UVSQ université PARIS-SACLAY

UFR Simone Veil - Santé

CAMPUS DE SAINT-QUENTIN-EN-YVELINES

U1296 Unit : Radiation
Defense, Health, Environment
Lyon & Brétigny-sur-Orge, France

Workshop Individual response to IR

Stockholm - Sept 1, 2022 - Session 1

Factors influencing the risk of tissue effects

Functional assays to predict individual patient response

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Individual response to ionizing radiation
Factors influencing the risk of tissue effects

Abnormal tissue response after high doses IR (radiotherapy) : radiosensitivity

≠ Cancer proneness after exposure to IR (high and low dose) : radiosusceptibility

≠ Tissue degeneration after exposure to IR (e.g., cataracts or cardiovascular effects): radiodegeneration

Radiosensitivity and radiosusceptibility
may be exclusive of each other (Li Fraumeni)

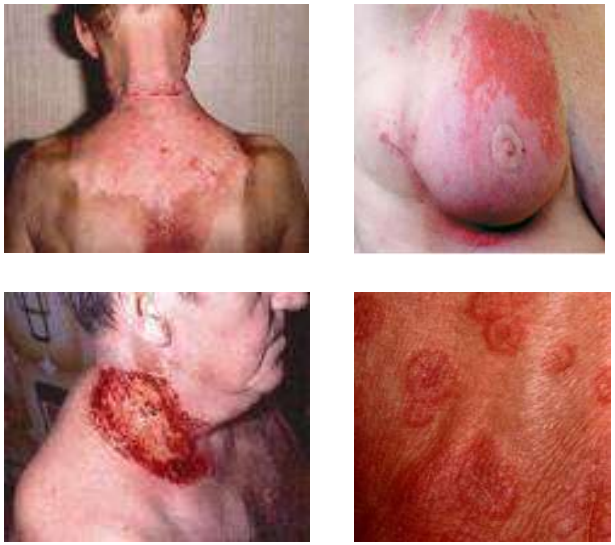
Foray N, Bourguignon M, Hamada N. Individual response to ionizing radiation. Mutation Research 2016-770:369-386

Abnormal tissue response after high doses IR (radiotherapy) = radiosensitivity (1)

- A clinical issue : radiation oncologists face early and late post-radiotherapy abnormal tissue response, i.e., adverse events with a variety of severity syndromes that quantify radiosensitivity
  - Not due to dosimetry or volume delivery errors
  - Grading systems : WHO (1979), CTC (1983), EORTC (1984), RTOG (1995) and CTCAE of NCI (Common Terminology Criteria for Adverse Events V5-2017 – V6 Fall 2022)

Grade	Clinical Characteristics
1	Asymptomatic or mild symptoms; intervention not indicated
2	Moderate; limiting ADLs; minimal, local, or noninvasive intervention indicated
3	Severe or medically significant; disabling; invasive intervention indicated
4	Life-threatening; urgent intervention required
5	Death related to adverse event

[a] ADLs, activities of daily living.

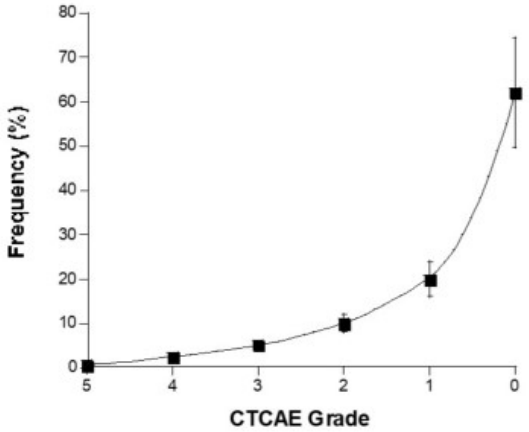


Frequency of tissue effects after radiation therapy
Calculation from COPERNIC cohort (INSERM 1296)
(Granzotto et al., 2016 = 117 patients + extension = 200)

CTCAE grades	0	1	2	3	4	5
% RT patients	65	17	10	5	2.5	0.5

(with a relative error of about 20% each)

Significant tissue effects
= 18 % of patients



Granzotto, A et al.. *International journal of radiation oncology, biology, physics* **2016,** 94, (3), 450-60).

