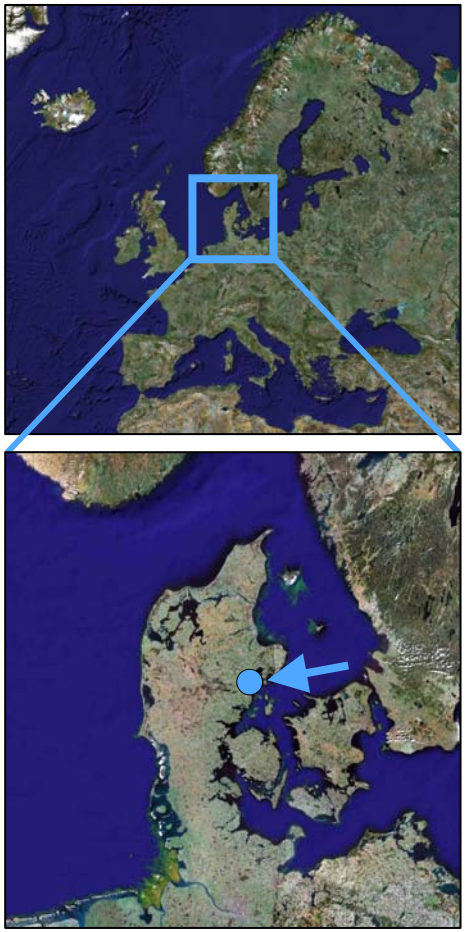
Predicting Individual Radiation Sensitivity:
Current and Evolving Technologies
Columbia University Kellogg Center, 2008

SNPs and late fibrosis



Jan Alsner

(Molecular biologist)

Department of Experimental Clinical Oncology

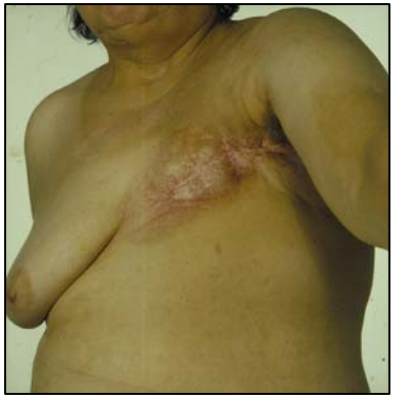
Aarhus University Hospital

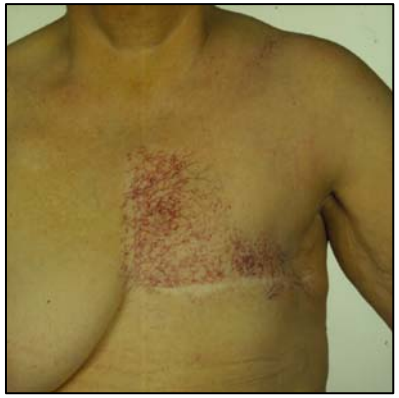
Denmark

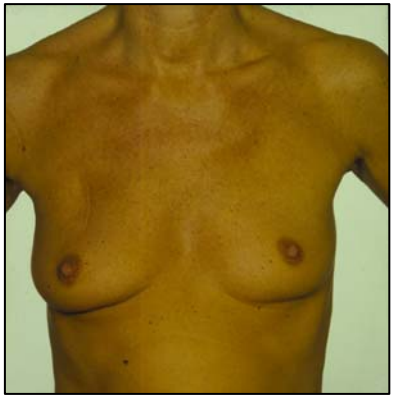
Supported by the Danish Cancer Society

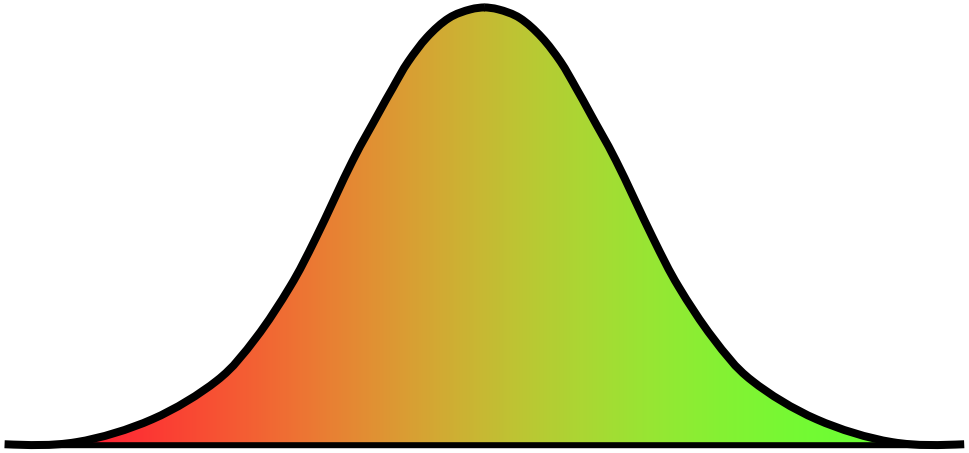
Variations in risk normal tissue toxicity

Within normal range (not over-reactors)









More sensitive

More resistant

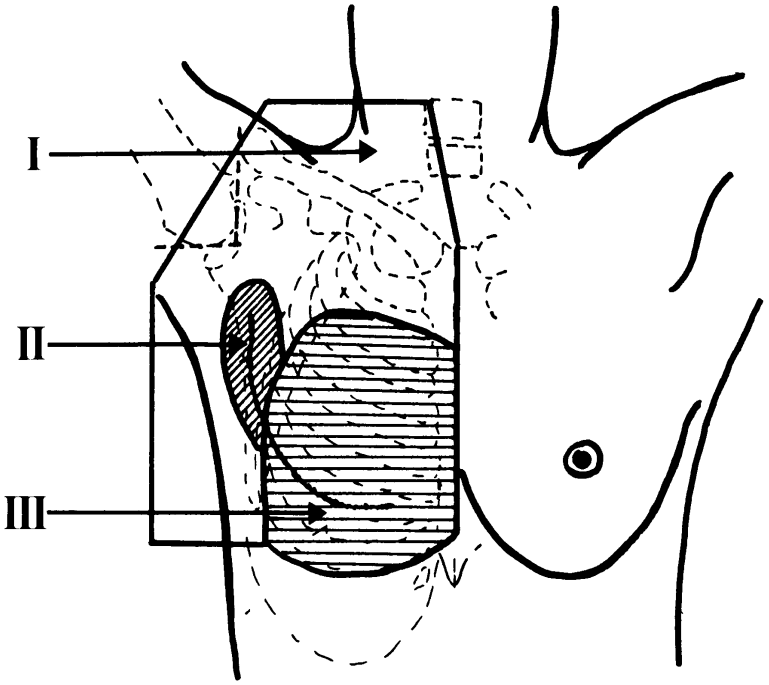
Background

- Aarhus post-mastectomy cohort
  - 319 patients treated '78-'82
  - Fibroblasts from 41 patients

