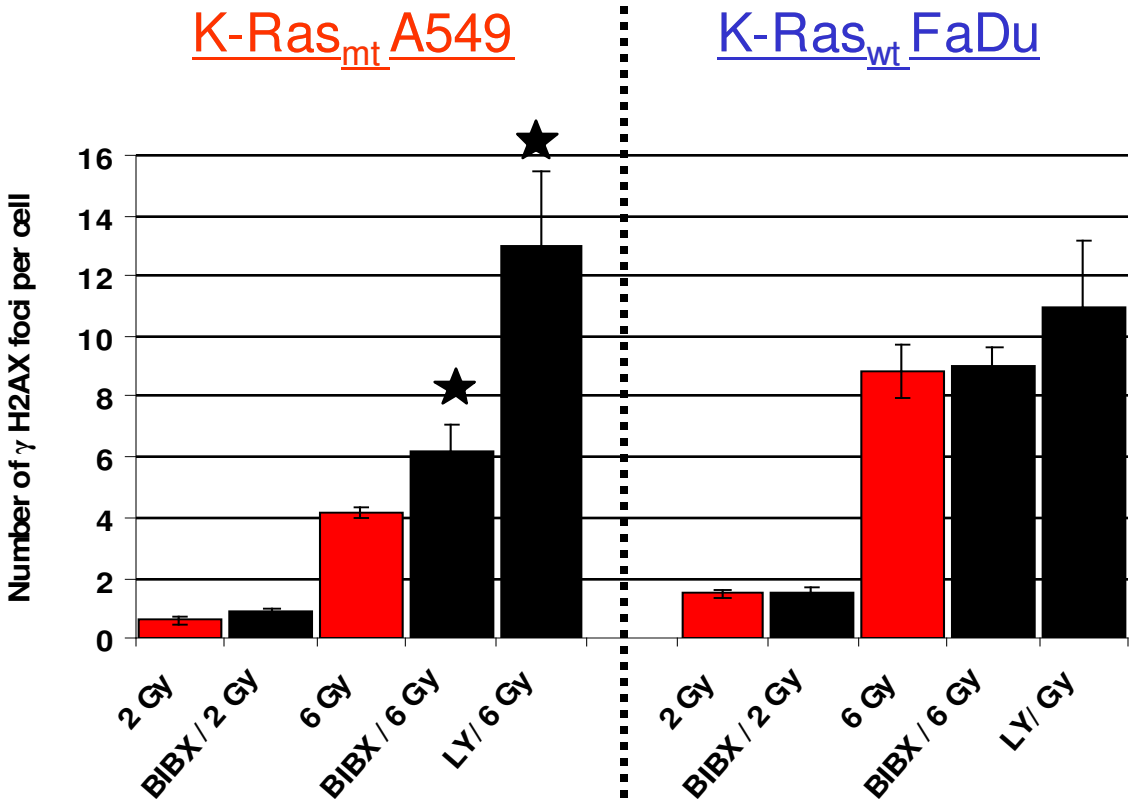
γ-H2AX-Foci indicating residual DNA-DSB 24h after IR

