Molecular imprinting of radiation induced cancer

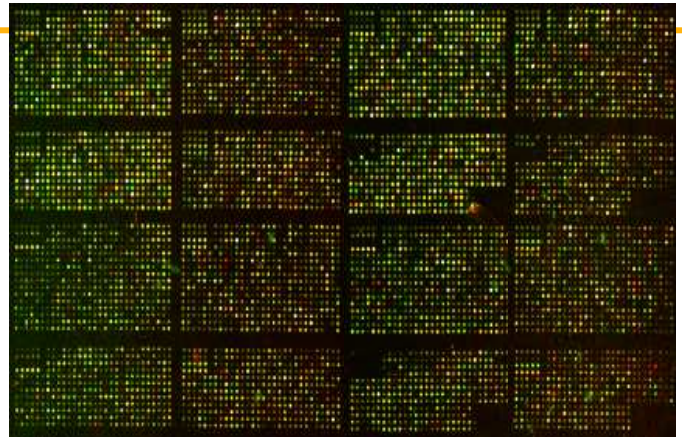
S Chevillard

The risk of cancer is clearly demonstrated at high doses and high dose rates

The risk at low doses remains highly controversial

The overall objective is to move from the extrapolation of the risk to the determination of the risk after low doses of radiation

Transcriptome analysis



for studying radiation induced tumors

- **thyroid**
- **sarcomas**
- **breast cancers**

Agilent microarrays with 25 000 oligonucleotides 50 bases in length

Differential hybridization with a pool of amplified normal thyroid RNA - same external reference for all hybridizations

Search for a signature of etiology by classifying a learning/training set of tumors
Validate the signature by blind prediction of etiology of an independent series of testing tumors

Post-radiotherapy thyroid tumors

57 thyroid tumors

Either sporadic or induced after radiotherapy for the treatment of a first cancer during childhood