Le Reun E et al.. *Int. J. Mol. Sci.* ***2022****, 23, 10434. https://doi.org/10.3390/ijms231810434*

Abnormal tissue response after high doses IR (radiotherapy) = radiosensitivity (2)

- **Adverse effects depends on the localization of cancer**

  - **Head and neck carcinoma**

- acute grade≥3 toxicities were mucositis 32%, pain 11%, xerostomia 7%, dysphagia 53%, radiodermatitis 44%, and osteonecrosis 1% and late grade≥3 toxicities were fibrosis 6%, dysphagia 21%, fistula 1%, and skin necrosis (*Santa Cruz O, Oncology. 2018)*
- at 2 years from baseline, the percentage of patients reporting moderate to severe complaints of dry mouth, sticky saliva, or changes in taste/smell was 30%, 22% and 18%, respectively, while the majority of patients had no or few complaints of swallowing (79%) or speech (64%). Quality-of-life after radiotherapy for advanced laryngeal cancer: Results of a phase III trial of the Dutch Head and Neck Society. (*Janssens, Radiotherapy and Oncology 2016*).

Abnormal tissue response after high doses IR (radiotherapy) = radiosensitivity (3)

- **Adverse effects depends on the localization of cancer**

• Breast cancer

- Many reviews indicate that 90-95% of breast cancer patient undergoing radiotherapy experience dermatitis. Dermatitis, although common, is considered a mild adverse reaction although severe reactions occur in 20-25% of patients.
- In a study of patients with breast cancer treated with excisional biopsy and primary RT, breast fibrosis was observed in 23% of patients, and the severity was dependent on daily radiation dose (*Clarke et al., Int J Radiat Oncol. 1983)*
- Dermatitis is a significant factor of alteration of Quality of life after radiotherapy of breast cancer *(Fuzissaki et al, 2019)*
- Irradiation of the breast on the left-side is associated with a higher incidence of myocardial infarction (RR 1.30) than when the right-side breast is irradiated (*Paszat et al, Journal of Clinical Oncology,1998; Cheng et al.,J Am Heart Assoc. 2017*) : link to radiosensitivity ?

Abnormal tissue response after high doses IR (radiotherapy) = radiosensitivity (4)

- **Adverse effects depends on the localization of cancer**

  - **Prostate cancer**

- 25% of patients developed Grade 2 or worse rectal bleeding with a median time of 11 months *(Akimoto at al., 2004. Int J Radiat Oncol Biol Phys)*
- late toxicity in prostate cancers 15% (GI) and 17% (GU). For severe effects, these values were 2% (GI) and 3% (GU) (*Ohri et al, 2012 Can J Urol)*
- late grade 2 GU toxicity 28% decreasing to 15% of patients at the end of follow-up (*Gadjar et al, 2008 Radiation Oncology)*
- The 10-year likelihood of developing grade 2 and 3 late genitourinary toxicity was 11% and 5% after IMRT *(Alicikus et al, 2011 Cancer)*

Abnormal tissue response after high doses IR (radiotherapy) = radiosensitivity (5)

- **Adverse effects depends on the localization of cancer**

  - **Rectal cancer**

- 5 to 10% of patients receiving radiation in the pelvis will develop severe intestinal complications within 10 years after treatment (*Chapel et al, 2013 World J Stem Cells)*
- late-stage rectitis occurs in 20% of patients, in general between 6 and 24 months after radiotherapy, and sometimes more than 10 years later *(Parades et al, 2007)*
- toxicity of radiotherapy of rectal cancer : pre-surgery radiotherapy is more toxic than post-surgery radiotherapy (grade 4/5 toxicity 34% versus 24%) (*Glimelius et al, 2002 British Medical Bulletin)*

Abnormal tissue response after high doses IR (radiotherapy) = radiosensitivity (6)