A549 + 6 Gy

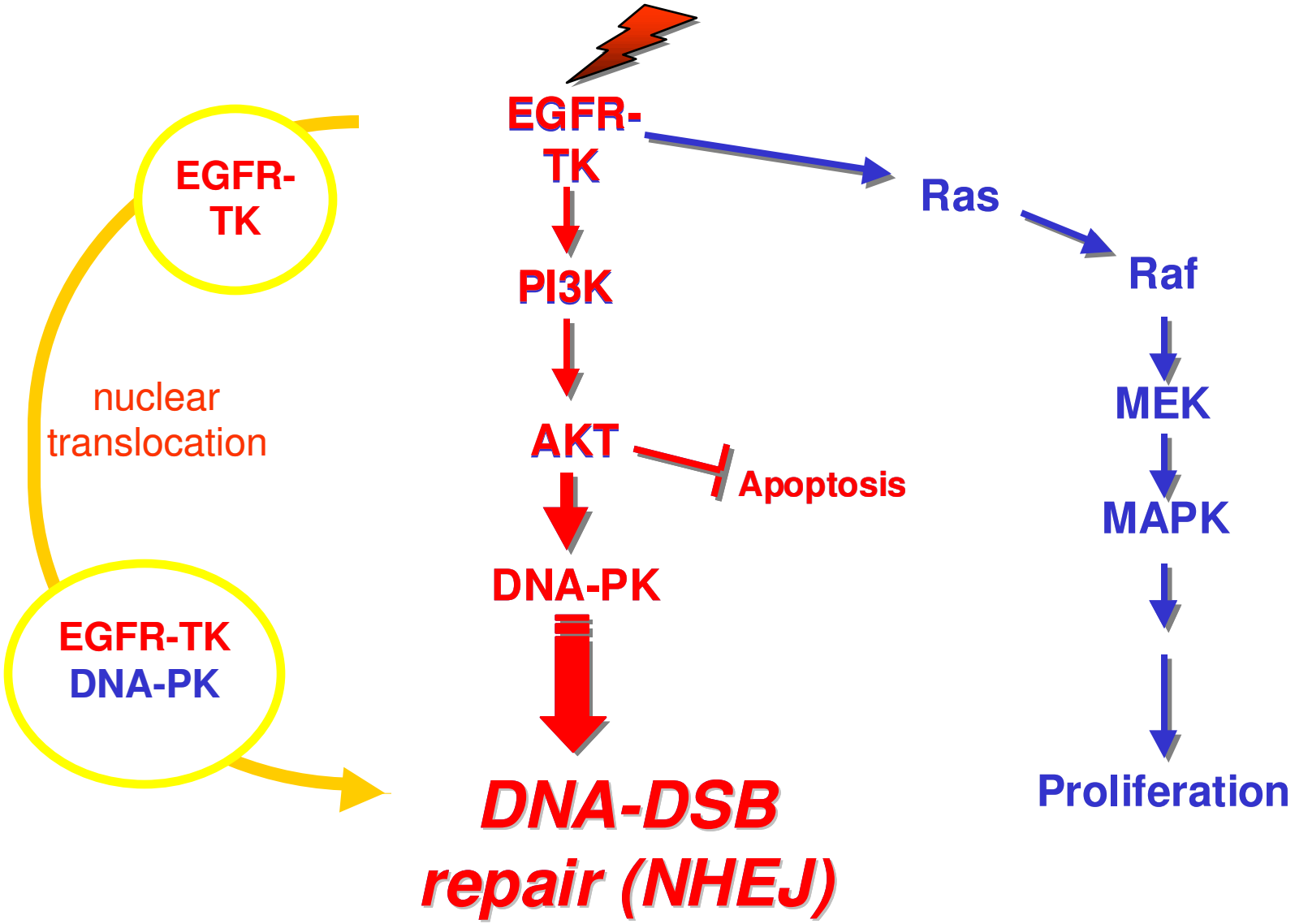


A549 + BIBX + 6 Gy





Importance of EGFR-signaling for DNA-DSB repair in K-Ras$_{mt}$ tumor cells



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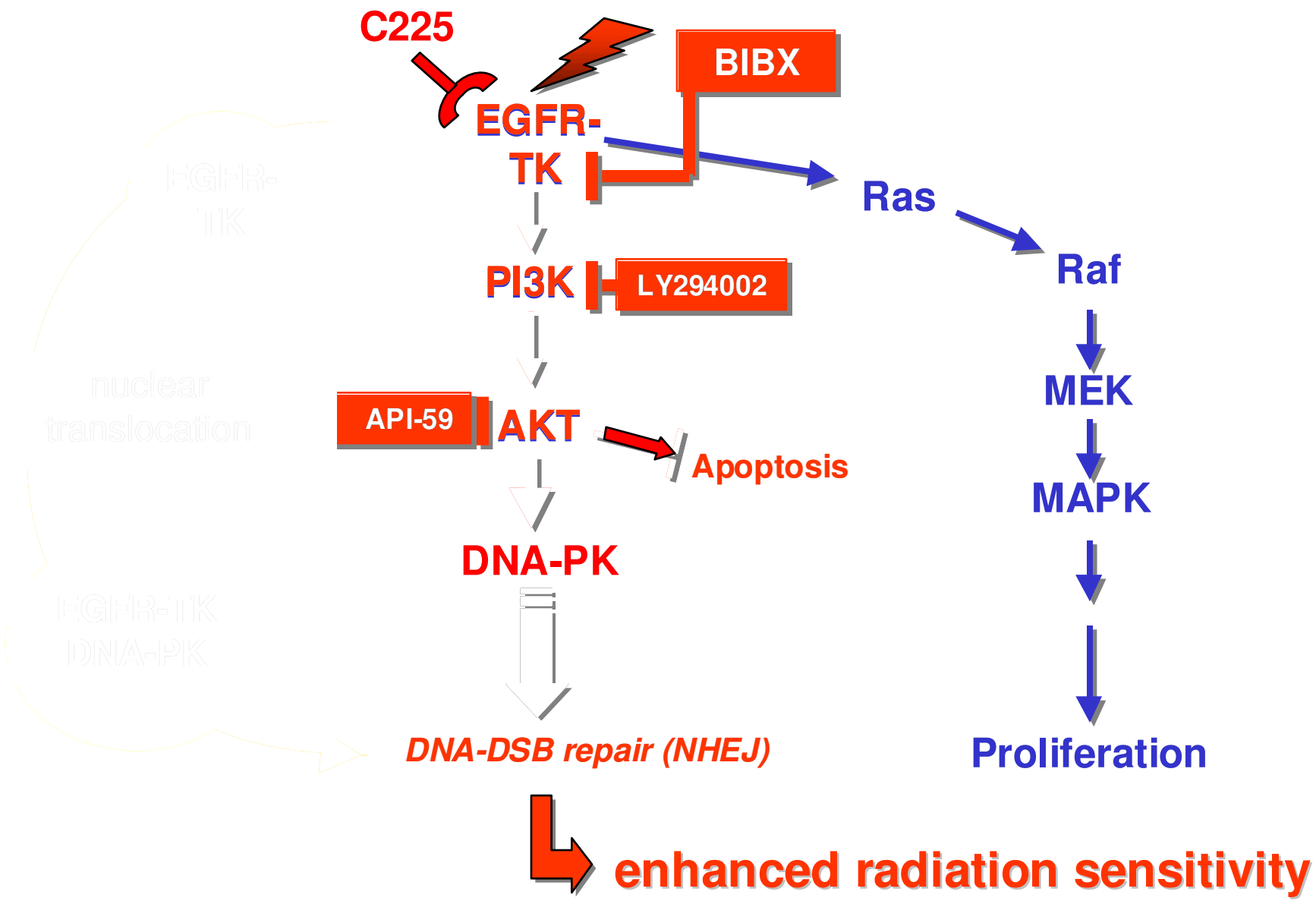
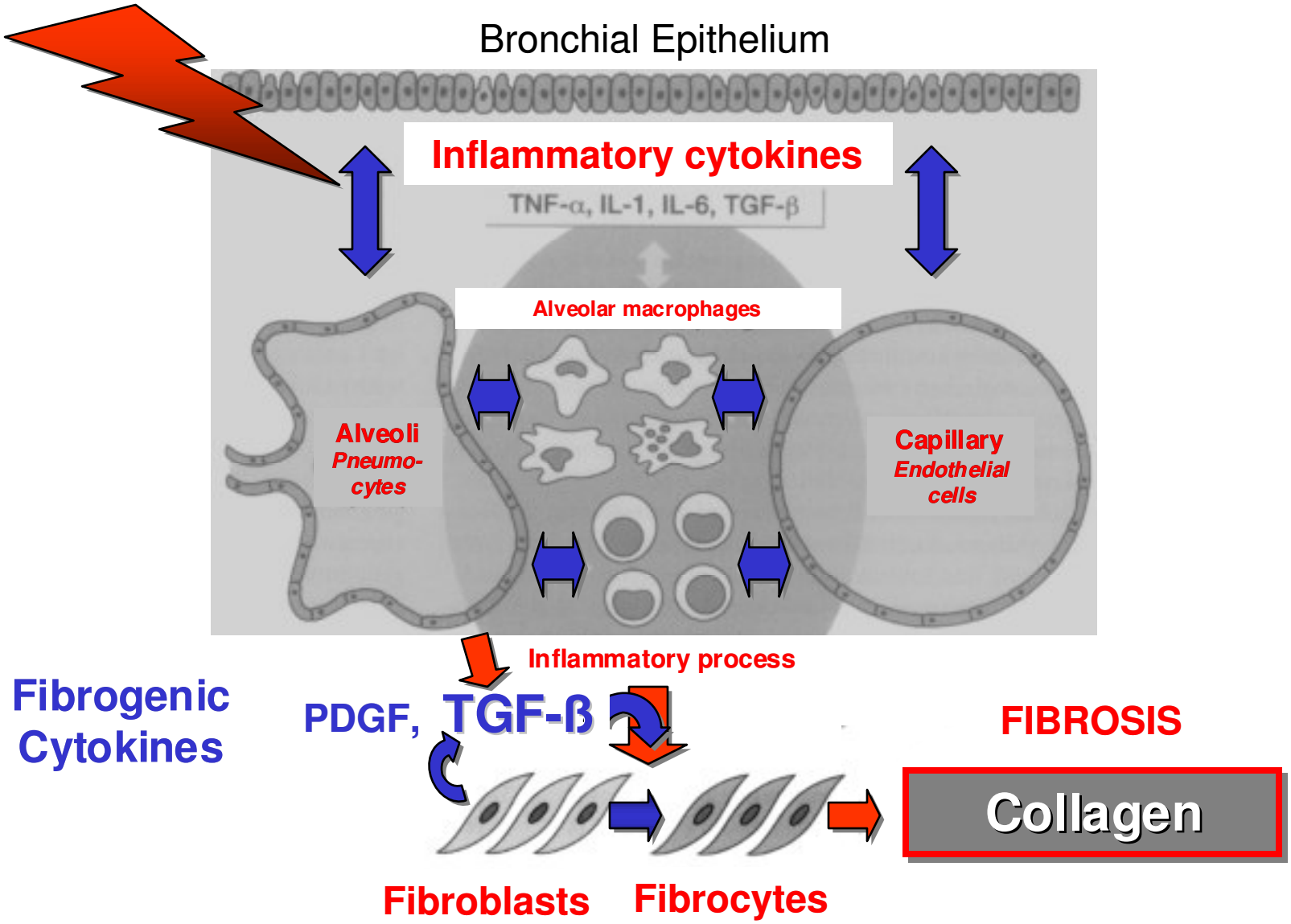