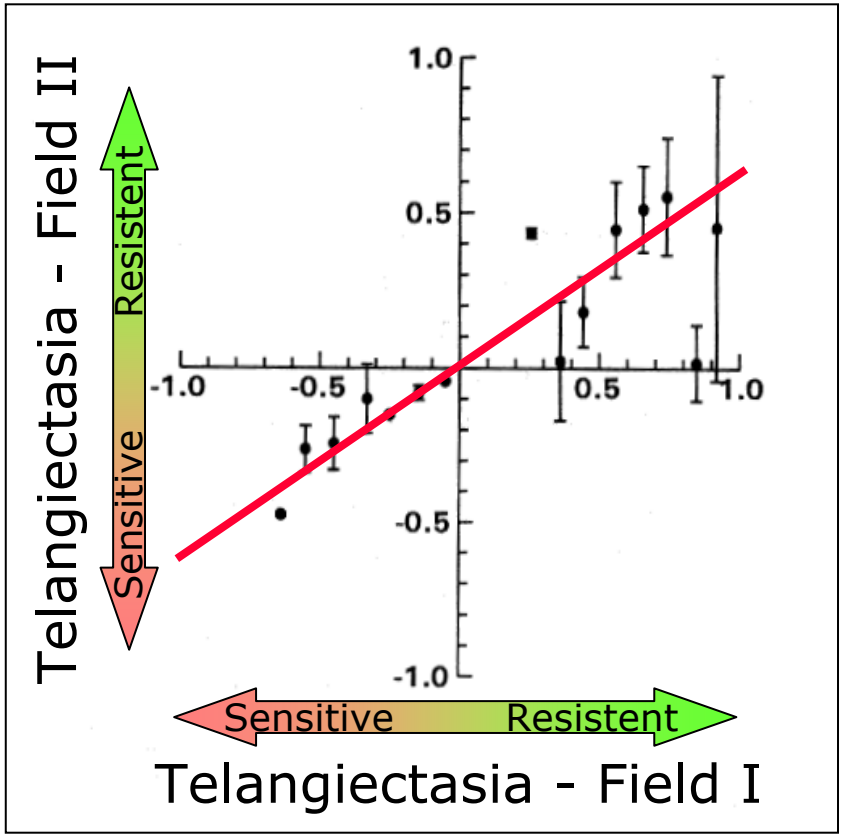
Photon field without bolus

”Scar” photon field with bolus

Chest wall electron field

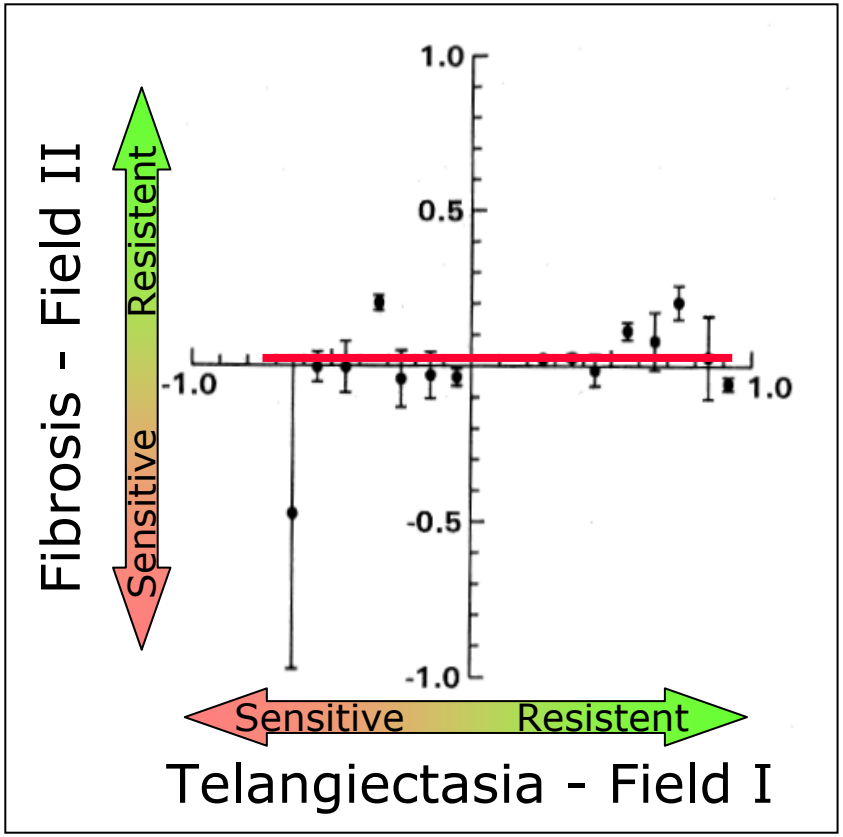


Intra- and inter-patient variation



Telangiectasia *versus* telangiectasia

Correlation

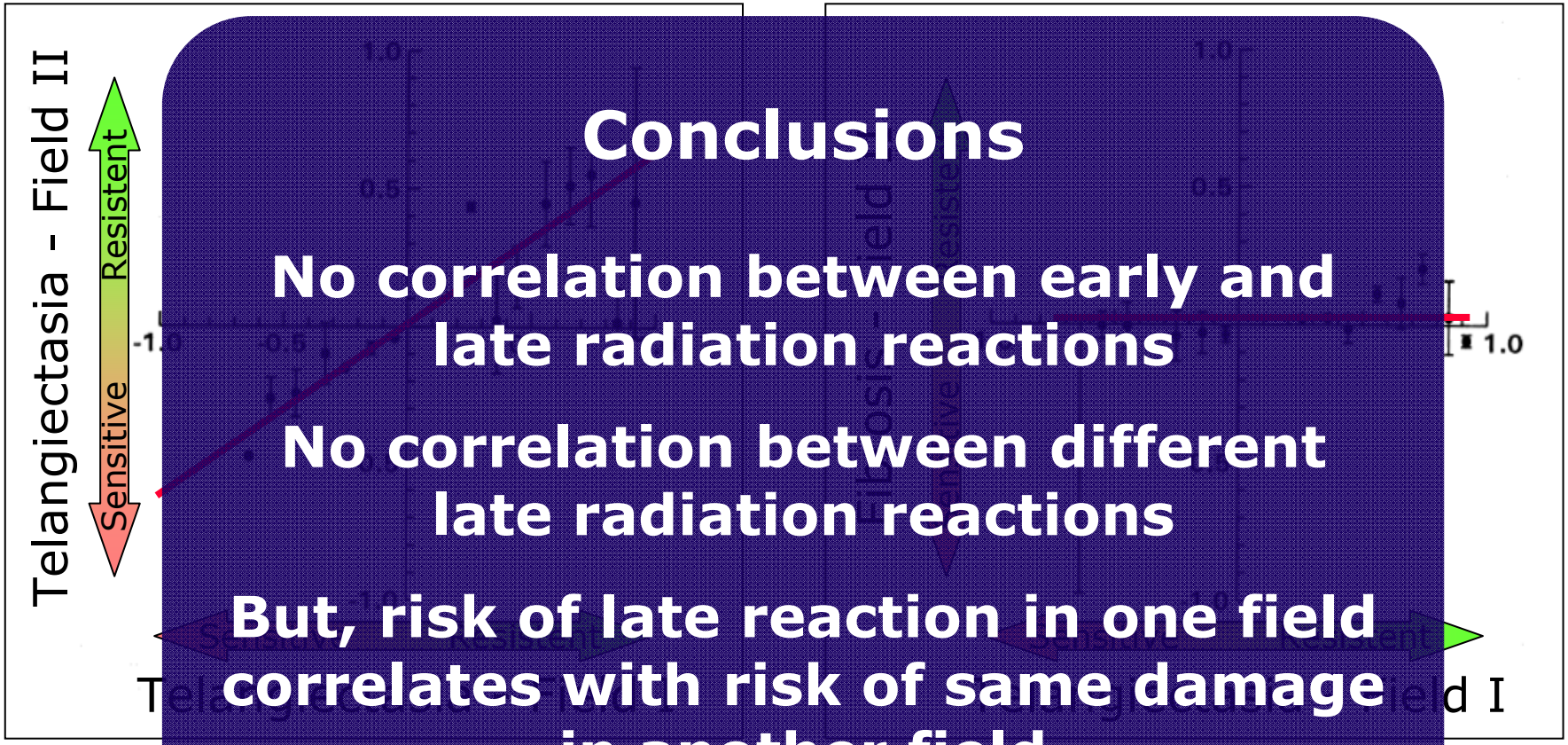


Telangiectasia *versus* fibrosis

No correlation

Bentzen and Overgaard (1991) Radiother Oncol 20:159-165
Bentzen and Overgaard's (1993) Eur J Cancer 29A:1373-1376

Intra- and inter-patient variation



Conclusions

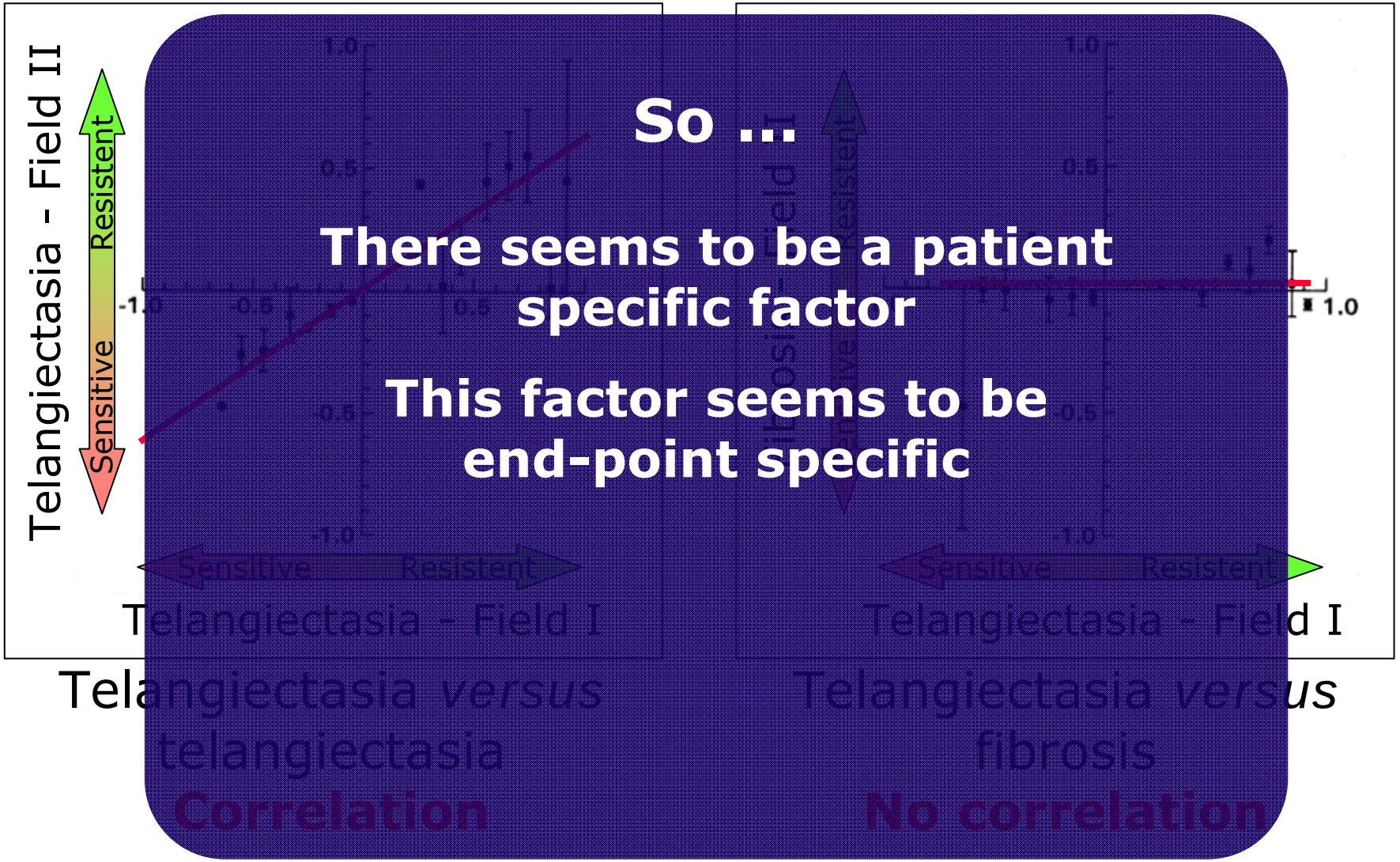
No correlation between early and late radiation reactions

No correlation between different late radiation reactions

But, risk of late reaction in one field correlates with risk of same damage in another field

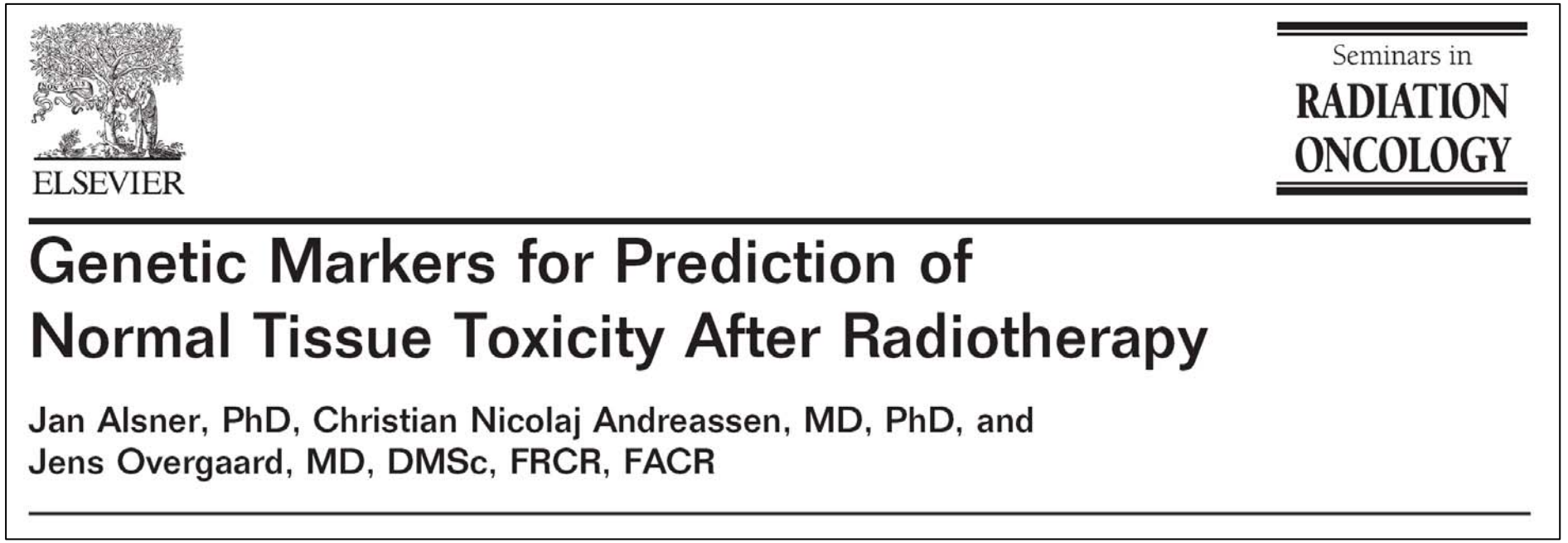
Bentzen and Overgaard (1991) Radiother Oncol 20:159-165
Bentzen and Overgaard's (1993) Eur J Cancer 29A:1373-1376

Intra- and inter-patient variation



Bentzen and Overgaard (1991) Radiother Oncol 20:159-165
Bentzen and Overgaard's (1993) Eur J Cancer 29A:1373-1376

Predicting normal tissue toxicity



ELSEVIER

Seminars in
RADIATION
ONCOLOGY

Genetic Markers for Prediction of Normal Tissue Toxicity After Radiotherapy

Jan Alsner, PhD, Christian Nicolaj Andreassen, MD, PhD, and
Jens Overgaard, MD, DMSc, FRCR, FACR

Alsner et al. (2008) Seminars in Radiation Oncology 18:126-135
April 2008 Issue: Prognostic and Predictive Markers in Oncology

Predicting normal tissue toxicity

- Candidate gene *versus* whole-genome association studies
- Methodological problems
  - Quantitative complex traits and endpoints
  - Clinical *versus* biological phenotypes
  - Tagging SNPs *versus* functional variants
  - Genetic haplotypes
  - Confounding factors
- Intermediate *versus* clinical phenotypes
  - Differential gene expression between patients with either low or high risk of radiation-induced fibrosis in patient-derived fibroblasts after irradiation

Genetic association studies

First author, year	Gene(s) investigated	N=	Conclusion
Ahn, 2006	*CAT, SOD2, MPO, eNOS*	446	No significant associations with acute skin toxicity. Association between obesity and skin toxicity possibly modified by *MPO* and *eNOS* SNPs
Ambrosone, 2006	*GSTA1, GSTP1, GSTM1, GSTT1*	446	*GSTP1* codon 105 Val allele significantly associated with increased risk of acute skin toxicity
Andreassen, 2003	*TGFB1, SOD2, XRCC1, XRCC3, APEX*	41	Risk of subcutaneous fibrosis significantly associated with the *TGFB1* position -509 T and codon 10 Pro alleles, *SOD2* codon 16 Ala and *XRCC1* codon 399 Arg alleles. *XRCC3* codon 241 Thr allele associated with risk of subcutaneous fibrosis and telangiectasia
Andreassen, 2005	*TGFB1, SOD2, XRCC1, XRCC3, APEX, ATM*	52	Risk of altered breast appearance significantly associated with the position –509 T and codon 10 Pro alleles in 26 matched case–control pairs
Andreassen, 2006	*ATM*	41	Risk of subcutaneous fibrosis significantly associated with the codon 1853 Asp/Asn and Asn/Asn genotypes
Andreassen, 2006	*TGFB1, SOD2, XRCC1, XRCC3, APEX, ATM*	120	No significant associations between the investigated SNPs and risk of radiation-induced subcutaneous fibrosis
Angele, 2003	*ATM*	24	Significant association between the *ATM* codon 1853 Asn/Asn genotype and risk of various acute and late adverse normal tissue reactions. intronic IVS22-77 CC genotype associated with reduced risk
Appleby, 1997	*ATM*	23	No *ATM* mutations detected in 23 patients with severe acute or late toxicity
Borgmann, 2002	*ATM, NBS, MRE11, RAD50, DNA ligase IV*	5	No mutations detected in five patients with severe late toxicity, possible *DNA ligase IV* polymorphism detected in one patient
Brem, 2006	*XRCC1*	247	Haplotype consisting of majority alleles in 4 polymorphic sites associated with increased radiosensitivity (mixed endpoint)
Bremer, 2003	*ATM*	10	No indications of increased acute or late radio-sensitivity in 10 patients being heterozygous for pathogenic *ATM* mutations
Cesaretti, 2005	*ATM*	37	Possession of missense mutations significantly associated with radiation-induced rectal bleeding and erectile dysfunction