- In fine, we are not equal with respect to IR = various tissue reactions in 5 -20% patients, possibly underestimated by clinicians (*Lupen et al, 2022 IJROBP*)
- New techniques are not even always superior to reduce adverse events (Prostate: *Pozniak-Balicka et al, 2020* + Breast: *Thomas et al, 2022* + Lung: *Le Reun et al, in press*)
- Epinal accidents (2006) : for the same excess of dose prostate cancer patients were cured or had grade 1-4 proctitis or died !
- Radiation oncologists already take into account known factors (diabetes, smoking…) and they wish to know unknown individual factors (abnormal DNA DR, genetic …)
- Radiation oncologists require predictive assays to adapt radiation therapy protocols to prevent AE

  ⟹ A significant medical, economic and societal issue

N.Foray, C.Colin, M.Bourguignon. 2012 - 100 years of individual radiosensitivity: how we have forgotten the evidence. Radiology, 2012, 264 :627-31

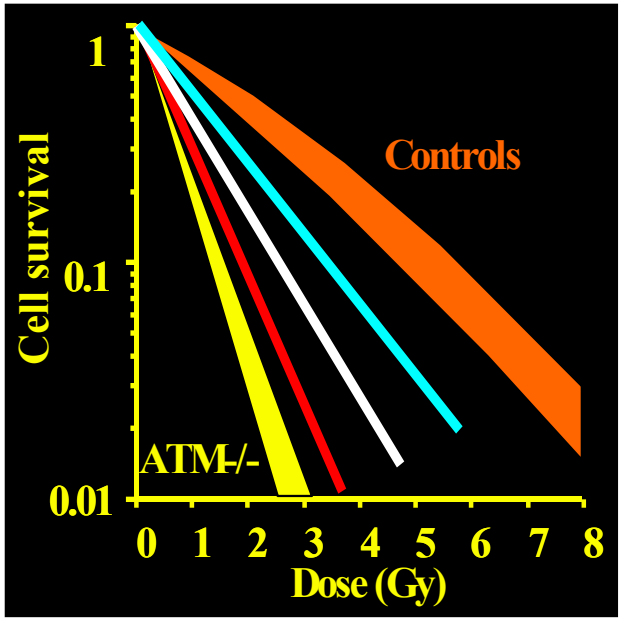
1981 : First correlations with individual radiosensitivity

Dr. EP Malaise
(1930-2013)

- Survival curves never cross : intrinsic radiosensitivity

- There is a continuum in radiation responses

- Quantitative correlation between survival fraction at 2 Gy (SF2) and local tumor control



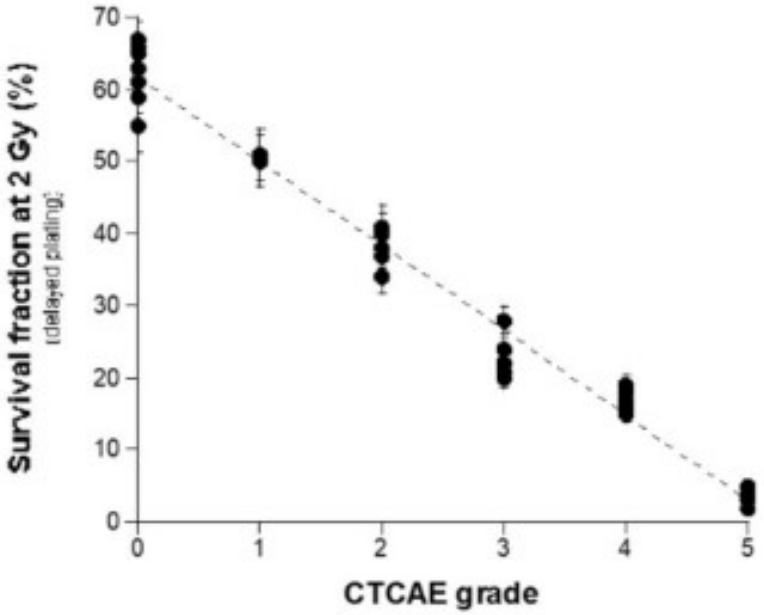
Clinical radiosensitivity =
in vitro surviving fraction at 2 Gy

Fertil and Malaise, Int J Radiat Biol Oncol Phys, 1981, 7(5):621-9.
Deschavanne and Fertil, Int J Radiat Biol Oncol Phys, 1996, 34(1):251-66.