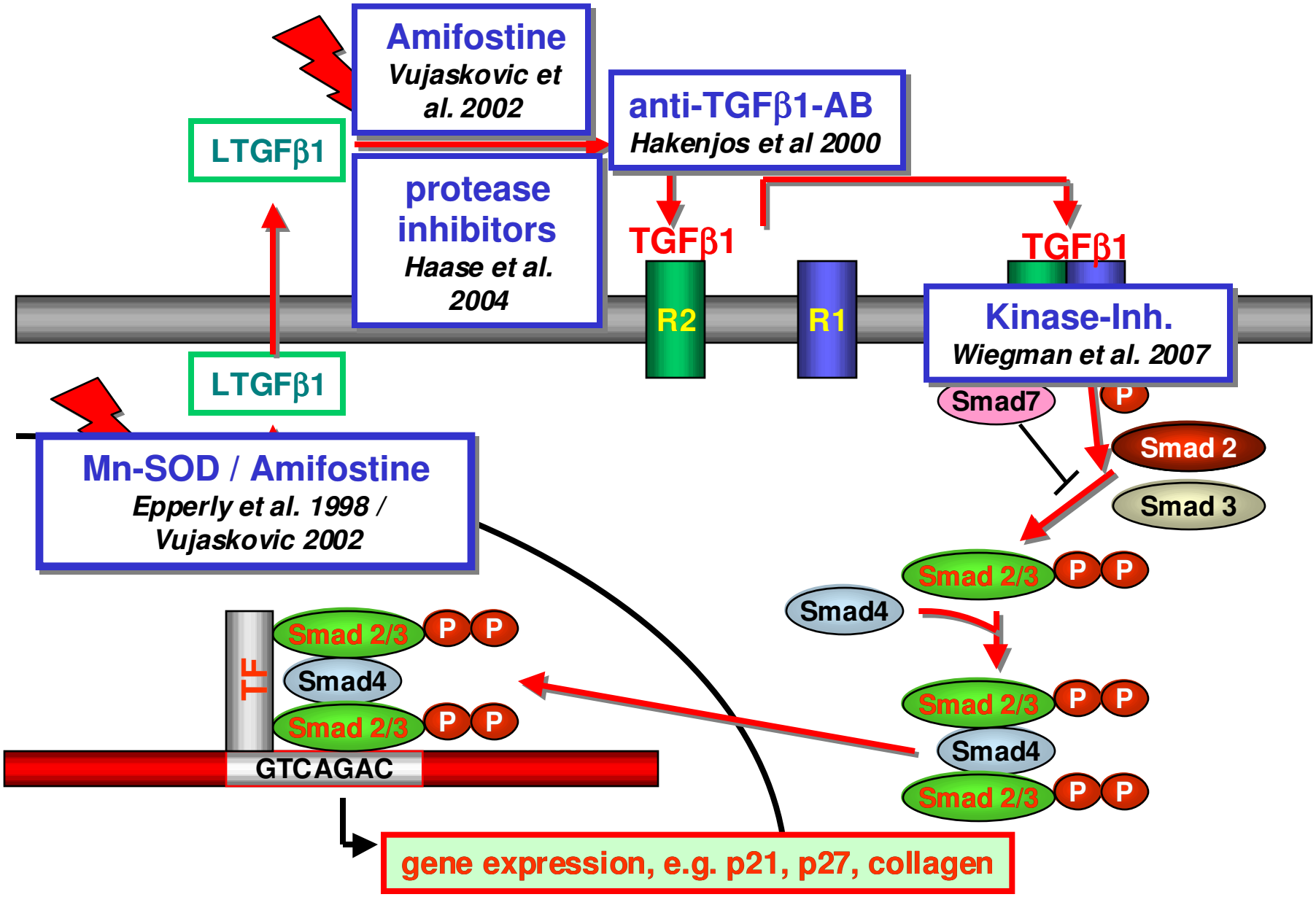
TGFß

Tissue responses resulting in radiation-induced fibrosis of the lung

(Rübe et al. 2004)