List of 40 association studies

(before summer 2007)

Alsner et al. (2008) Seminars in Radiation Oncology 18:126-135

Whole genome association studies

- Stage design

Vol 447|28 June 2007|doi:10.1038/nature05887 nature

ARTICLES

Genome-wide association study identifies novel breast cancer susceptibility loci

Douglas F. Easton[1], Karen A. Pooley[2], Alison M. Dunning[2], Paul D. P. Pharoah[2], Deborah Thompson[1], Dennis G. Ballinger[3], Jeffery P. Struewing[4], Jonathan Morrison[2], Helen Field[2], Robert Luben[5], Nicholas Wareham[5], Shahana Ahmed[2], Catherine S. Healey[2], Richard Bowman[6], the SEARCH collaborators[2*], Kerstin B. Meyer[7], Christopher A. Haiman[8], Laurence K. Kolonel[9], Brian E. Henderson[8], Loic Le Marchand[9], Paul Brennan[10], Suleeporn Sangrajrang[11], Valerie Gaborieau[10], Fabrice Odefrey[10], Chen-Yang Shen[12], Pei-Ei Wu[12], Hui-Chun Wang[12], Diana Eccles[13], D. Gareth Evans[14], Julian Peto[15], Olivia Fletcher[16], Nichola Johnson[16], Sheila Seal[17], Michael R. Stratton[17,18], Nazneen Rahman[17], Georgia Chenevix-Trench[19], Stig E. Bojesen[20], Børge G. Nordestgaard[20], Christen K. Axelsson[21], Montserrat Garcia-Closas[22], Louise Brinton[22], Stephen Chanock[23], Jolanta Lissowska[24], Beata Peplonska[25], Heli Nevanlinna[26], Rainer Fagerholm[26], Hannaleena Eerola[26,27], Daehee Kang[28], Keun-Young Yoo[28,29], Dong-Young Noh[28], Sei-Hyun Ahn[30], David J. Hunter[31,32], Susan E. Hankinson[32], David G. Cox[31], Per Hall[33], Sara Wedren[33], Jianjun Liu[34], Yen-Ling Low[34], Natalia Bogdanova[35,36], Peter Schürmann[36], Thilo Dörk[36], Rob A. E. M. Tollenaar[37], Catharina E. Jacobi[38], Peter Devilee[39], Jan G. M. Klijn[40], Alice J. Sigurdson[41], Michele M. Doody[41], Bruce H. Alexander[42], Jinghui Zhang[4], Angela Cox[43], Ian W. Brock[43], Gordon MacPherson[43], Malcolm W. R. Reed[44], Fergus J. Couch[45], Ellen L. Goode[45], Janet E. Olson[45], Hanne Meijers-Heijboer[46,47], Ans van den Ouweland[47], André Uitterlinden[48], Fernando Rivadeneira[48], Roger L. Milne[49], Gloria Ribas[49], Anna Gonzalez-Neira[49], Javier Benitez[49], John L. Hopper[50], Margaret McCredie[51], Melissa Southey[50], Graham G. Giles[52], Chris Schroen[53], Christina Justenhoven[54], Hiltrud Brauch[54], Ute Hamann[55], Yon-Dschun Ko[56], Amanda B. Spurdle[19], Jonathan Beesley[19], Xiaoqing Chen[19], kConFab[57*], AOCS Management Group[19,57*], Arto Mannermaa[58,59], Veli-Matti Kosma[58,59], Vesa Kataja[58,60], Jaana Hartikainen[58,59], Nicholas E. Day[5], David R. Cox[3] & Bruce A. J. Ponder[2,7]

Stage 1
>225,000 SNPs
~400 cases
~400 controls

Stage 2
>10,000 SNPs
~4,000 cases
~4,000 controls

Stage 3
30 SNPs
~22,000 cases
~22,000 controls

Whole genome association studies

- Direct design

Vol 447|7 June 2007|doi:10.1038/nature05911 nature

ARTICLES

Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls

The Wellcome Trust Case Control Consortium*

>500,000 SNPs

Quantitative complex traits

- A biological trait that has measurable phenotypic variation
- Genetic basis often involves the effect of several genes
  - Some affect the phenotype in an almost qualitative "all-or-none" way
  - Usually each causal gene only makes a small contribution to overall susceptibility
- Quantitative complex traits are often under environmental influences

Endpoints

- Most common diseases + risk of radiation-induced morbidity: quantitative complex traits
- Binary phenotypes
  (like disease occurrence "yes or no")
- Complex phenotypes (radiation-induced morbidity)
  - Severity and the time factor (late effects)
  - Clinical *versus* biological phenotypes
  - Confounding factors
  - Genotype-phenotype associations

Clinical *versus* biological phenotypes

- Presumably, differences exist between the genetic component of various types of radiation-induced morbidity in unselected patients
  - Example: Data from the Aarhus post-mastectomy cohort
- Even if the same overall endpoint is evaluated, clinically defined phenotypes might represent a different underlying molecular pathology
  - Example: alterations in breast appearance after irradiation and late radiation morbidity assessed by palpation of subcutaneous induration might not reflect exactly the same biological mechanisms

Confounding factors

- Potential confounding factors are differences in
  - radiation dose and type
  - target volume
  - target dose specification (especially when at a variable depth like tumor location)
  - overall treatment time
  - fractionation
  - concomitant chemotherapy
  - juxtaposed skin surfaces
  - immobilizing and dose-modifying device
  - comorbidity (e.g. connective tissue diseases)

Genotype-phenotype associations

- Understanding the biological function of genotype-phenotype associations
  - Tagging SNPs *versus* functional variants
  - Genetic haplotypes

Haplotypes

Carcinogenesis vol.27 no.12 pp.2469–2474, 2006
doi:10.1093/carcin/bgl114
Advance Access publication July 8, 2006

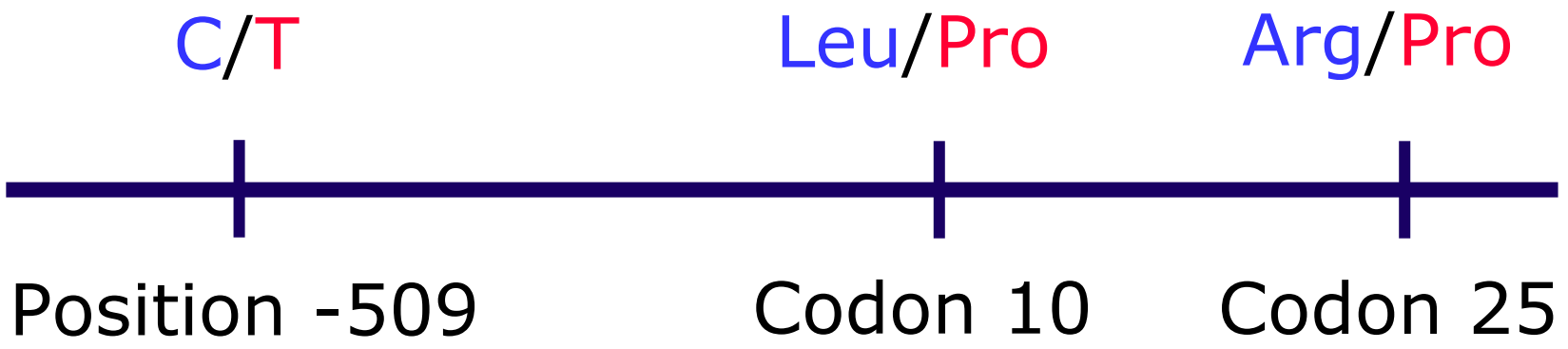
The *XRCC1 −77T→C* variant: haplotypes, breast cancer risk, response to radiotherapy and the cellular response to DNA damage

Reto Brem[1], David G.Cox[2], Brigitte Chapot[1], Norman Moullan[1], Pascale Romestaing[3], Jean-Pierre Gérard[4], Paola Pisani[1] and Janet Hall[1,5,*]

[1]International Agency for Research on Cancer, 150 cours Albert Thomas, 69372 Lyon, France, [2]Department of Epidemiology, Harvard School of Public Health, Boston, MA 02115, USA, [3]Centre Hospitalier Lyon-Sud, Radiothérapie, Curiethérapie, Oncologie, Chemin du Grand-Revoyet, 69496, Pierre Bénite, France and [4]Centre Antoine-Lacassagne, 33 Avenue de Valombrose, 06189 Nice, Cedex 2, France

Haplotypes

TGFβ1 ?



SNP association studies

NATURE|Vol 447|7 June 2007 nature

FEATURE

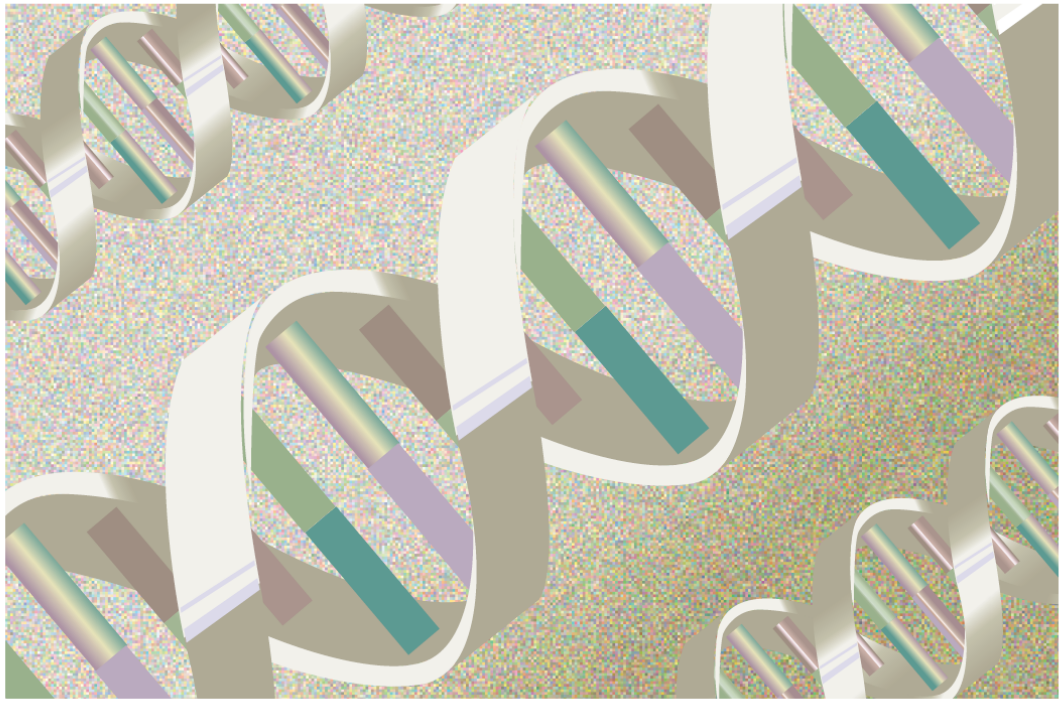
Replicating genotype–phenotype associations

What constitutes replication of a genotype–phenotype association, and how best can it be achieved?

NCI-NHGRI Working Group on Replication in Association Studies

The study of human genetics has recently undergone a dramatic transition with the completion of both the sequencing of the human genome and the mapping of human haplotypes of the most common form of genetic variation, the single nucleotide polymorphism (SNP)[1–3]. In concert with this rapid expansion of detailed genomic information, cost-effective genotyping technologies have been developed that can assay hundreds of thousands of SNPs simultaneously. Together, these advances have allowed a systematic, even 'agnostic', approach to genome-wide interrogation, thereby relaxing the requirement for strong prior hypotheses.