Clonogenic cell survival (SF2) vs CTCAE grades

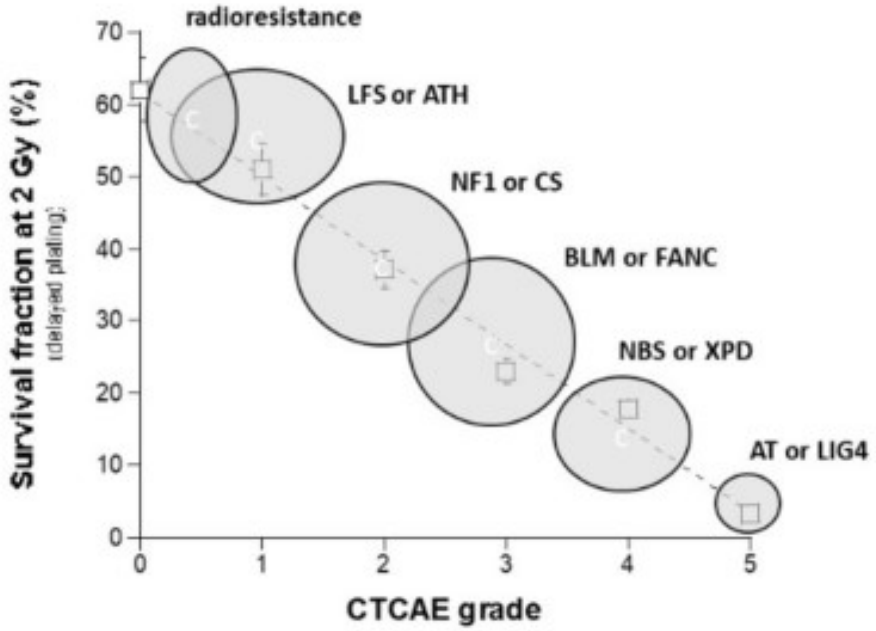
A

36 Copernic patients



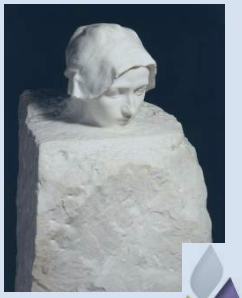
B

Radiosensitivity of genetic origin



SF2% = 61.55 – 11.72 x grade, $r^2 = 0.98$

UMR 1296

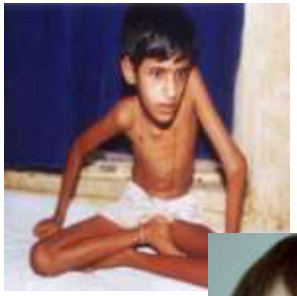


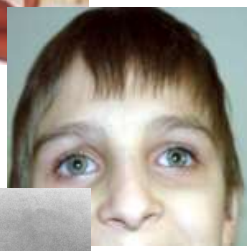
THE CELLINE PROJECT

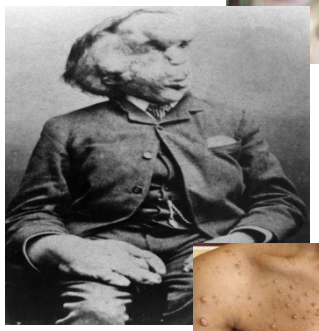
Groupe de Radiobiologie
Centre de Recherche en Cancérologie de Lyon

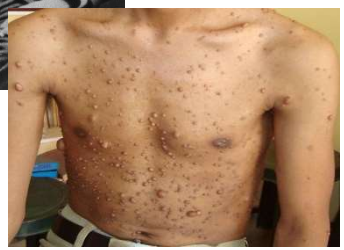
Prediction of radiosensitivity from genetic diseases