TGFß1-signaling and potential *targets* for intervention



Radiation-induced lung fibrosis

(Vujaskovic et al. 2002)

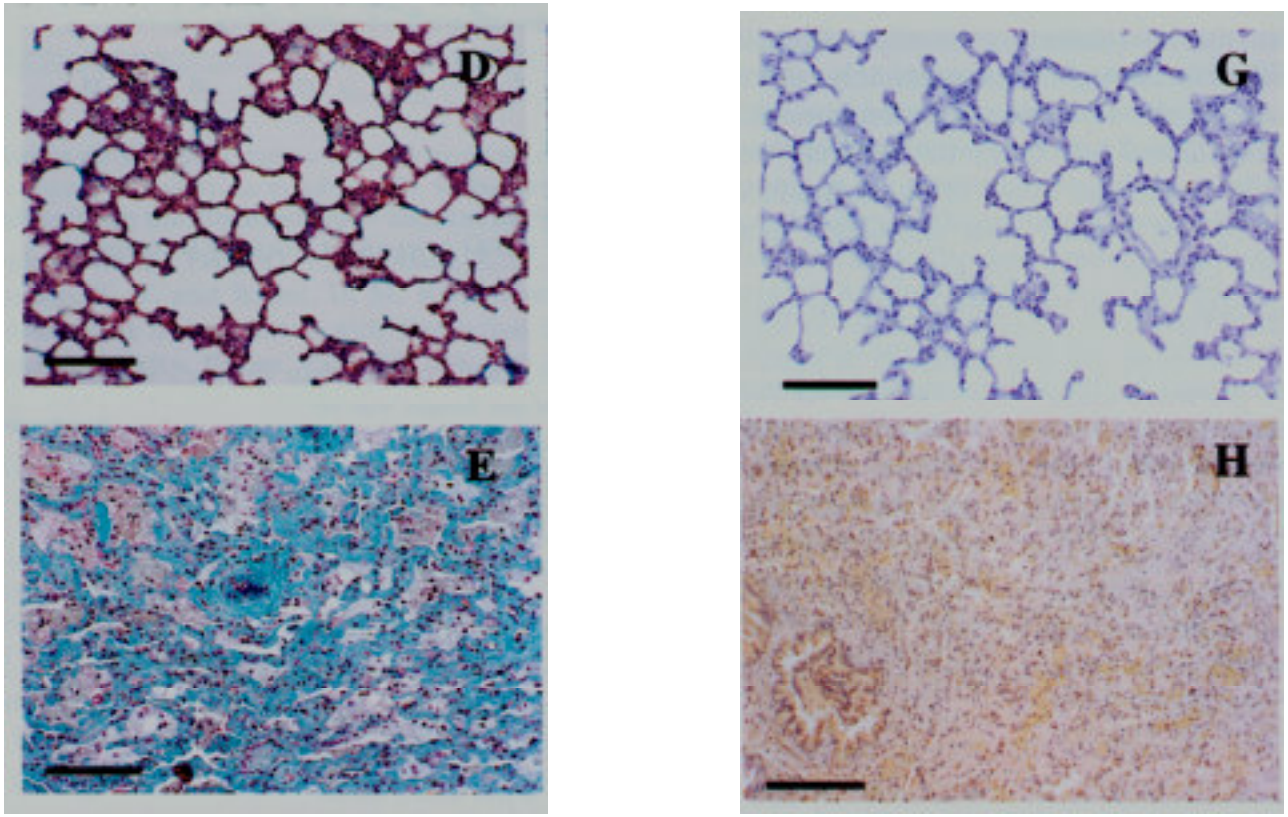
Rat lung

Total collagen production

TGFß1 production

before RT

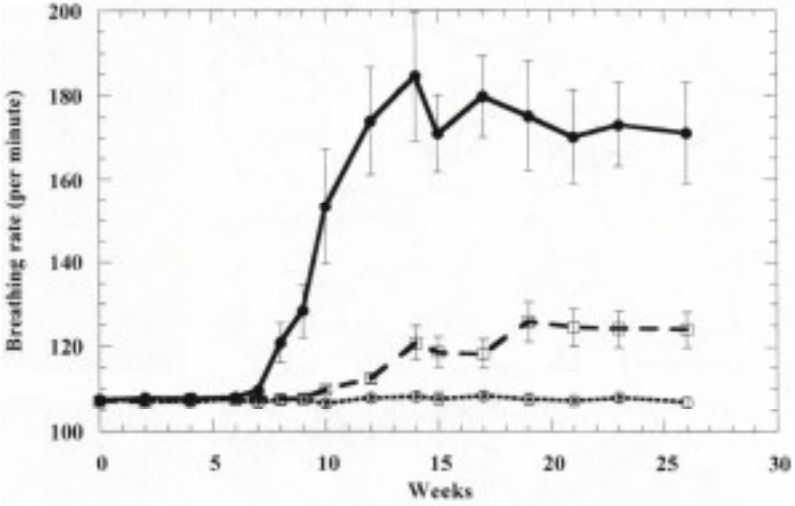
6 month after IR (SD 28 Gy)