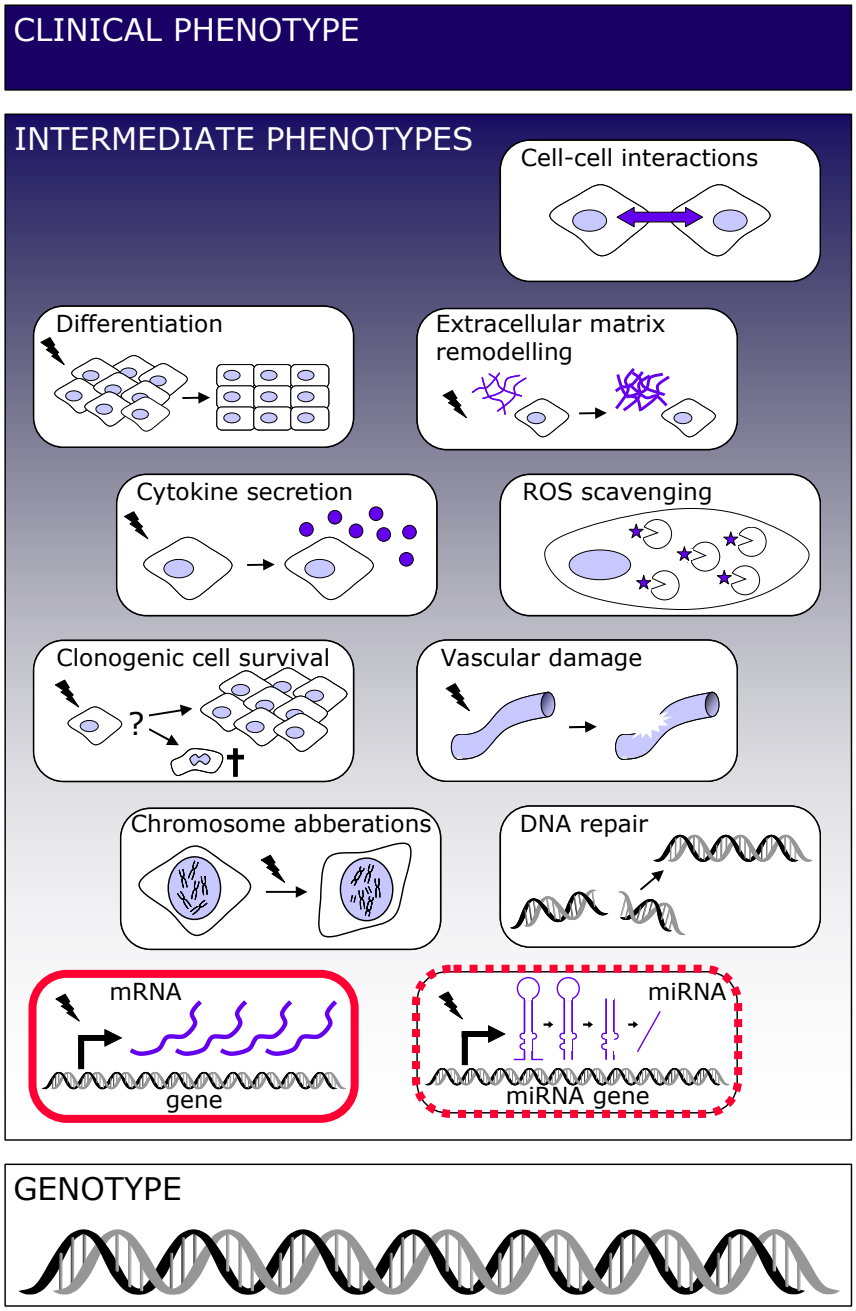
So far, comprehensive reviews of the published literature, most of which reports work based on the candidate-gene approach, have demonstrated a plethora of questionable genotype–phenotype associations, replication of which has often failed in independent studies[4–7]. As the transition to genome-wide asso-



Endophenotypes (Intermediate)

- The concept of dividing a clinical phenotype (*quantitative complex trait*) into more stable phenotypes which
  - correlate with the clinical phenotype
  - associate more closely with genetic variants
  - may be portrayed by gene/protein expression patterns
  - may be applicable to pathway analysis

Various normal tissue damage endpoints



SNPs, CNVs, and other types of sequence variation

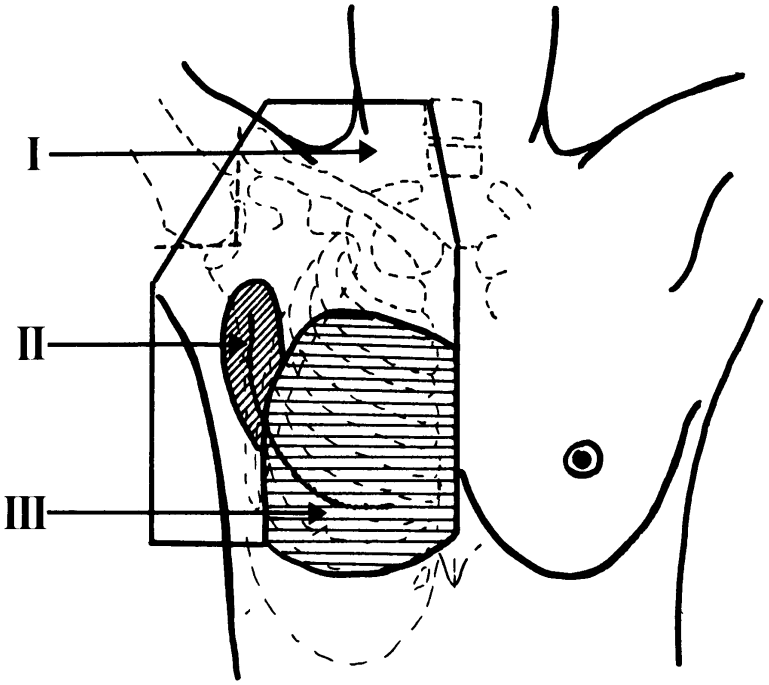
Irradiated fibroblasts

- Aarhus post-mastectomy cohort
  - 319 patients treated '78-'82
  - Fibroblasts from 41 patients