28 tumors for the Learning / training set

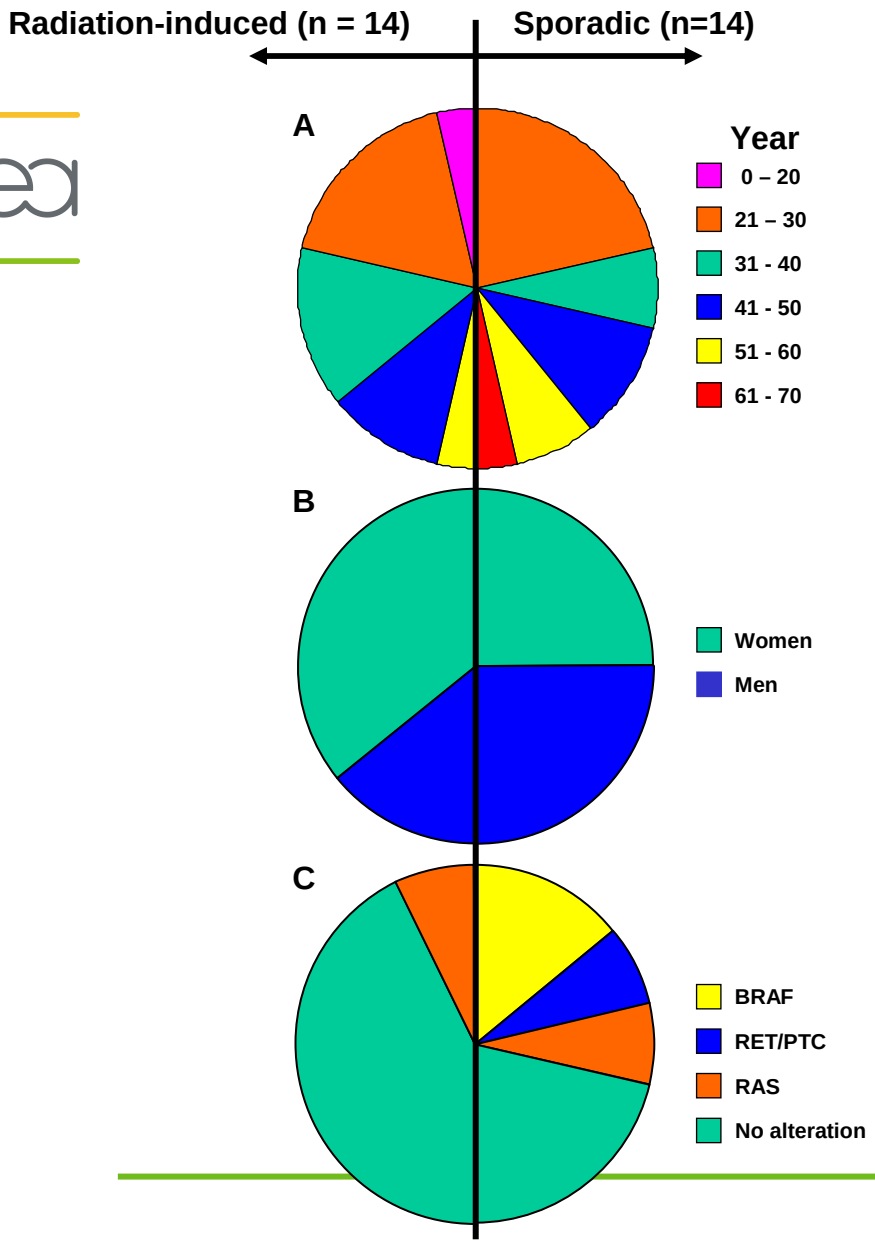
14 RI-induced

14 sporadic

Validation set : 29 tumors

Unknown etiology at time of analysis

Characteristics of radiation-induced tumors in the learning set



As function of :