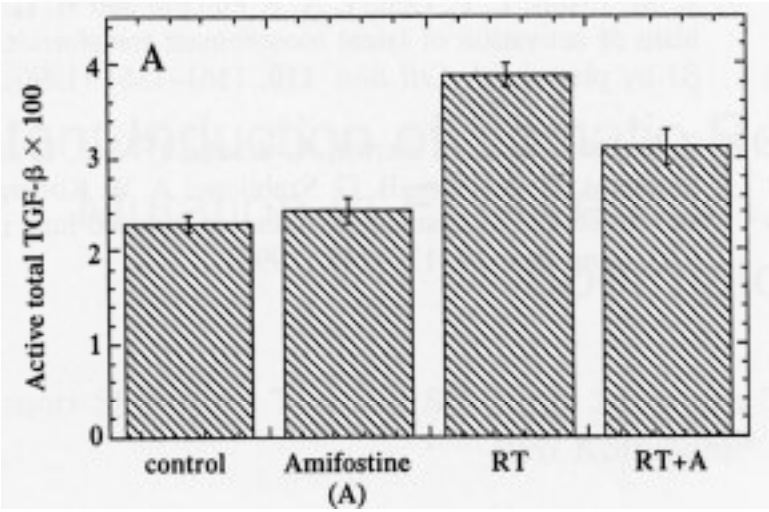


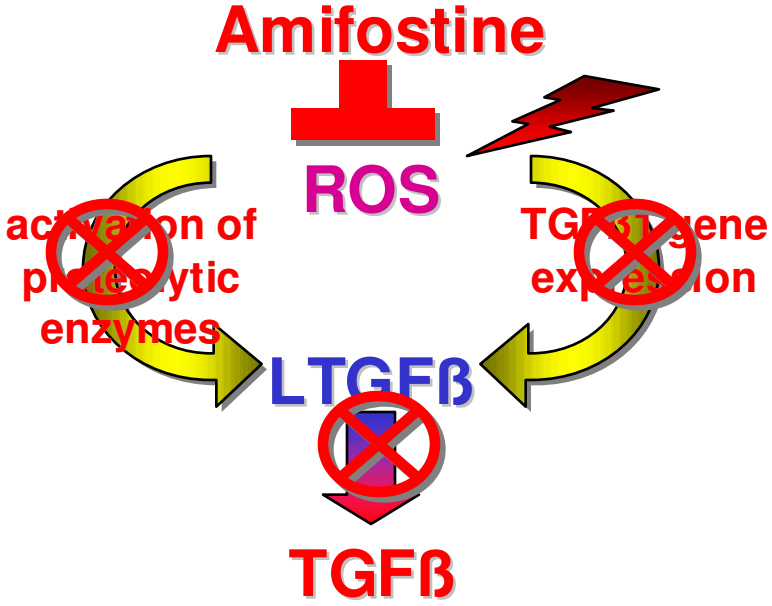
Amifostine prevents manifestation of radiation-induced fibrosis of the lung by inhibiting TGF-ß production

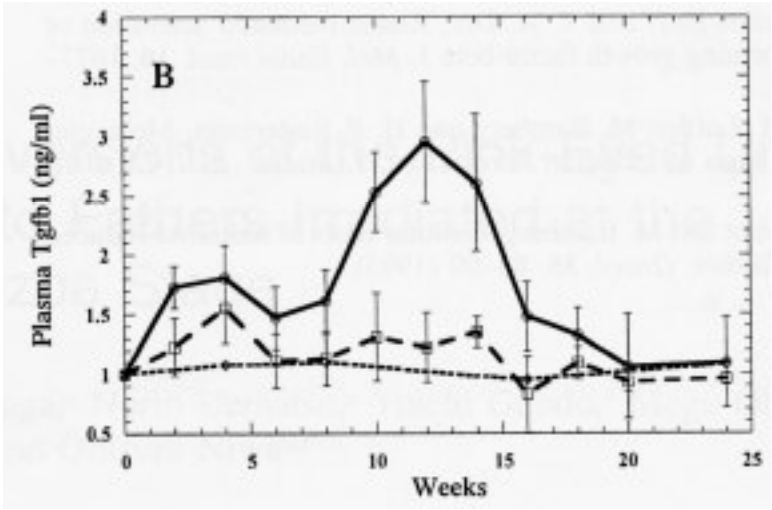
(Vujaskovic et al. 2002)