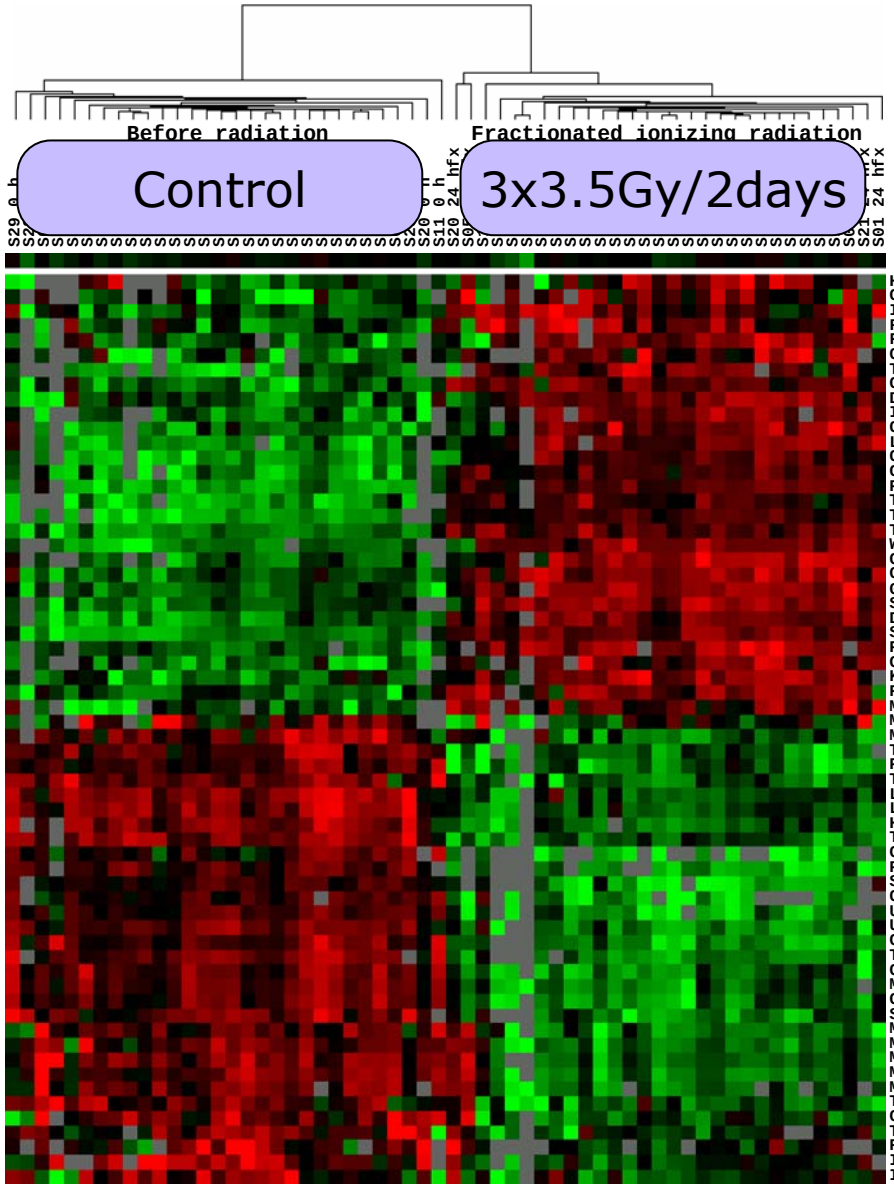
Photon field without bolus

”Scar” photon field with bolus

Chest wall electron field



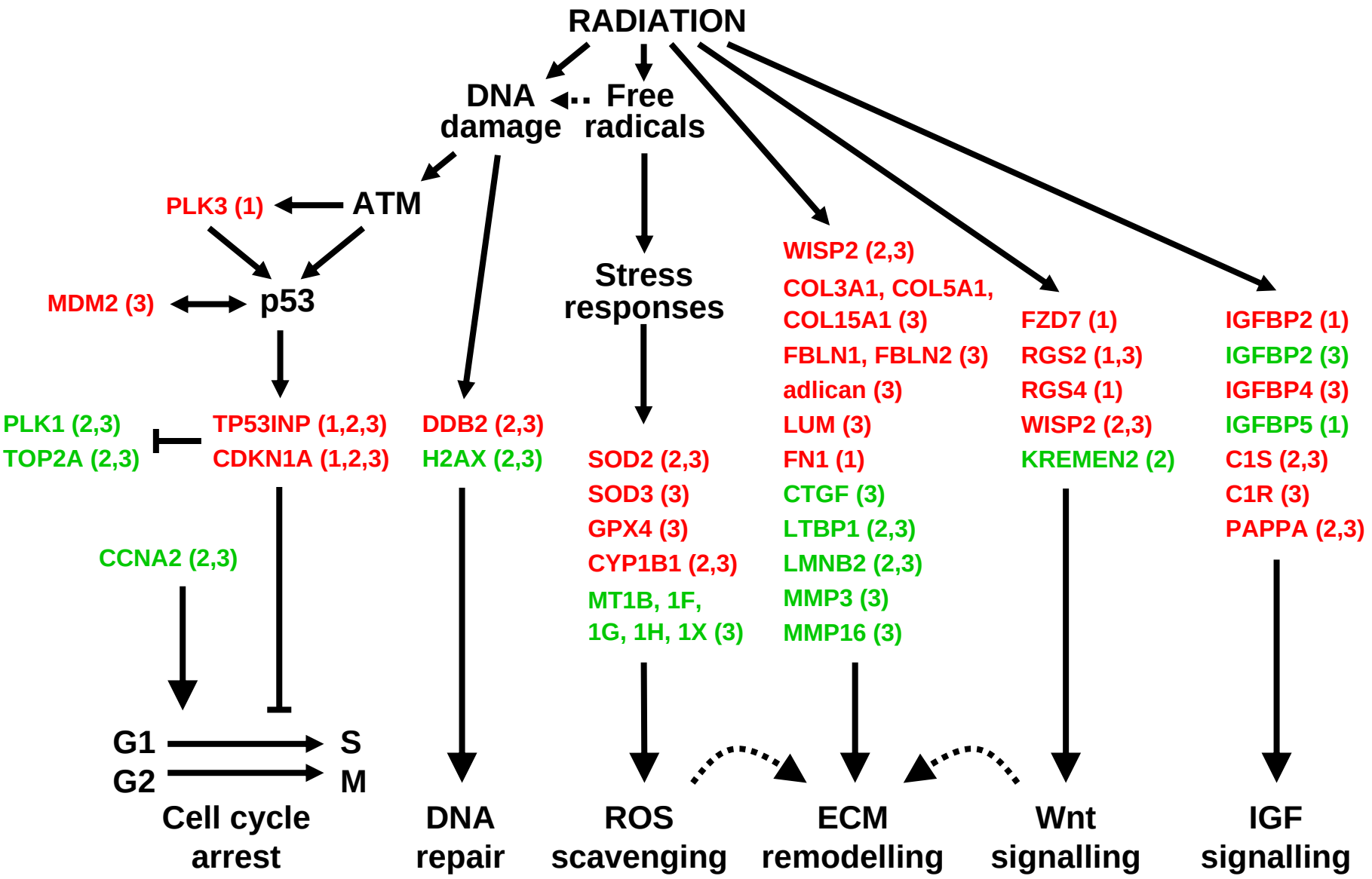
Genome-wide approach



Selection of genes
(total: 225)
SAM analysis

Rødningen et al (2005) Radiother Oncol 77:231–240

1) 1 x 3.5 Gy; 2 h 2) 1 x 3.5 Gy; 24 h 3) 3 x 3.5 Gy / 2 days; 2 h



Rødningen et al (2005) Radiother Oncol 77:231–240

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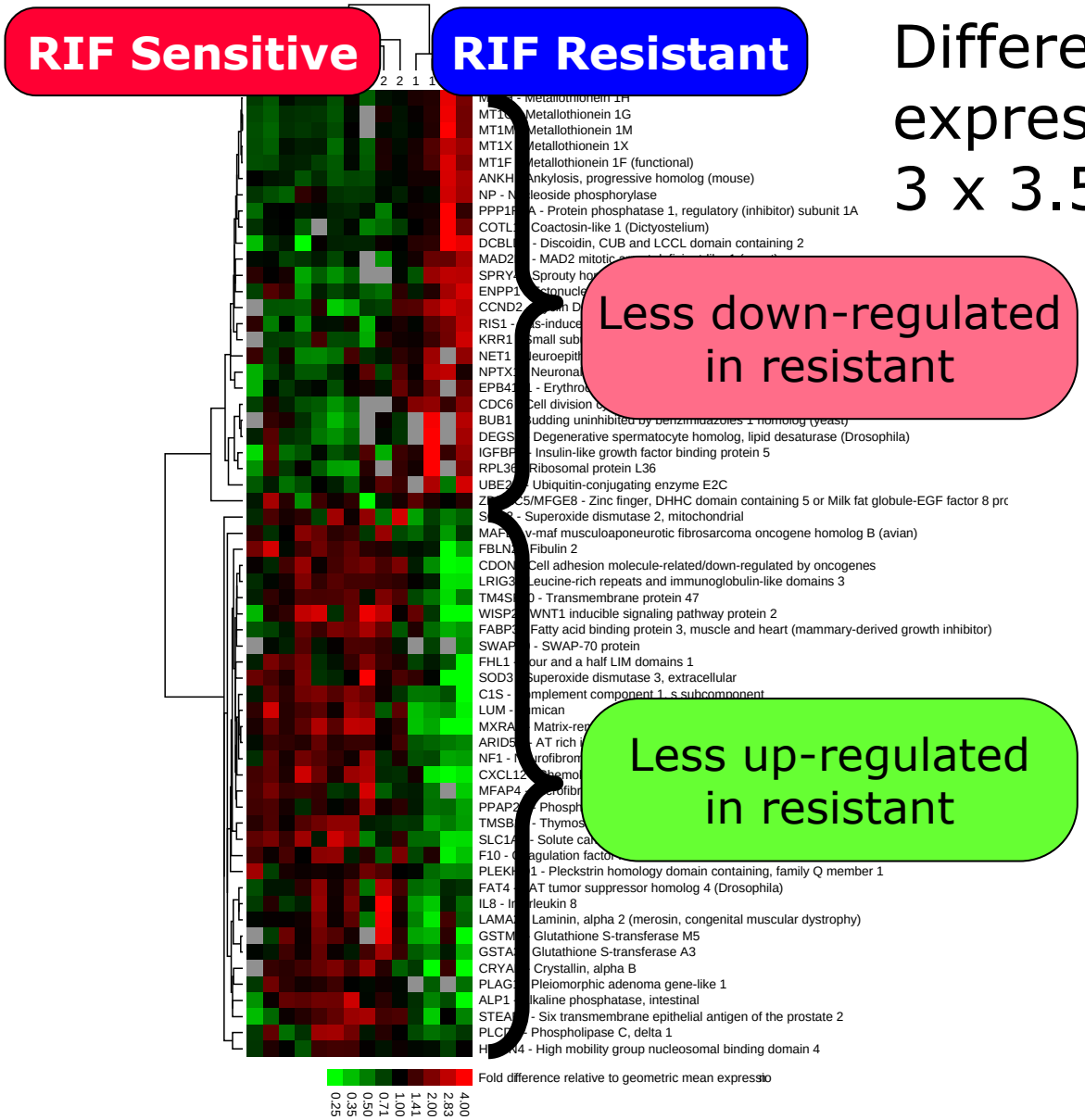


Differentially expressed genes after 3 x 3.5 Gy /2 days (total: 60)

Rødningen et al (2008) Radiother Oncol 86:314–320

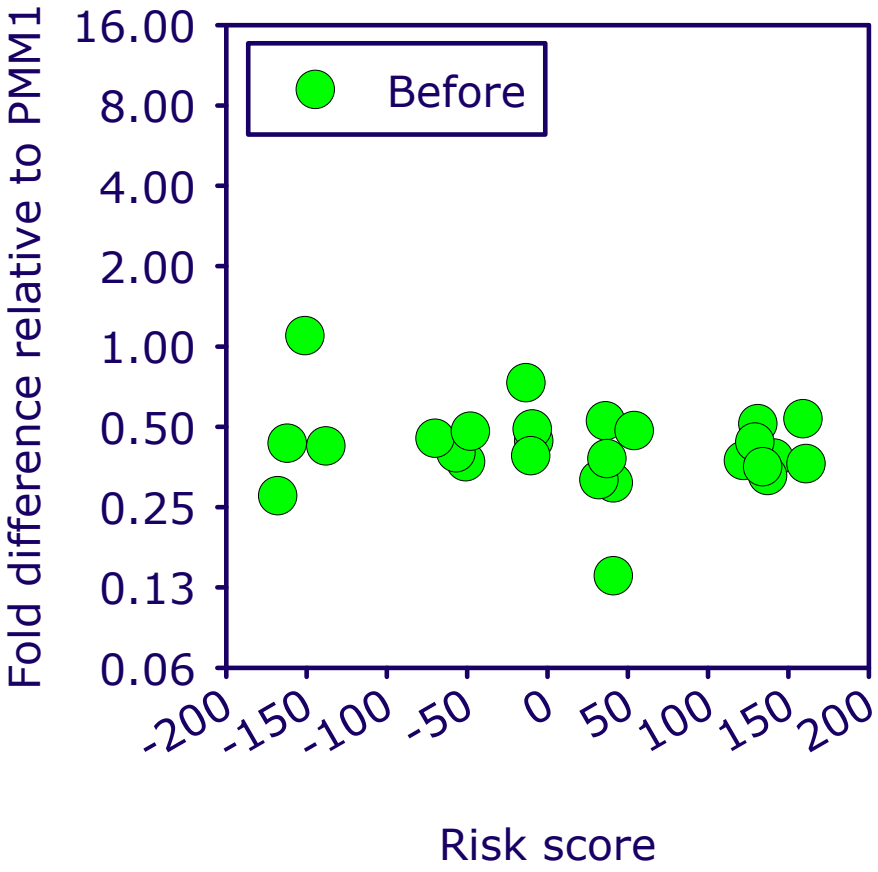
'Independent' validation - real-time PCR

- Same and more cell lines (but different batches)
- New irradiation - reference gene: *PMM1*
- Before and after 3 x 3.5 Gy / 2 days

Alsner et al (2007) Radiother Oncol 83:261–266

FAP, Fibroblast activation protein α

Before and after 3 x 3.5 Gy / 2 days



- Member of the cell surface dipeptidyl peptidase (DPP) family of serine proteases
- Important during embryonic development
- Induction of FAPα expression in fibroblast during wound healing, in fibrotic processes, and in tumour stroma

Alsner et al (2007) Radiother Oncol 83:261–266

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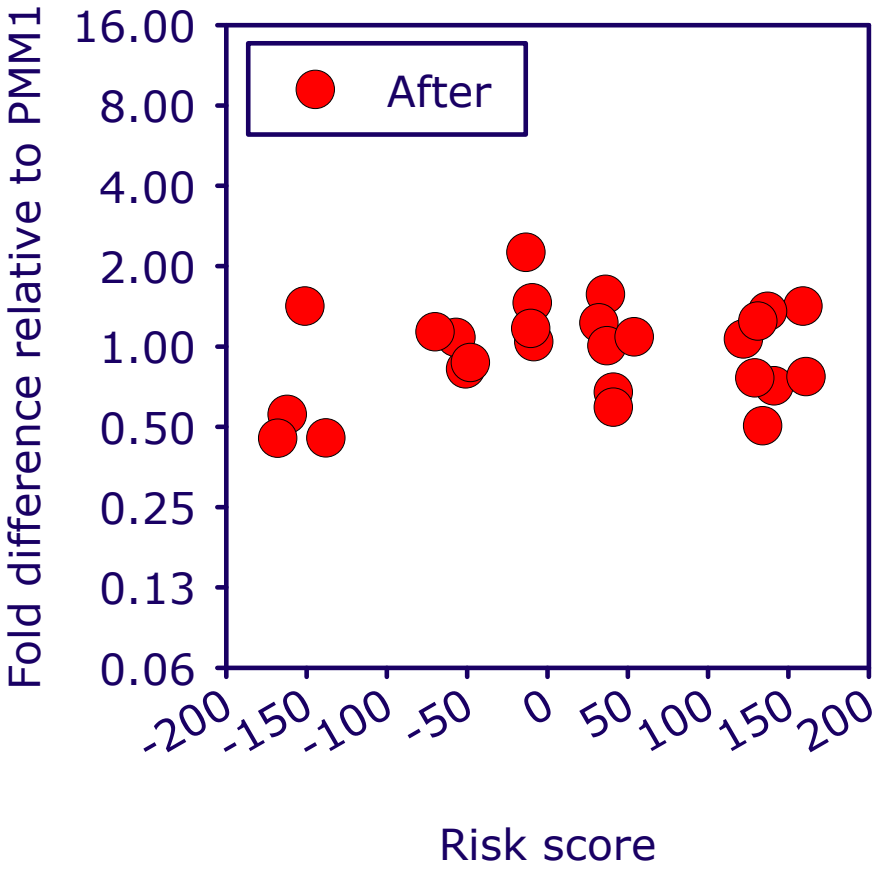

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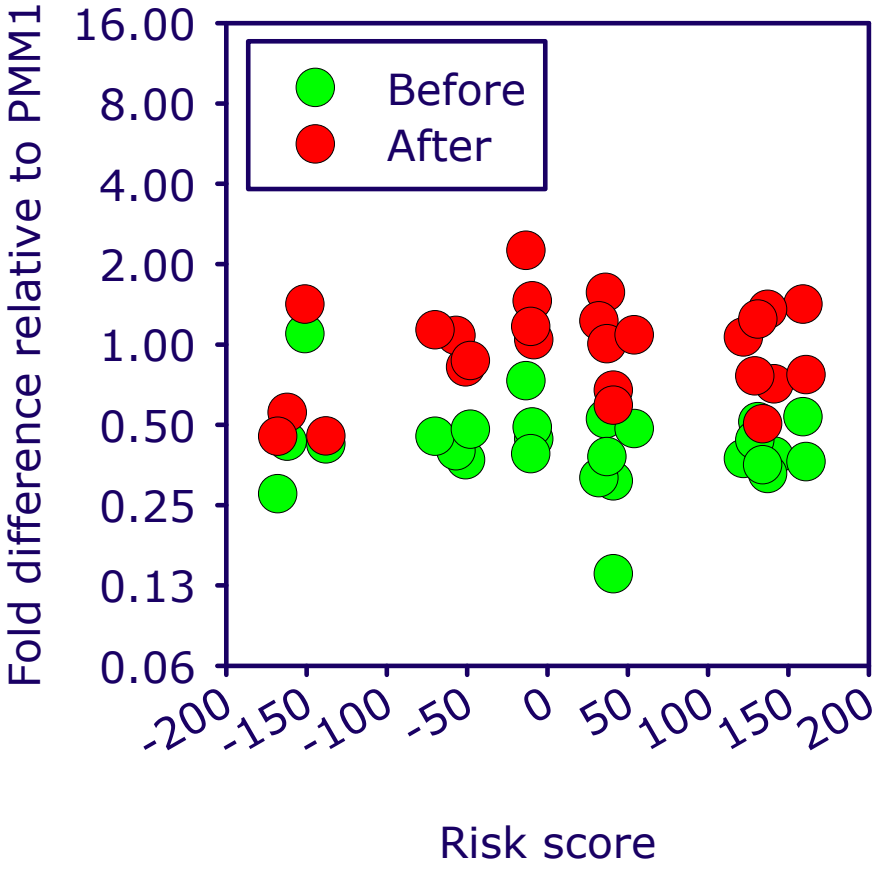
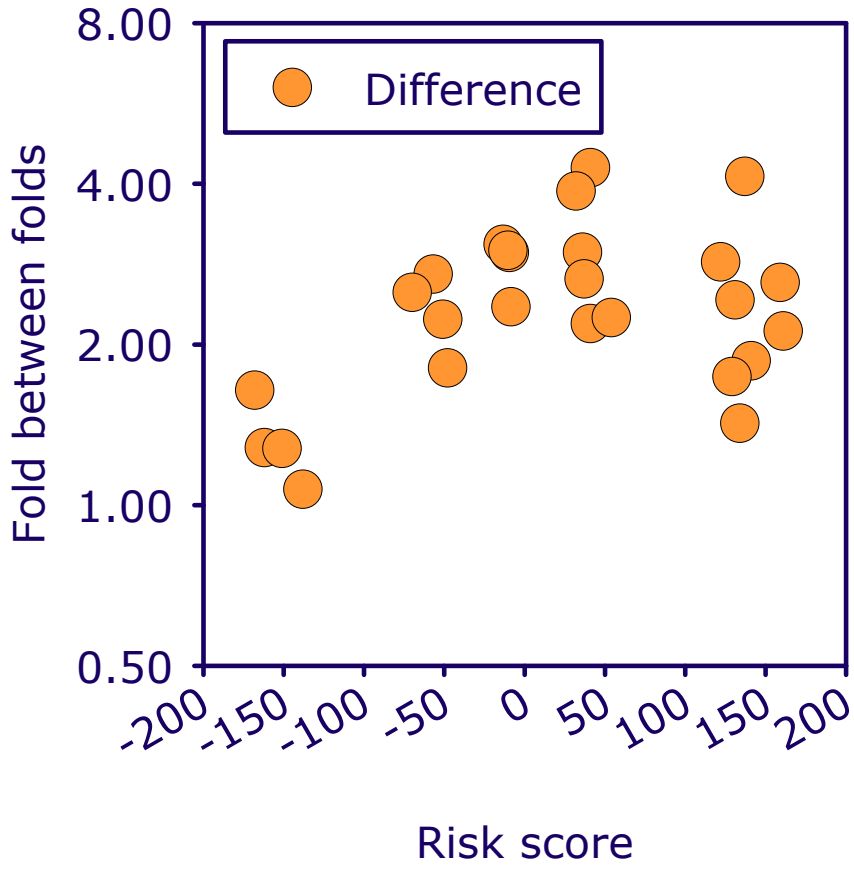
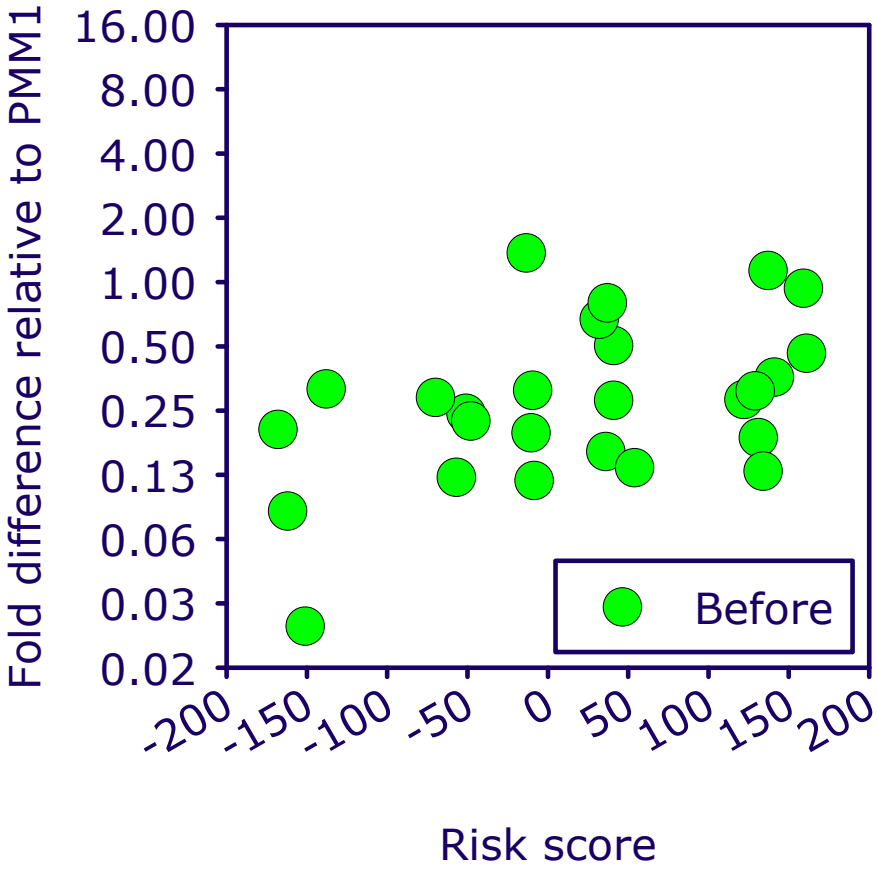