Age

Mean age
35 years old for R tumors
37 years old for S tumors

2 groups were matched histology, TNM, sex, ethnicity and age at tumor diagnosis

Gender

No bias when comparing the two subgroups

Gene mutations

Gene expression analysis

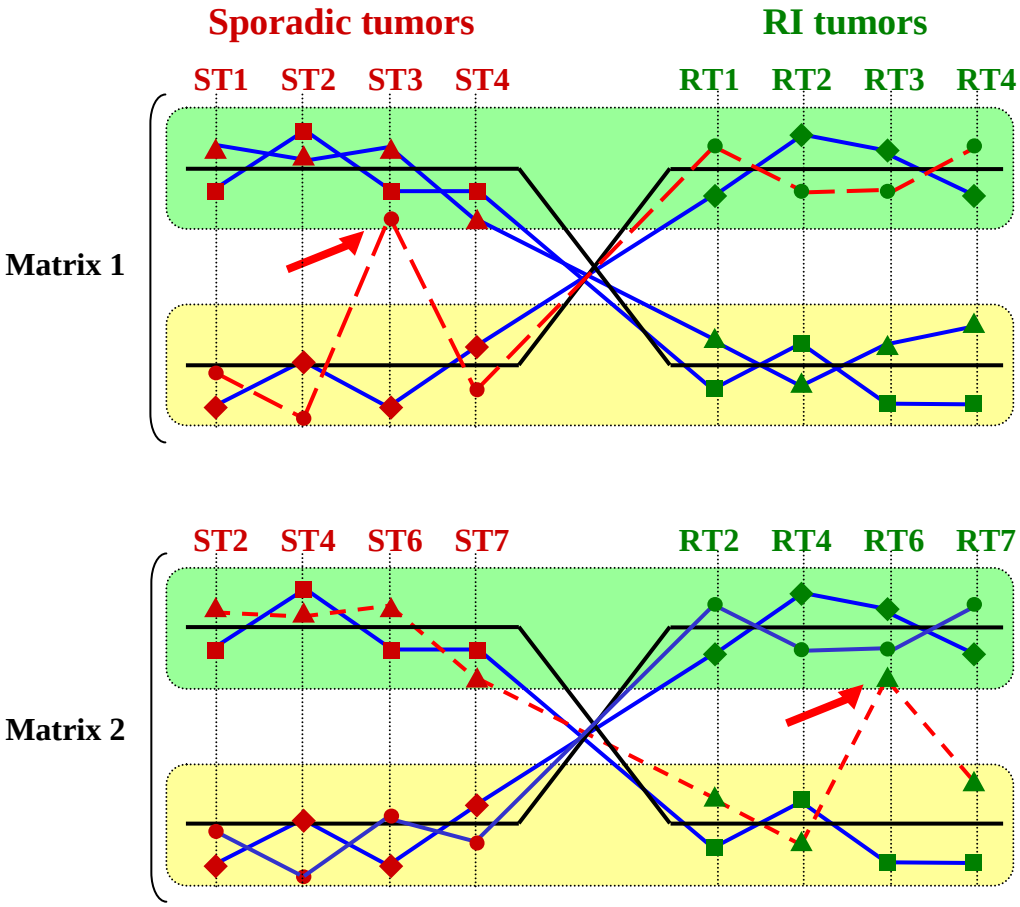
14 S — **4 tumors**

Learning / training set

14 R — **4 tumours**

Included at least one tumour of each histology
At least a 50% difference in their tumor compositions (per aetiology)