150 mg/kg amifostine 30 min before IR



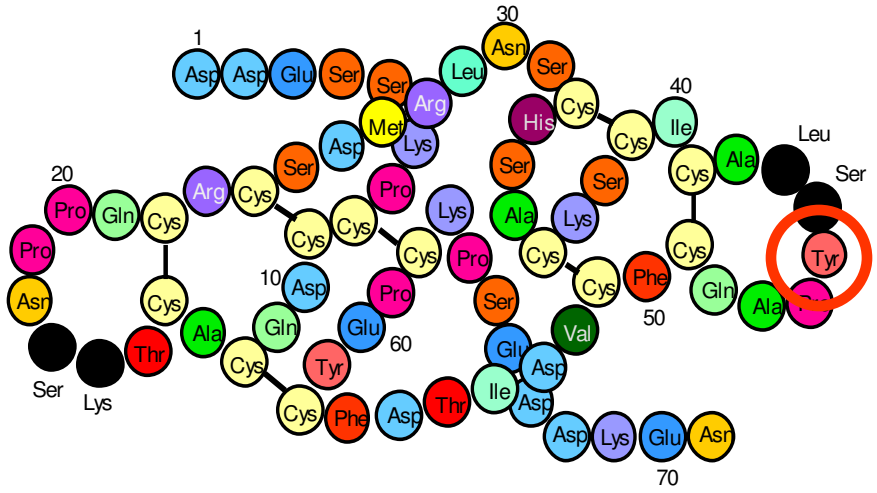






BBI / pTyr

Bowman-Birk Proteinase Inhibitor (BBI) and pTyr

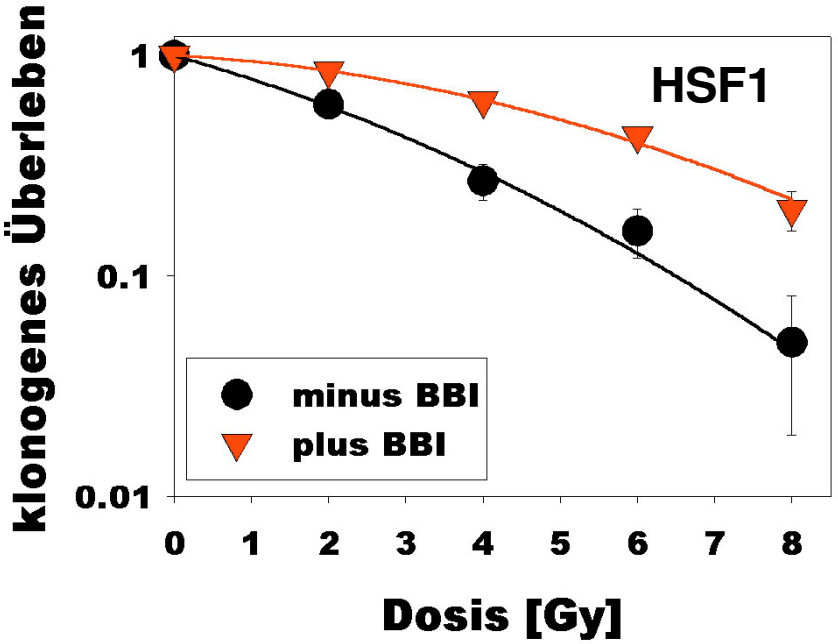


phospho-tyrosine (pTyr)

BBI affects normal and tumorigenic human fibroblasts differentially

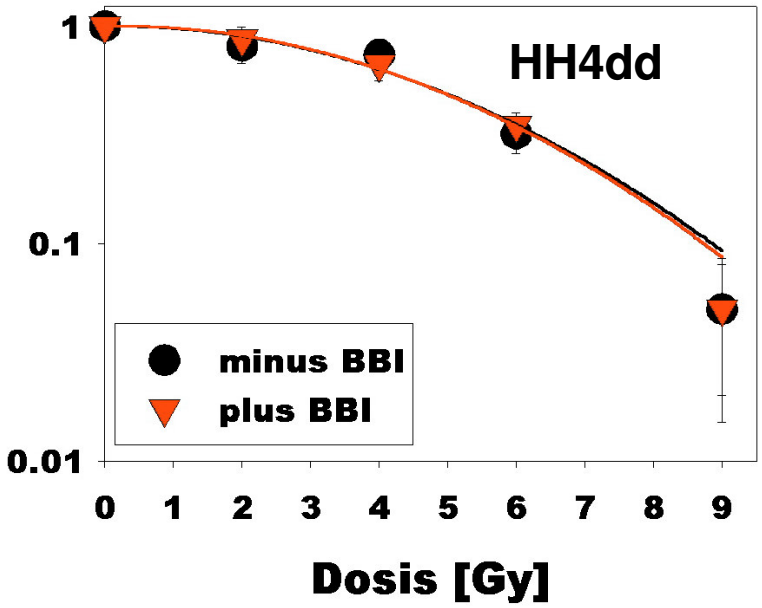
(Dittmann et al.1995)

Normal human skin fibroblasts



wildtype p53

Transformed, tumorigenic human skin fibroblasts

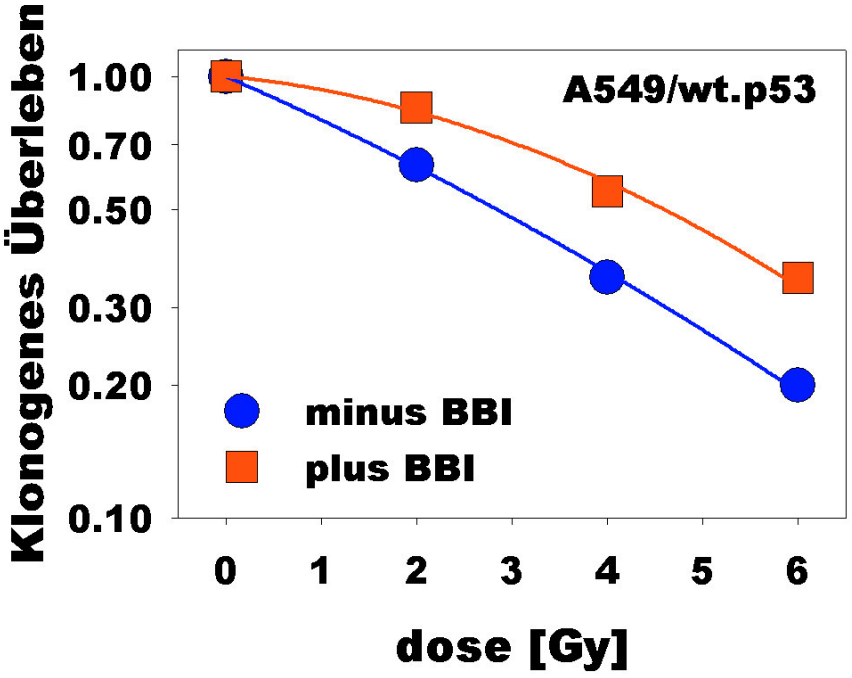


mutated p53

BBI affects $p53_{wt}$ and $p53_{mt}$ tumor cells differentially

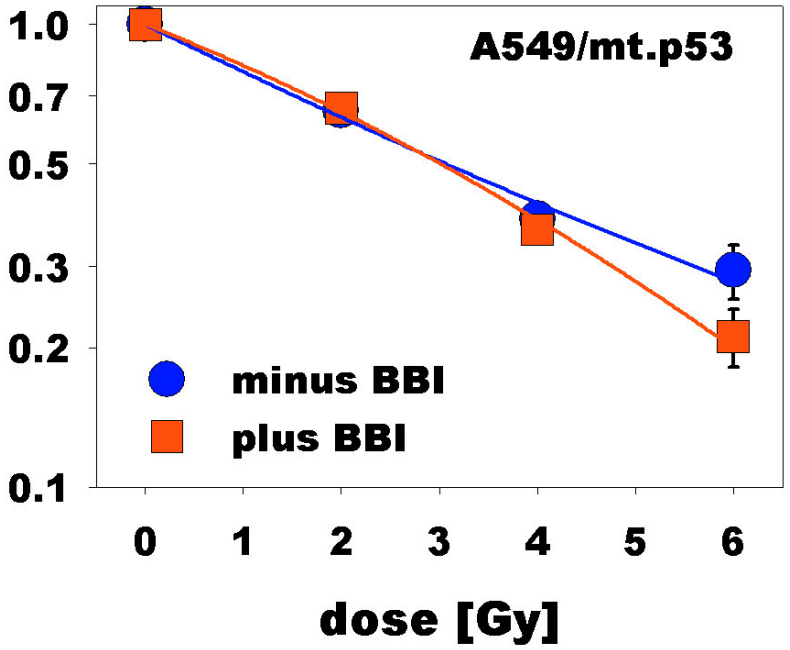
(Dittmann et al.1998)