A routine in the lab since 2003 : N>100









SYNDROMES	GENES	SF2 (%)
Ataxia telangiectasia	ATM	1-5
Ligase 4 syndrome	LIG4	2-6
Progeria	Lamin A	5-10
Nijmegen's syndrome	NBS1	5-10
ICF syndrome	DNMT3B	10-15
Bruton's syndrome	BTK	15-20
Agammaglobulinemia	LIG1	15-20
Oxoprolinuria	GSS	15-30
Huntington's syndrome	HTT	15-30
Proteus syndrome	PTEN AKT1	15-30
Mac Cune Albright syndrome	GNAS	15-30
Tuberous sclerosis	TSC	15-30
Xeroderma Pigmentosum D	XPD	15-30
Cockayne's syndrome	CS	15-30
Usher syndrome	USH	15-30
Rothmund-Thomson	RECQL4	15-30
Neurofibromatosis type 1	NF1	15-30
Neurofibromatosis type 2	NF2	15-30
Turcot's syndrome	MMR gene	15-30
Bloom's syndrome	BLM	15-30
Fanconi anemia	FANC	15-30
Retinoblastoma	Rb	15-30

Individual radiosensitivity ?

Clinical radiosensitivity = continuous phenomenon

Predictive functional assays should :

- reflect the continuous spectrum of responses and the dose dependence over the relevant clinical dose range
- establish a quantitative relationship between clinical radiosensitivity (from CTCAE grade 0 to grade 5 whatever the early or late nature of tissue reaction) and cellular radiosensitivity
- identify patients with moderate radiosensitivity (up to 20% of the population) / hypersensitive patients with a known genetic disease (1-5%)

Deschavanne et al 1996

The major assays used to quantify individual radiosensitivity (1/9)

1- Clonogenic cell survival assays (SF2)

- The gold standard of radio-sensitivity
- Radio-sensitivity considered as the consequence of cell death
- Fibroblasts and lymphocytes from patients (AT)
- Quantitative correlation established between clinical responsiveness (CTCAE grades) and cellular radiosensitivity (in vitro clonogenic assay)
- Too time consuming

Puck et Markus1996, Taylor 1975, Huo 1994, Fertil and Malaise 1981, Foray 1997

The major assays used to quantify individual radiosensitivity (2/9)