After permutations, 143 combinations have been retained

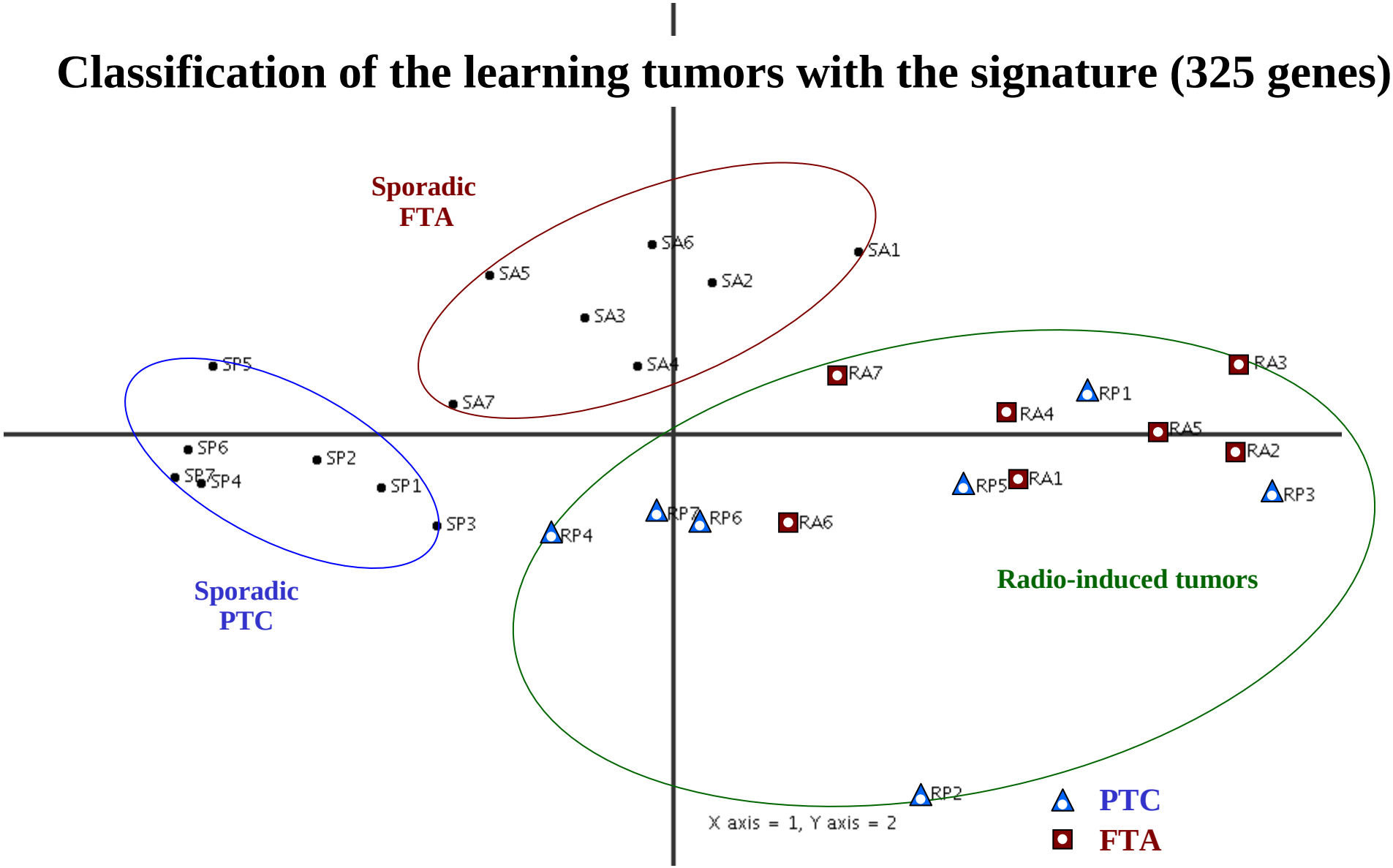


Search for potential signature
Classification of the 20 remaining training tumors
To retain a matrix
- at least one tumor well classified
- and zero miss classified