lung adeno carcinoma cell line A549 presenting wildtype p53



wildtype p53: *functional*

lung adeno carcinoma cell line A549 transfected w/ mutated p53



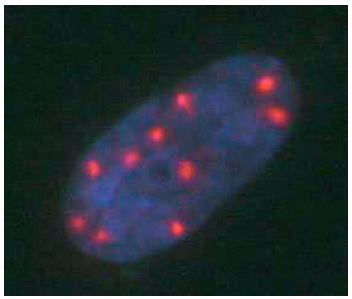
mutated p53: *non-functional*

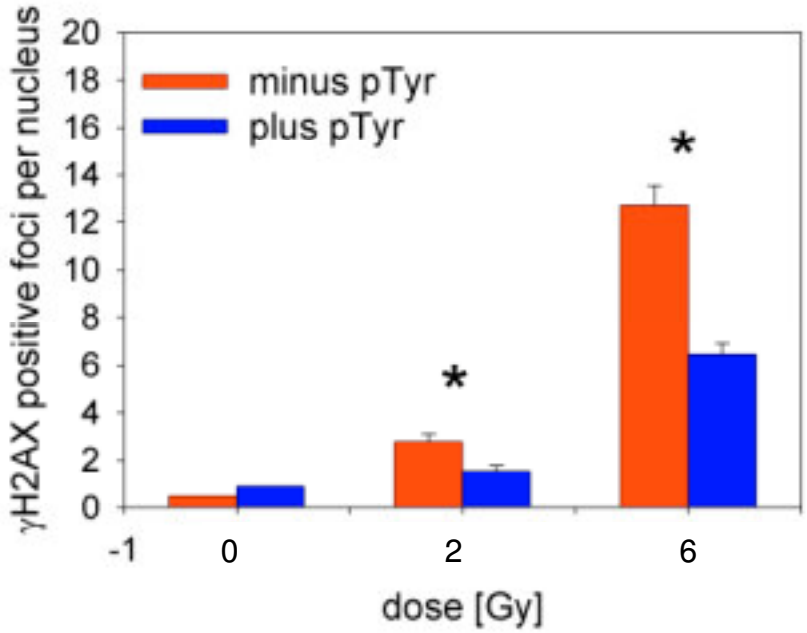
Stimulation of DNA-DSB-repair by P-Tyr in $p53_{wt}$ cells

(Dittmann et al. 2006)

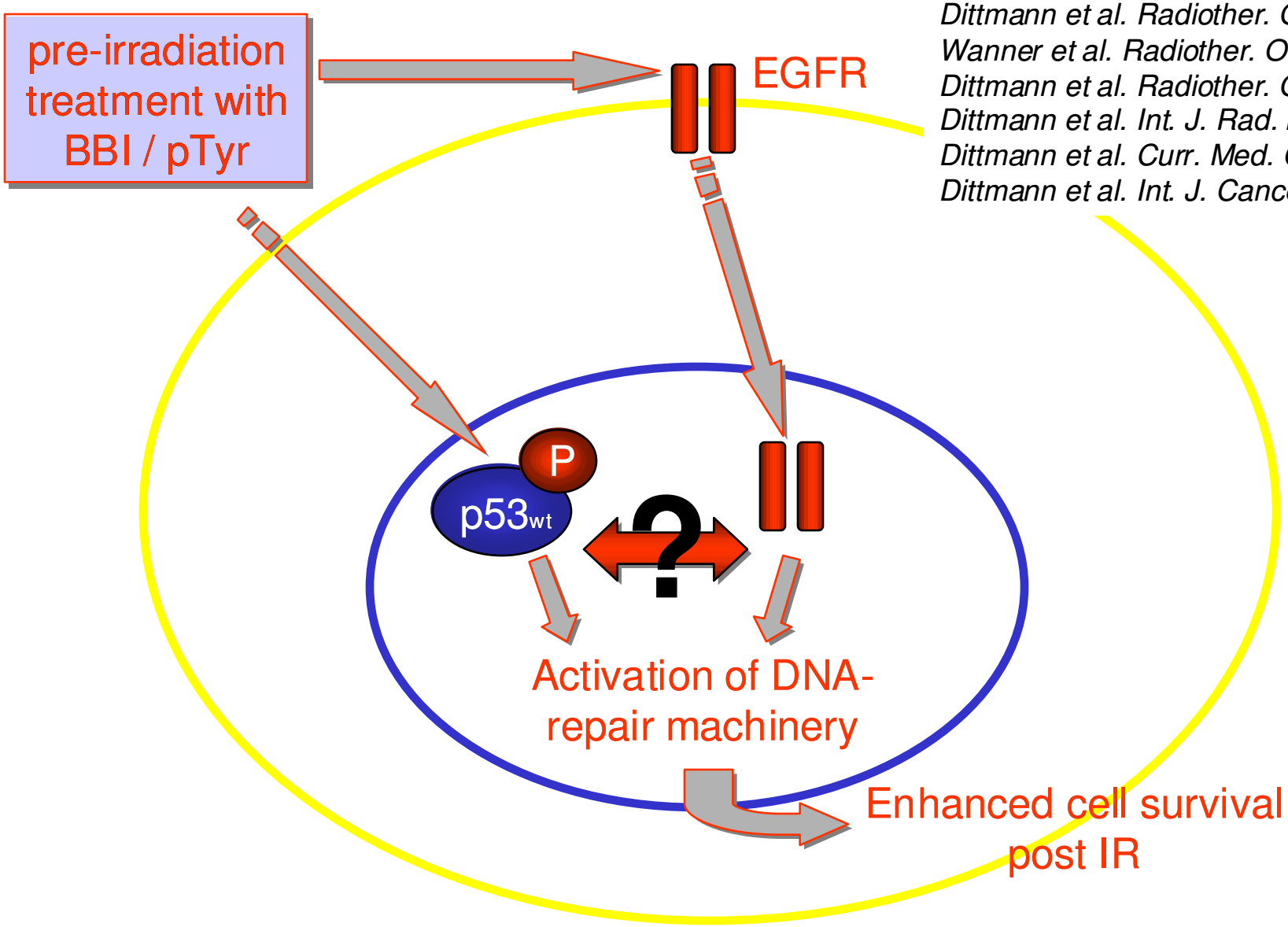
γ-H2AX-focus assay was used for determination of residual DNA-DSB 24 h post IR