Alsner et al (2007) Radiother Oncol 83:261–266

MXRA5, Matrix-remodelling associated 5 (Adlican)

Before and after 3 x 3.5 Gy / 2 days



- Adhesion protein with leucine-rich repeats and immunoglobulin domains related to perlecan (large extracellular matrix proteoglycan)
- Upregulated during senescence, in skin fibroblasts from centenarians, and in cancer

Alsner et al (2007) Radiother Oncol 83:261–266

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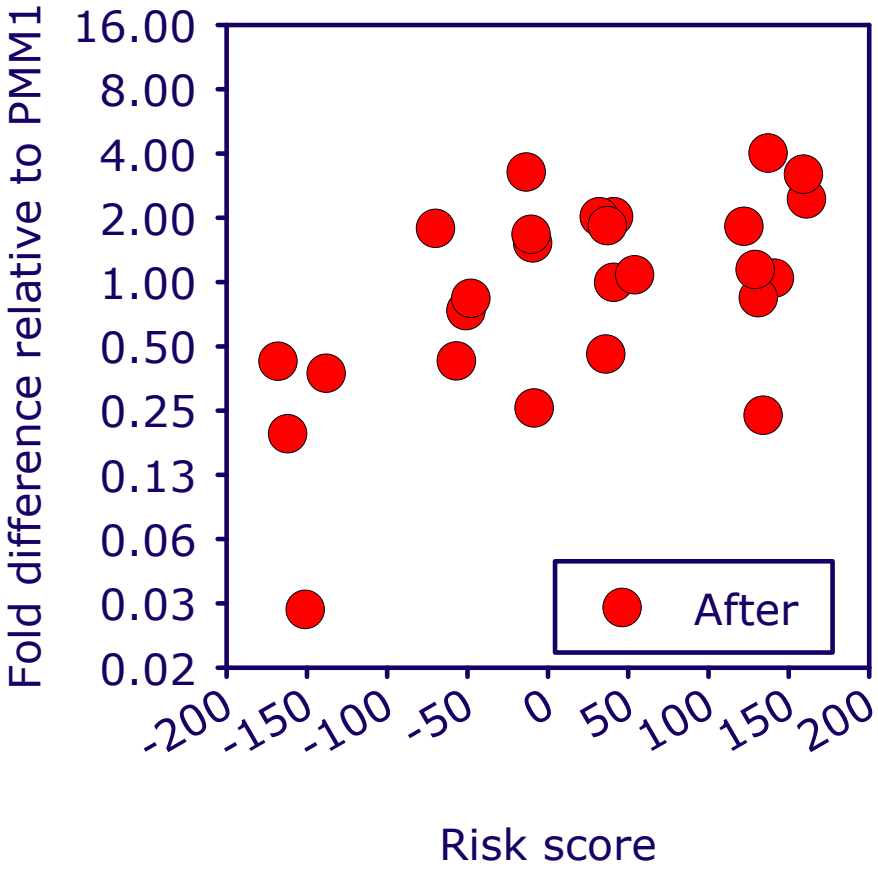

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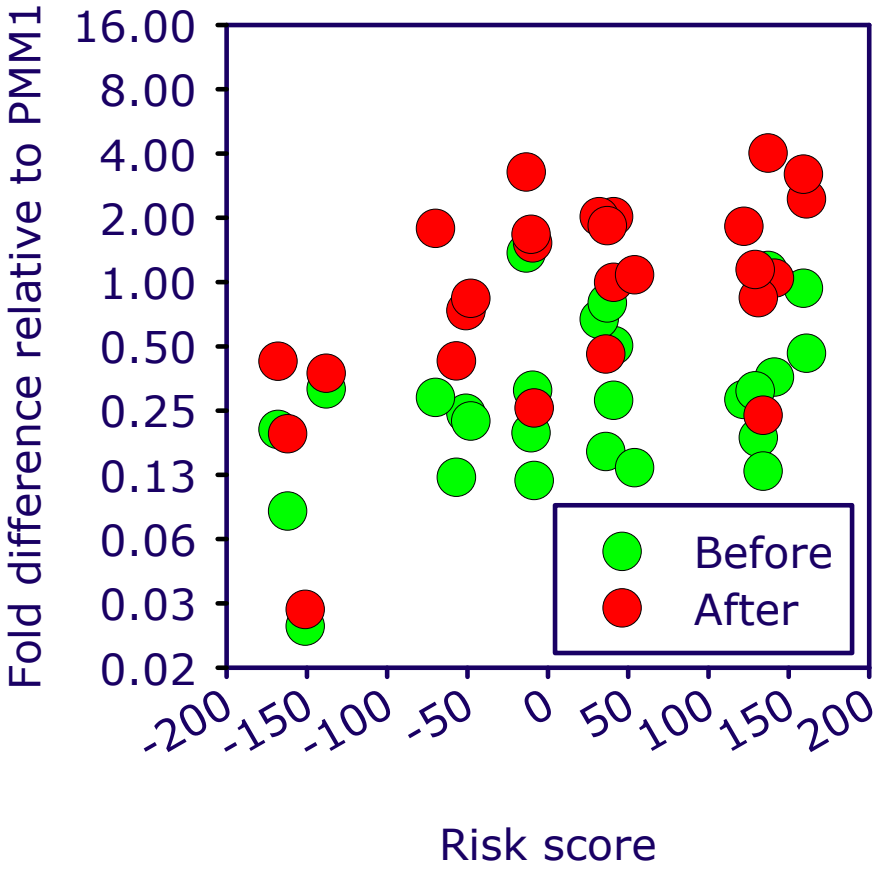
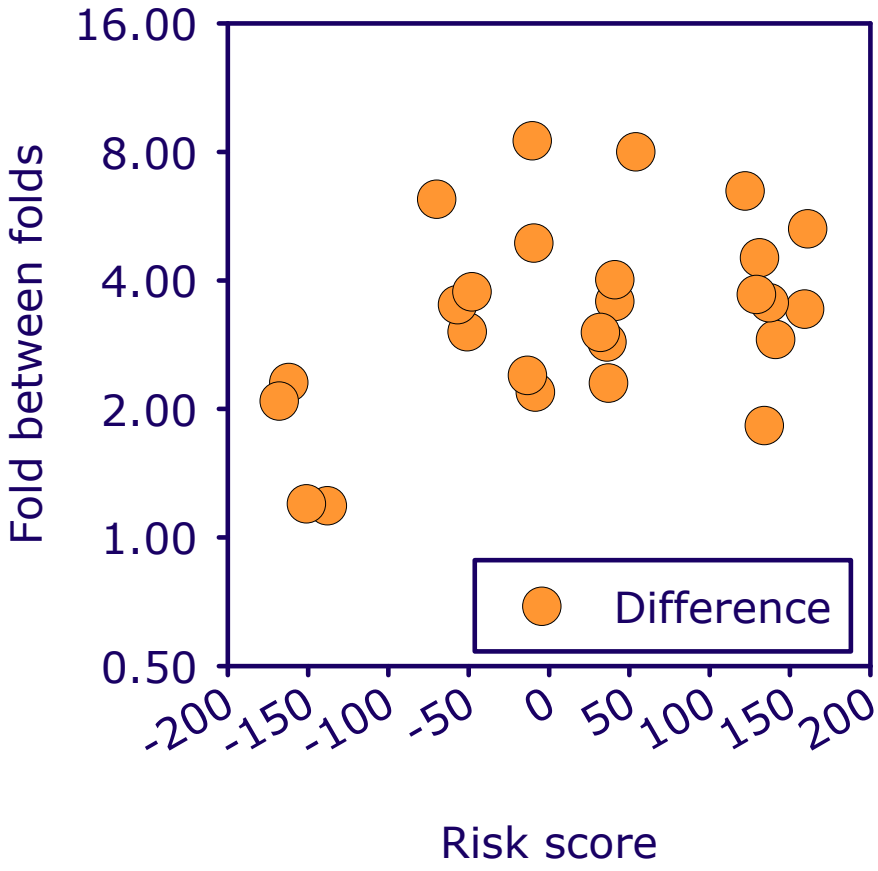
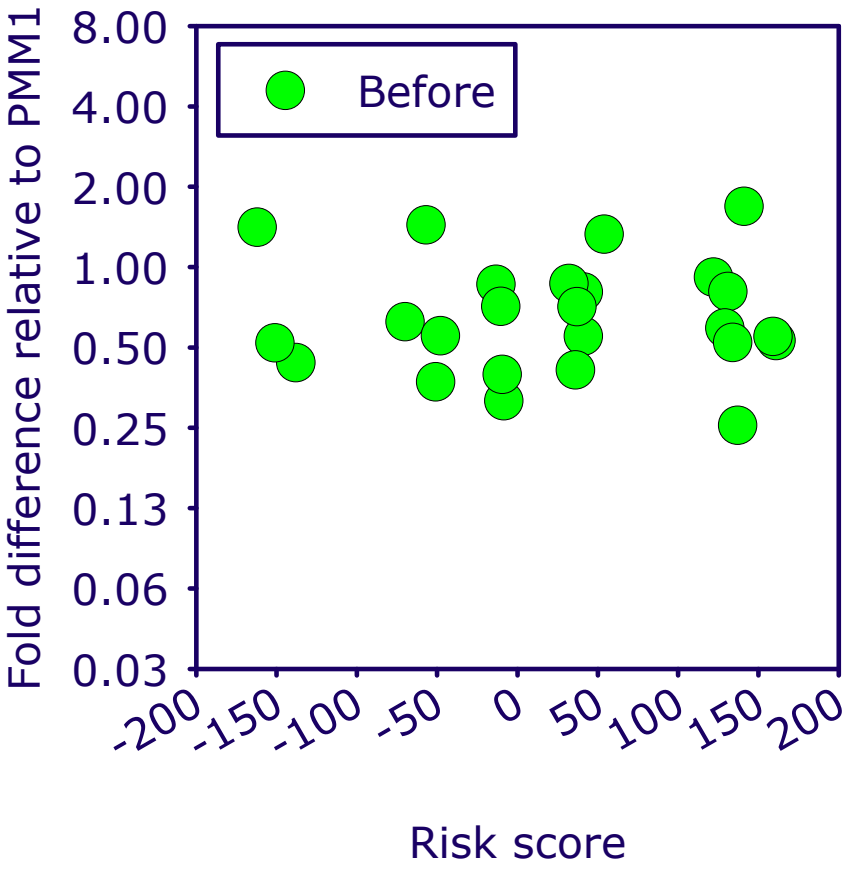