Genes identified as discriminating in more than 70% of the retained combinations

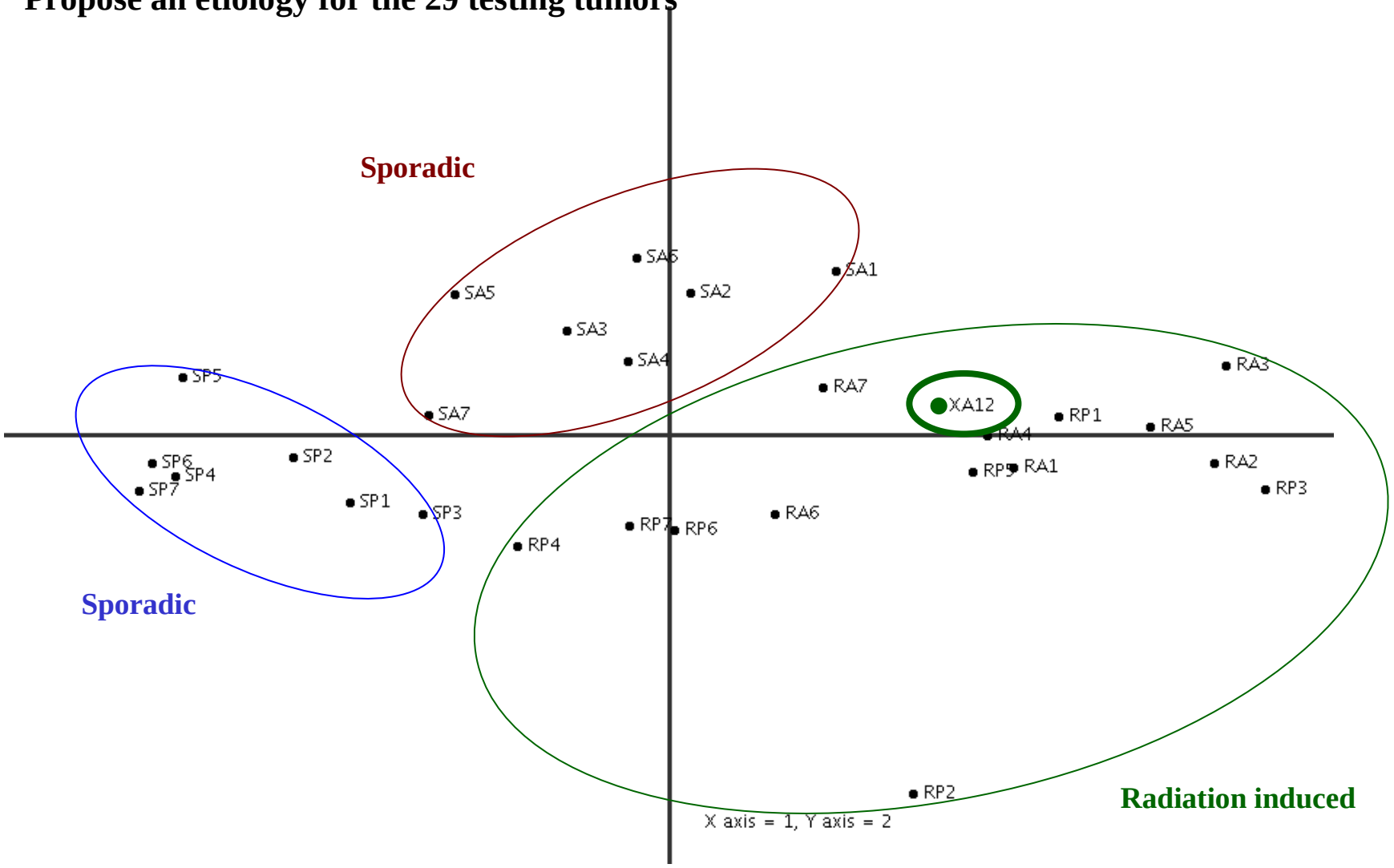
SIGNATURE all RI vs all S
325 genes

Classification of the learning tumors with the signature (325 genes)



Blind validation of the 325 genes signature

Propose an etiology for the 29 testing tumors



Blind validation of the 325 gene R/S signature

Validation 29 tumors

Clinical data	Signature prediction
16 S	14 S 2 R
13 R	12 R 1?

26/29 well classified
2/29 miss-classified
1/29 undetermined etiology

	RI	S
+ test (RI)	12	2
- test (S)	0	14

Ory K,Endoc Relat Cancer, 2011

Sensitivity	12/13	0.92	Proportion of RI tumors well concluded among RI tumors
Specificity	14/16	0.87	Proportion of S tumor well concluded among S tumors
Positive predictive value	12/14	0.85	Proportion of tumor with + test (R), well concluded
Negative predictive value	14/14	1	Proportion of tumors with – test (S), well concluded

Post-Chernobyl tumors from CTB (n=27)

Detours et al. (2005) => Absence of a specific radiation signature in post-Chernobyl thyroid cancers

Detours et al. (2007) => identified a set of 256 genes almost classifying post-Chernobyl tumors
Misclassified 17% of the post-Chernobyl PTC

A signature of H2O2 lymphocyte exposure… classifies the Chernobyl tumors

Problem of methodology?