fibroblasts, $p53_{wt}$





Mode of action of BBI and pTyr

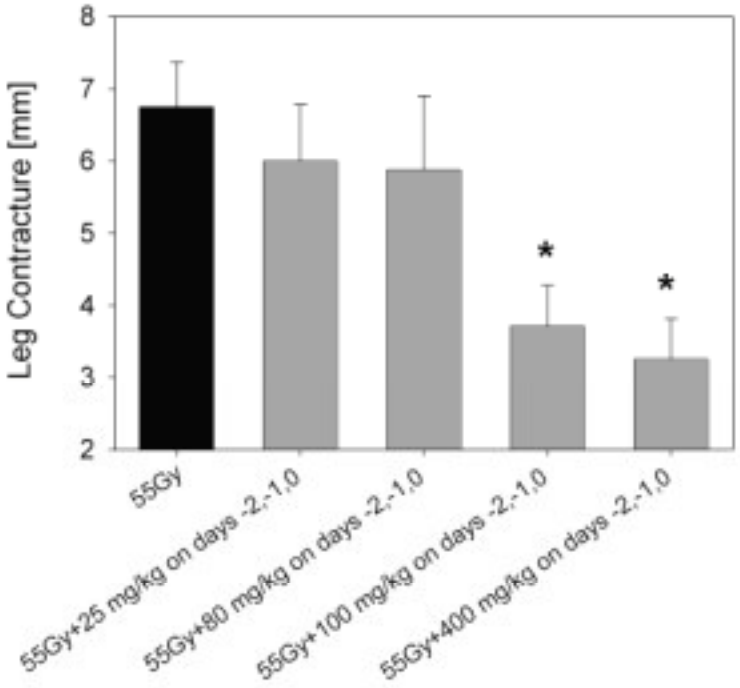


Dittmann et al. Radiother. Oncol 2008
Wanner et al. Radiother. Oncol 2008
Dittmann et al. Radiother. Oncol 2007
Dittmann et al. Int. J. Rad. Biol. 2003
Dittmann et al. Curr. Med. Chem. 2003
Dittmann et al. Int. J. Cancer 2001

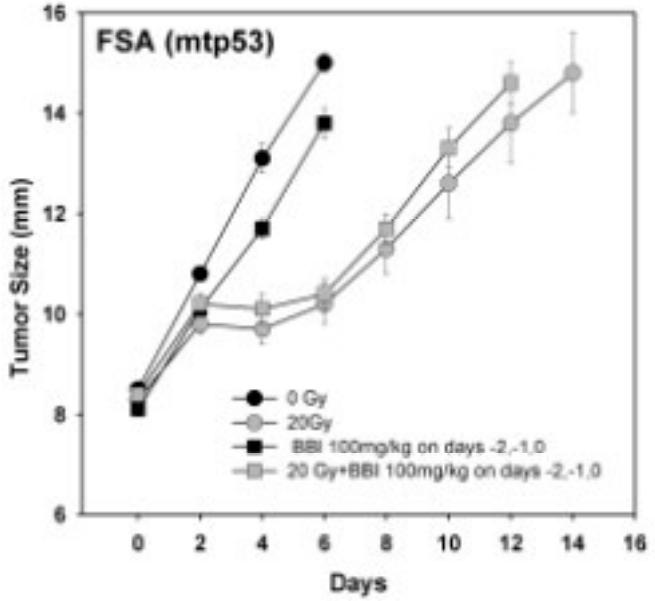
BBI effect in vivo: normal tissue vs. tumor response

(Dittmann et al. 2005)

Leg contracture assay



Tumor growth delay assay



C3H-mice were treated 3x with different doses of BBI at day –2, day -1, and day 0 before IR; both hind legs were irradiated w/ SD of 55 Gy. Leg contracture was quantified up to 120 days post IR.

Current impact of proposed perspectives

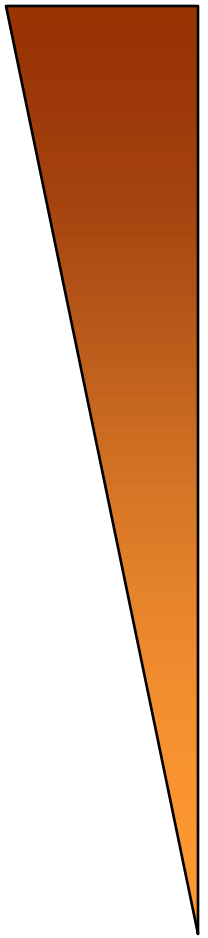
Identification of molecular targets for radiosesitization / radioprotection in the context of their normal and pathological mechanisms

Identification of tissue specific target structures on the basis of biological / molecular imaging

Identification of genetic markers of individual radiation sensitivity (*Genomics / Proteomics*)

Development of molecular prediction for RT (*Theranostics*)

Application of stem cells to rescue damaged normal tissue



Thanks to the co-workers ....