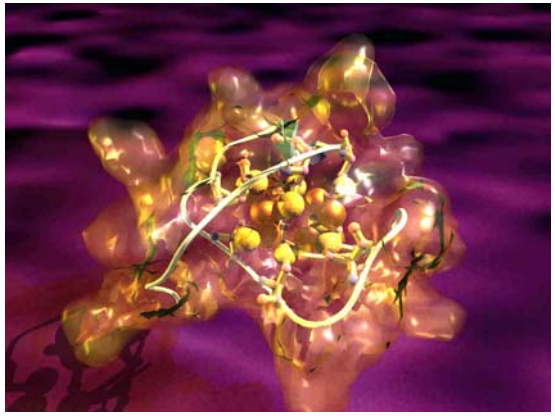
Alsner et al (2007) Radiother Oncol 83:261–266

MT1X, Metallothionein 1X

Before and after 3 x 3.5 Gy / 2 days



- Family of small proteins, 61-62 amino acids with 20 cysteines
- Involved in
  - zinc homeostasis
  - protection against heavy metal toxicity and oxidative damage



Alsner et al (2007) Radiother Oncol 83:261–266

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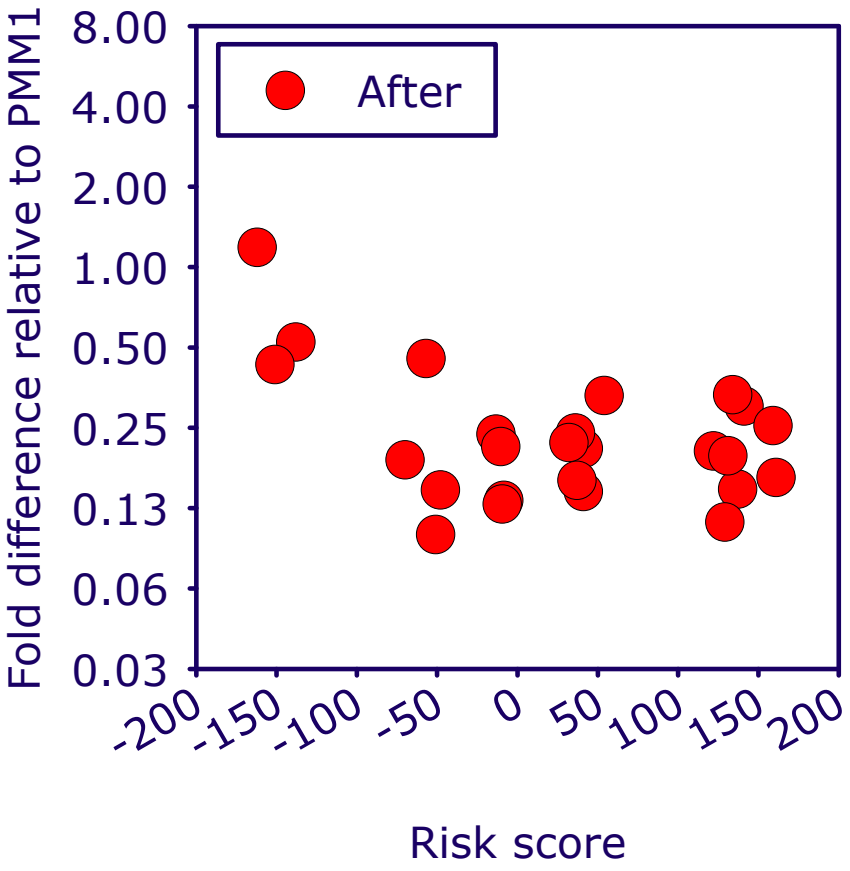

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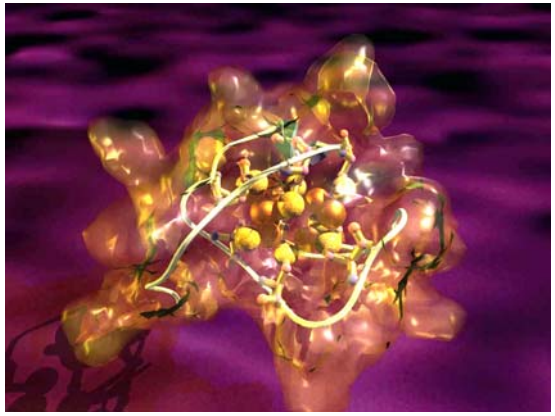
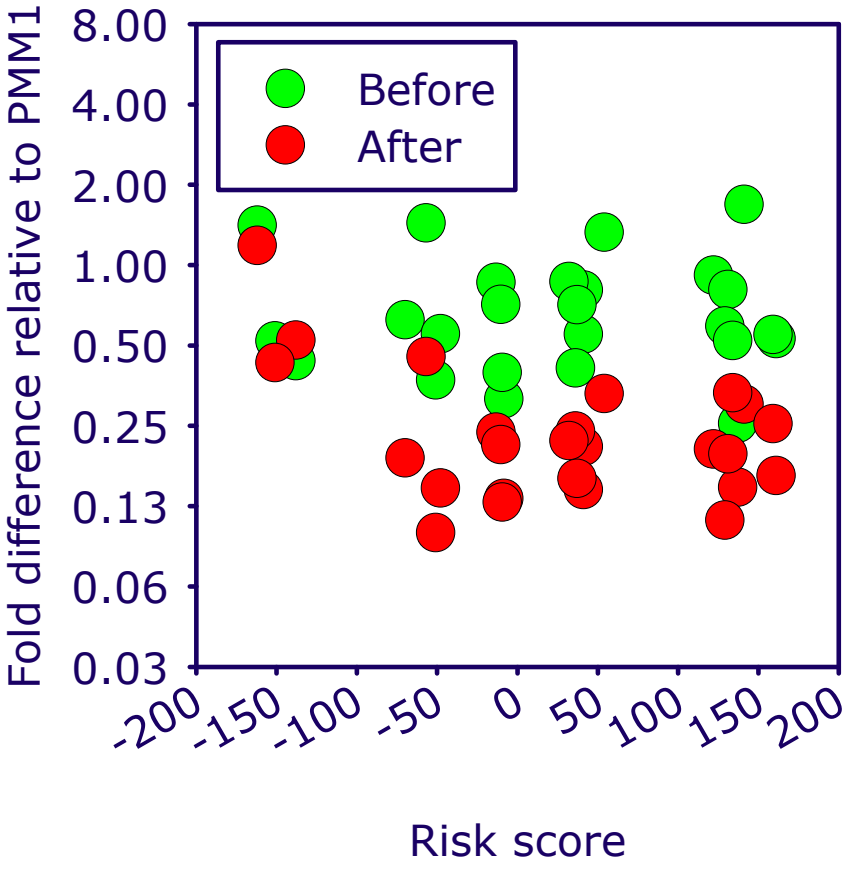
  - zinc homeostasis
  - protection against heavy metal toxicity and oxidative damage