7 C and 7 S tumors matched in age at tumor diagnosis for Searching a signature

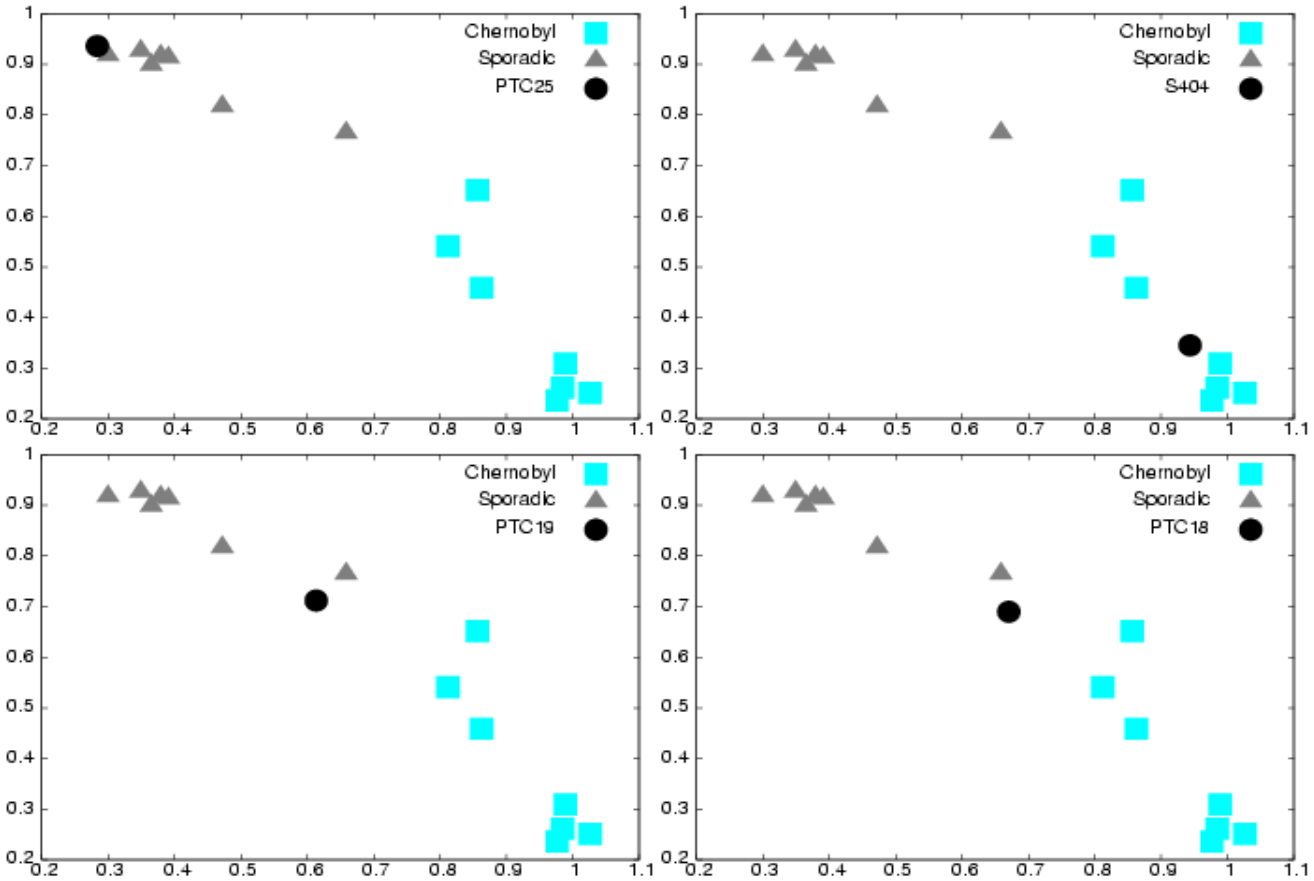
Testing group 13 remaining tumors

Sporadic PTC vs Chernobyl PTC

Series of Detours et al 2005 2007

Chernobyl PTC
S PTC
N = 14 **Learning**

Chernobyl PTC
S PTC
N = 13 **Testing**

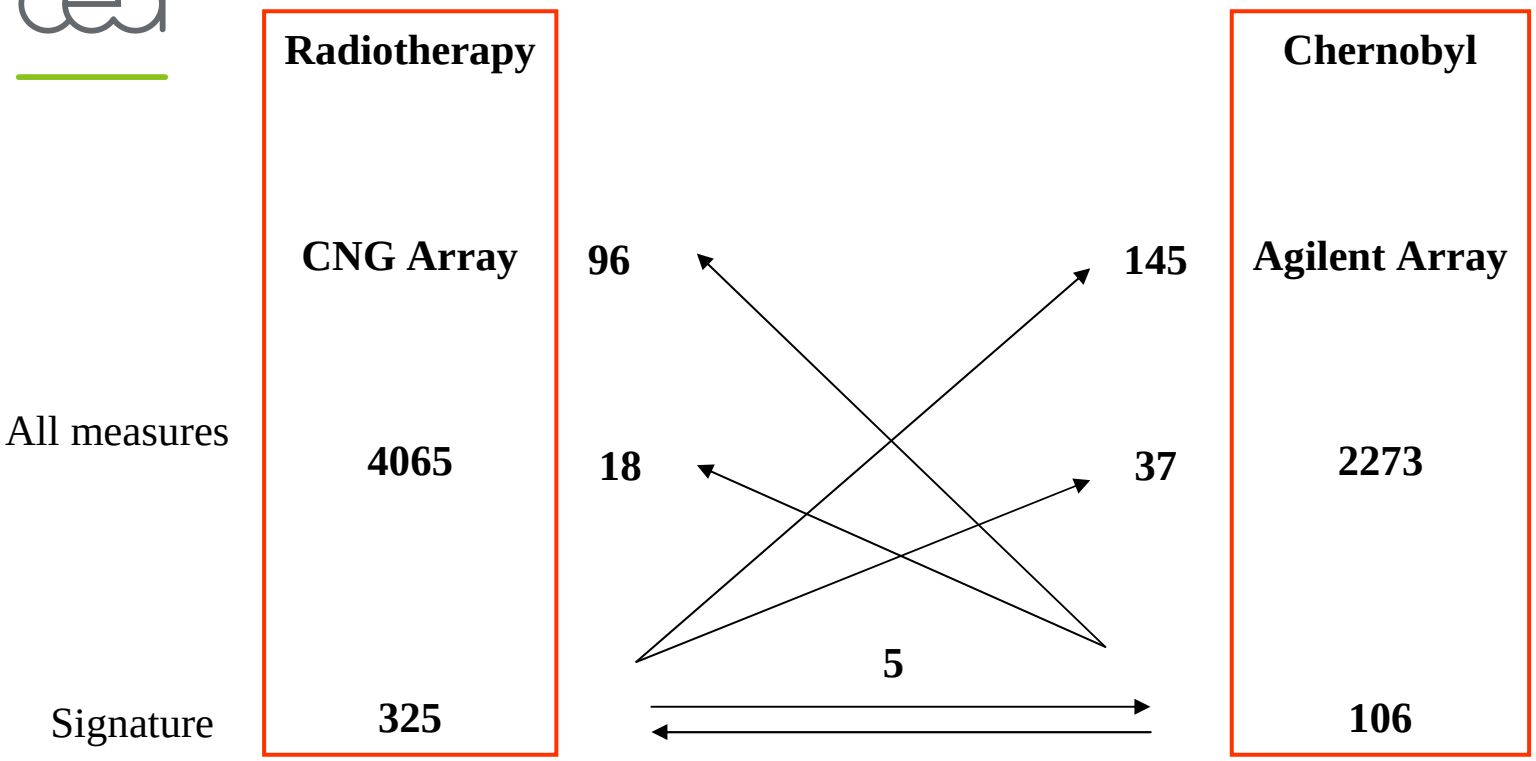


106 genes

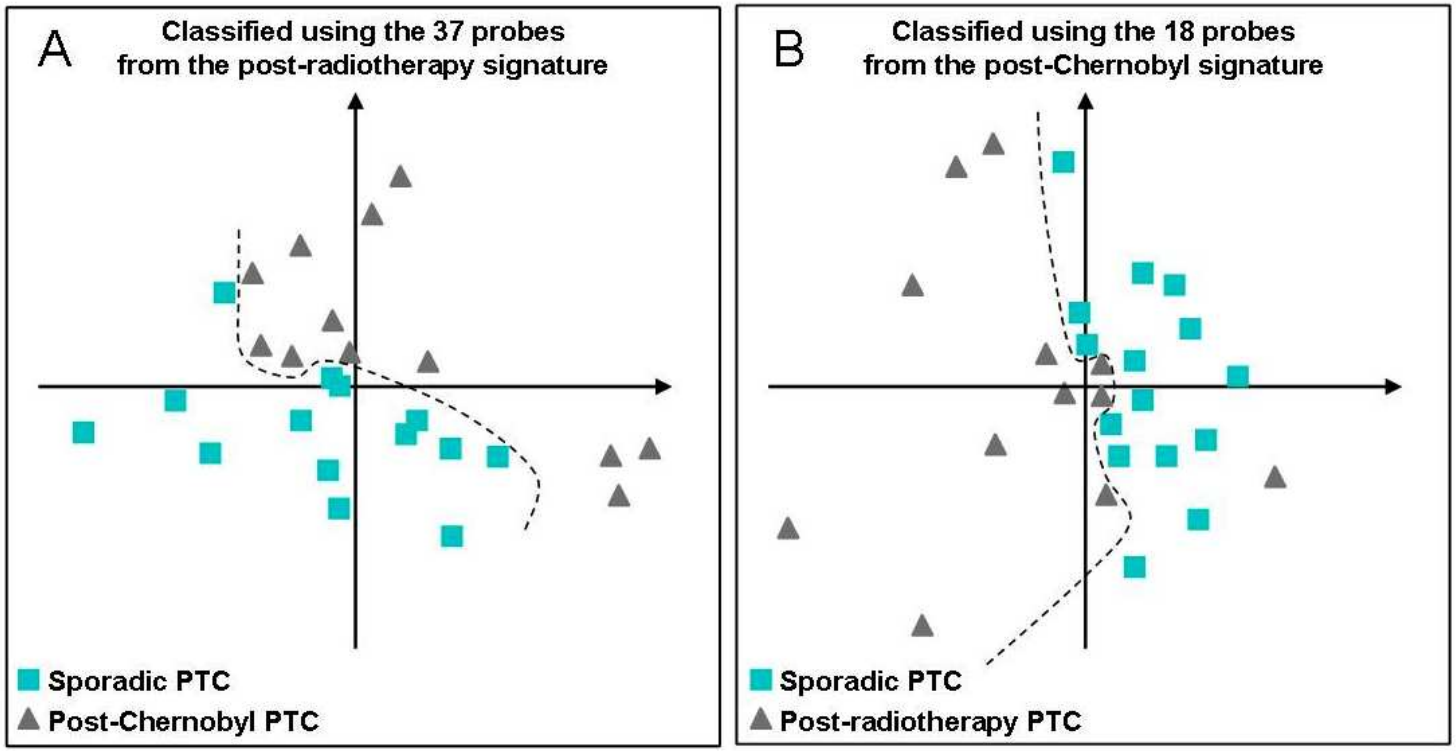
Ugolin N, PLoS One 2011

11 well predicted
2 non-determined

Comparison of post-radiotherapy and post-Chernobyl signatures



Does the Chernobyl signature classify postradiotherapy K and reciprocally?



Cross-classification of the post-radiotherapy and the post-Chernobyl series of tumors

Since the genes separate R and S tumors of the other series, it means that are deregulated in both series however without « weight » for tumor prediction

The 5 common genes

- PABPC1 stability and splicing of RNA
- SERPINE1 protease inhibitor
- GTF2H2 transcription factor DNA repair
- DHCR24 cholesterol synthesis
- CLU cell death and tumor progression

These genes were already described as involved in cellular radiation response

SERPINE 1 and GTF2H2 were previously found deregulated in

-normal human thyroid tissue from Graves'disease , 2 weeks after exposure either with Fission neutron at 0.2Gy - 0.2Gy/hr or Cs-g rays at 1 Gy -1.2 Gy/mi (Mut Res 2010 Adachi S)

-human epithelial thyroid immortalized cell line exposed at 10 cGy of HZE particles in the form of iron ions (Rad Res 2009, Sanzari JK)

Conclusions

We found a signature
of post-radiotherapy induced thyroid tumors (325 genes)
of Chernobyl tumors (106 genes)

The 5 common genes separate sporadic and each radiation induced series

Each signature classifies the other series

External radiation and internal contamination derived tumors seem to have something in common regarding the gene expression imprinting.. Different doses, dose rates, acute/chronic

Among the 5 common genes, 2 were already described as participating in short term radiation response

Molecular tools for analyzng radiation induced tumors at doses lower than 0.1 Gy?

- **Specific molecular mechanisms of radiation-induced carcinogenesis?**

- **Genetic susceptibility to radiation-induced cancers?... To individual radiation sensitivity? ... SNP analysis of normal DNA**

- **Molecular imprinting of past history of radiation exposure… normal irradiated tissue….. Epigenetic modulation through for example specific methylation profiles…. We have already information on radiation induced genomic instability and methylation alteration**

- **We aim to analyze a series of Chernobyl tumors together with the normal thyroid l tissue for transcriptome, miRNome and SNP analyses**

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