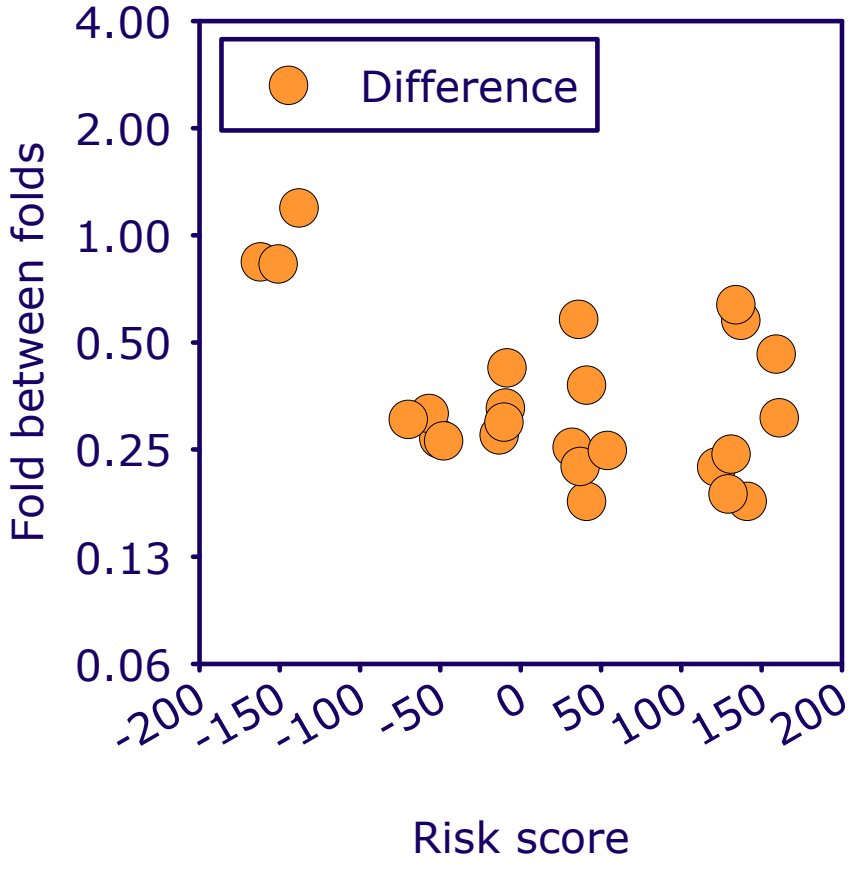


Alsner et al (2007) Radiother Oncol 83:261–266

MT1X, Metallothionein 1X

Before and after 3 x 3.5 Gy / 2 days

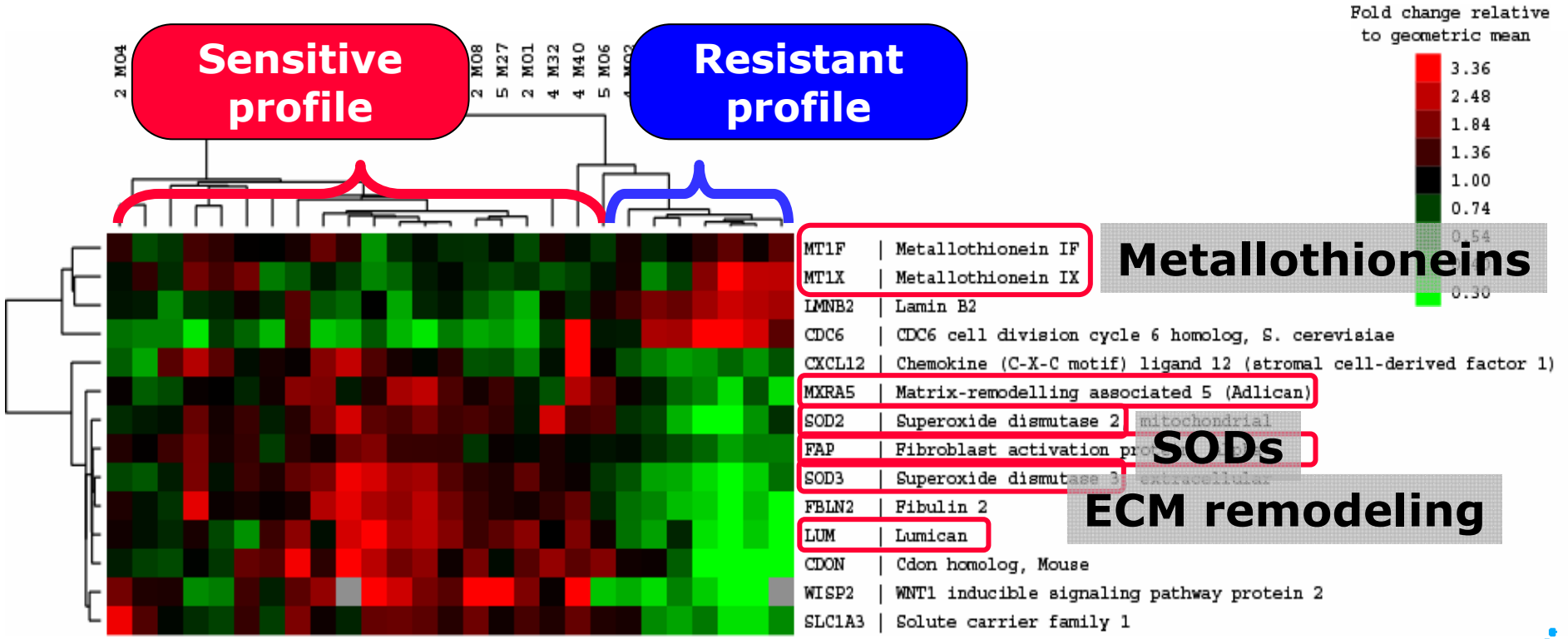




Alsner et al (2007) Radiother Oncol 83:261–266

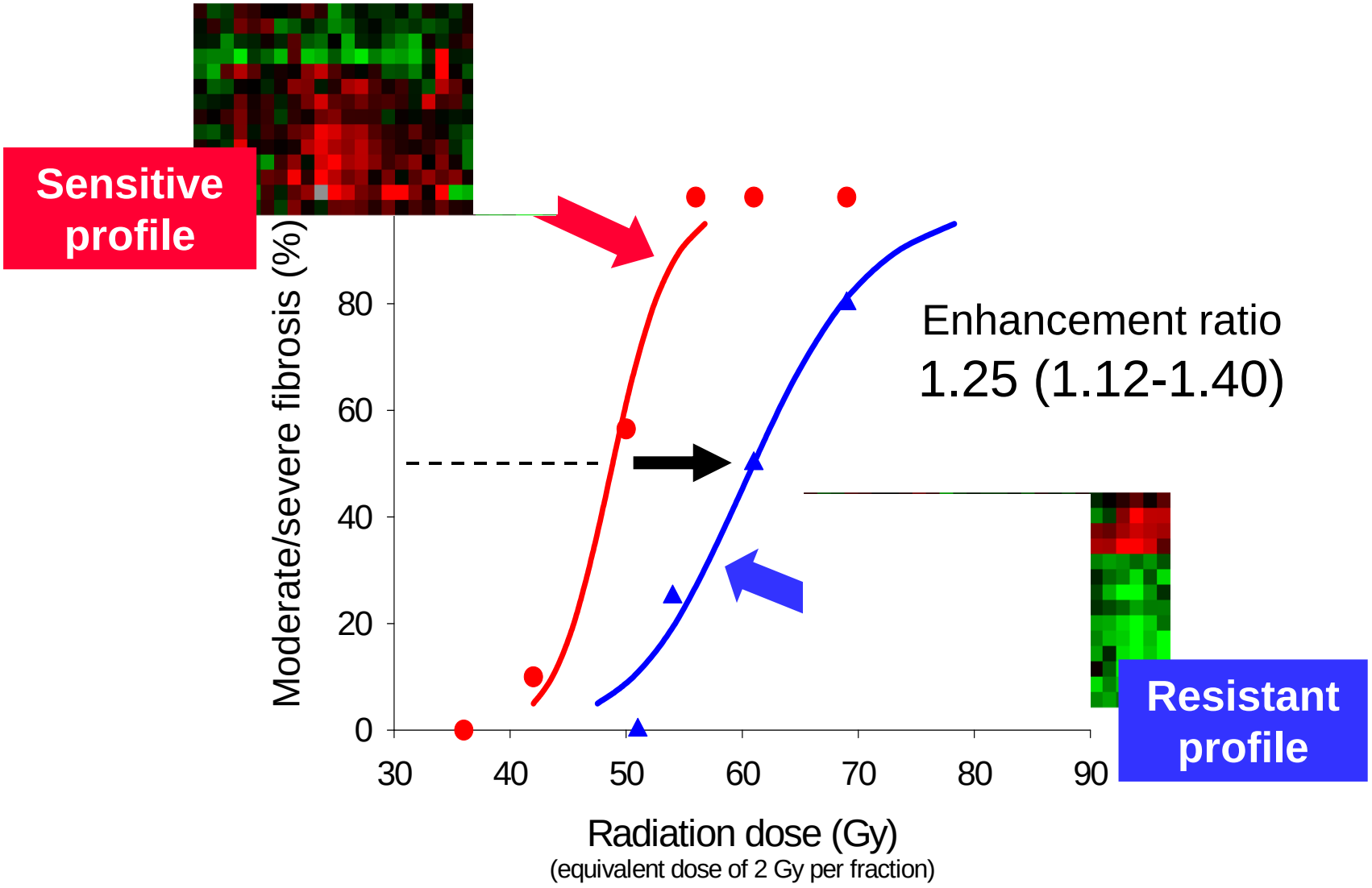
'Independent' validation - real-time PCR

- Same and more cell lines (but different batches)
- New irradiation - reference gene: *PMM1*
- Data based on delta values (before and after IR)



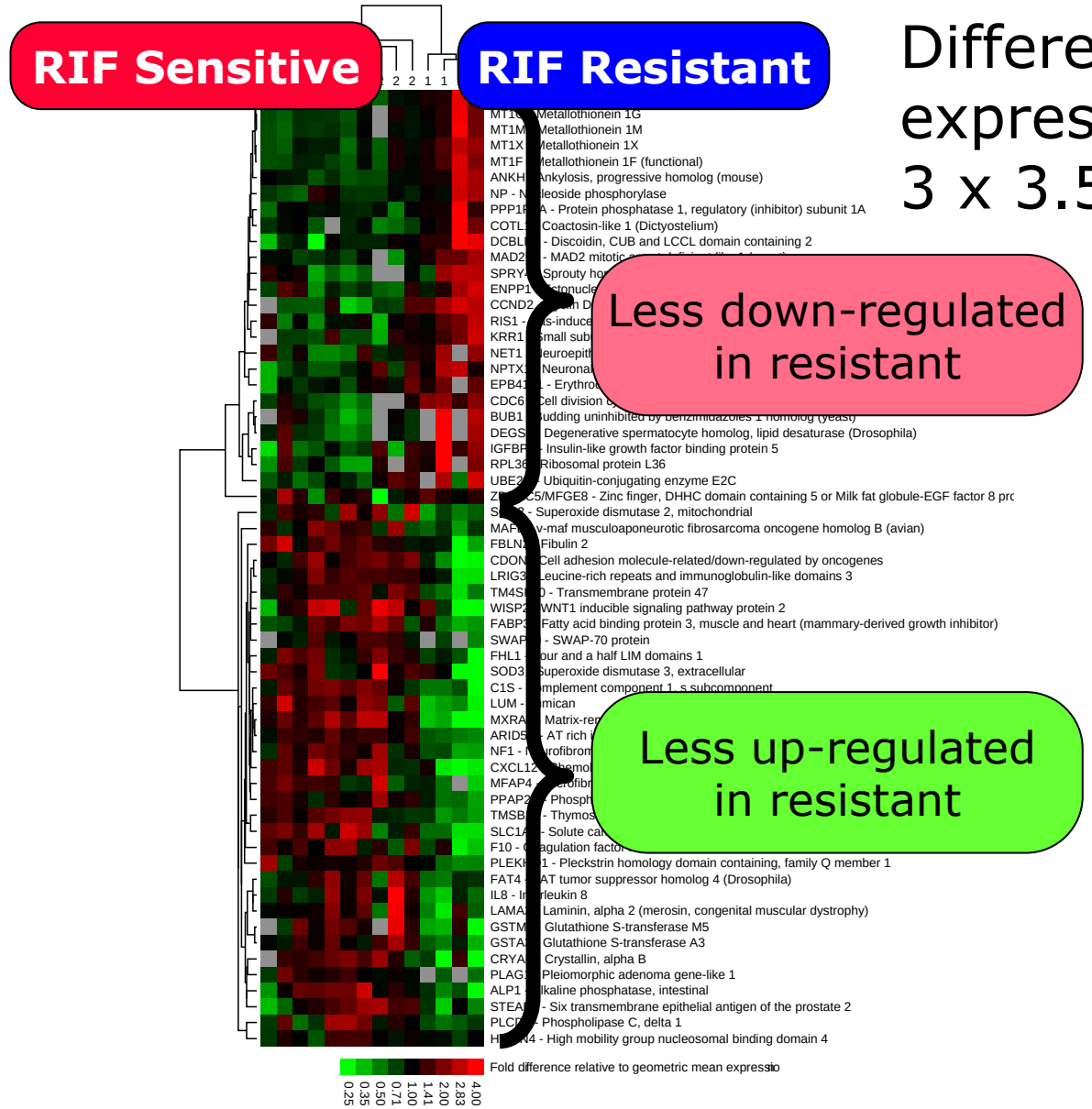
Alsner et al (2007) Radiother Oncol 83:261–266

Genetic profile and risk of subcutaneous fibrosis



Alsner et al (2007) Radiother Oncol 83:261–266

Genome-wide approach



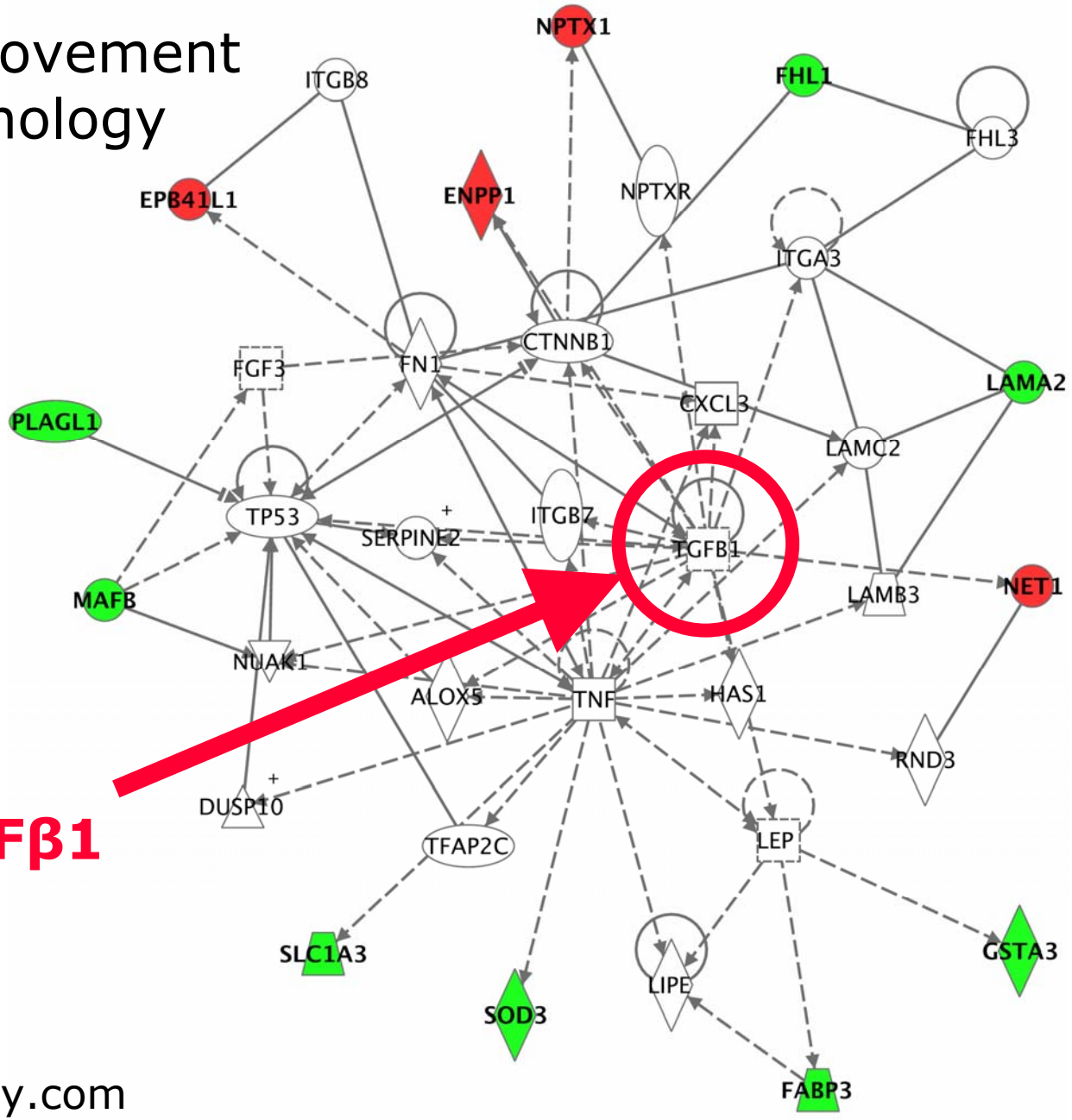
Differentially expressed genes after 3 x 3.5 Gy /2 days (total: 60)

↓

Pathway analysis (Ingenuity)

Ingenuity pathway analysis

Cellular movement
Cell morphology
Cell death



TGFβ1

www.ingenuity.com

Future studies

- Functional studies on fibroblasts
  - Validate differential mRNA expression after irradiation
  - Differential miRNA expression after irradiation ?
  - Pathway analysis (siRNA)
  - SNP arrays ?
- Animal studies
  - Subcutaneous fibrosis (leg extension)
  - Lung fibrosis

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