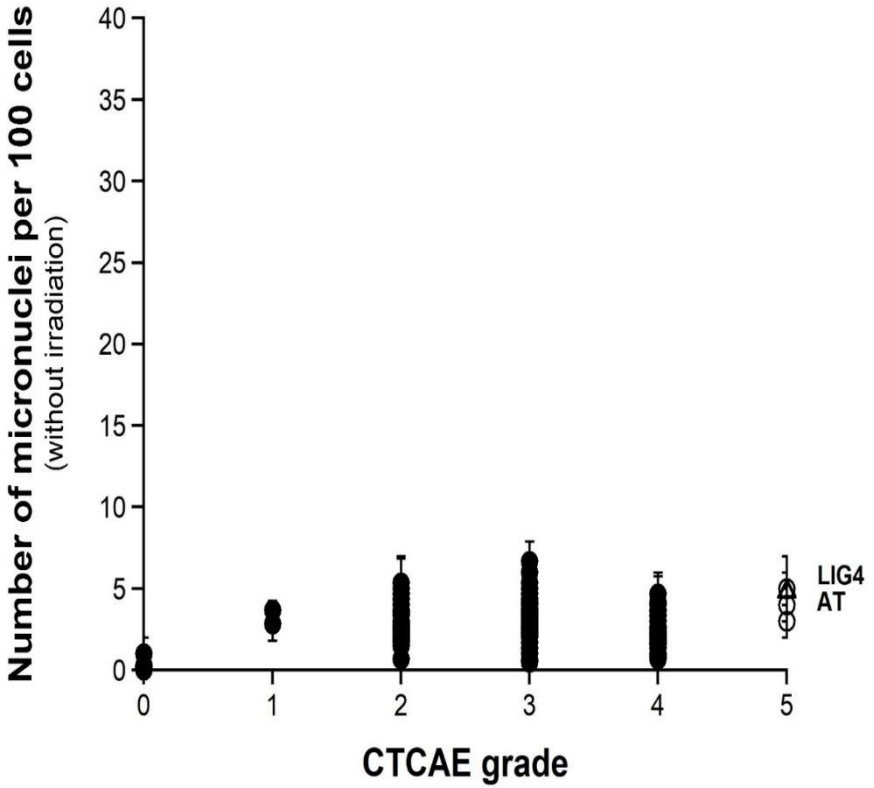
2- Cell death assays

- Mitotic death / micronucleus assay
  - Micronucleus frequency correlated with radiation-induced clonogenic inactivation
  - Too time consuming
- Cellular senescence, i.e., permanent G1 arrest
  - No general correlation between senescence and radiosensitivity
- Apoptosis
  - The most documented death mode
  - Significant cell type dependence : lymphocytes (+) but fibroblasts (-)
  - No general correlation between apoptosis and radiosensitivity
  - One inverse correlation reported in CD8 T-lymphocytes at 8 Gy, i.e., the lower the apoptosis yield, the higher the radiosensitivity !

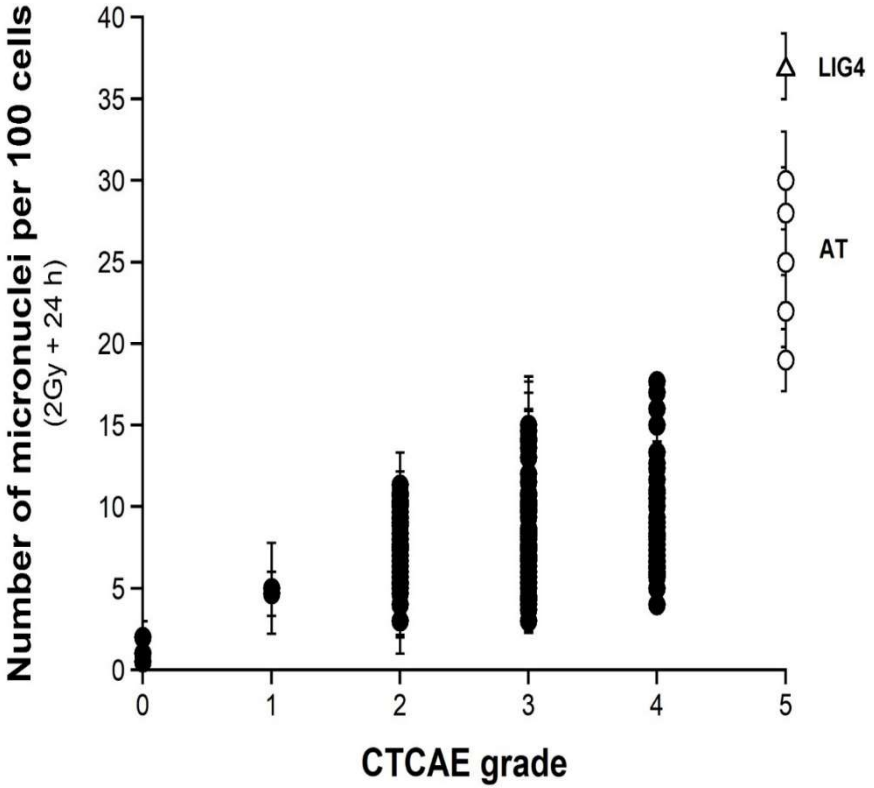
Grote et al 1981, Di Leonardo et al1994, Fenech 2000, Foray et al 1999, Joubert et al 2008, Schmitz et al 2003 & 2007, Finnon et al 2012, Ozsahin et al 1997 & 2005, Azria et al 2015, Lapierre et al 2022

Micronuclei vs CTCAE grades

200 Copernic patients



Before irradiation



24h after 2Gy

UMR 1296

UK Health Security Agency

Patients with marked (31cases) or mild (28 controls) late adverse reaction to adjuvant breast radiotherapy

Radiotherapy and Oncology 105 (2012) 329–336

Contents lists available at SciVerse ScienceDirect

Radiotherapy and Oncology

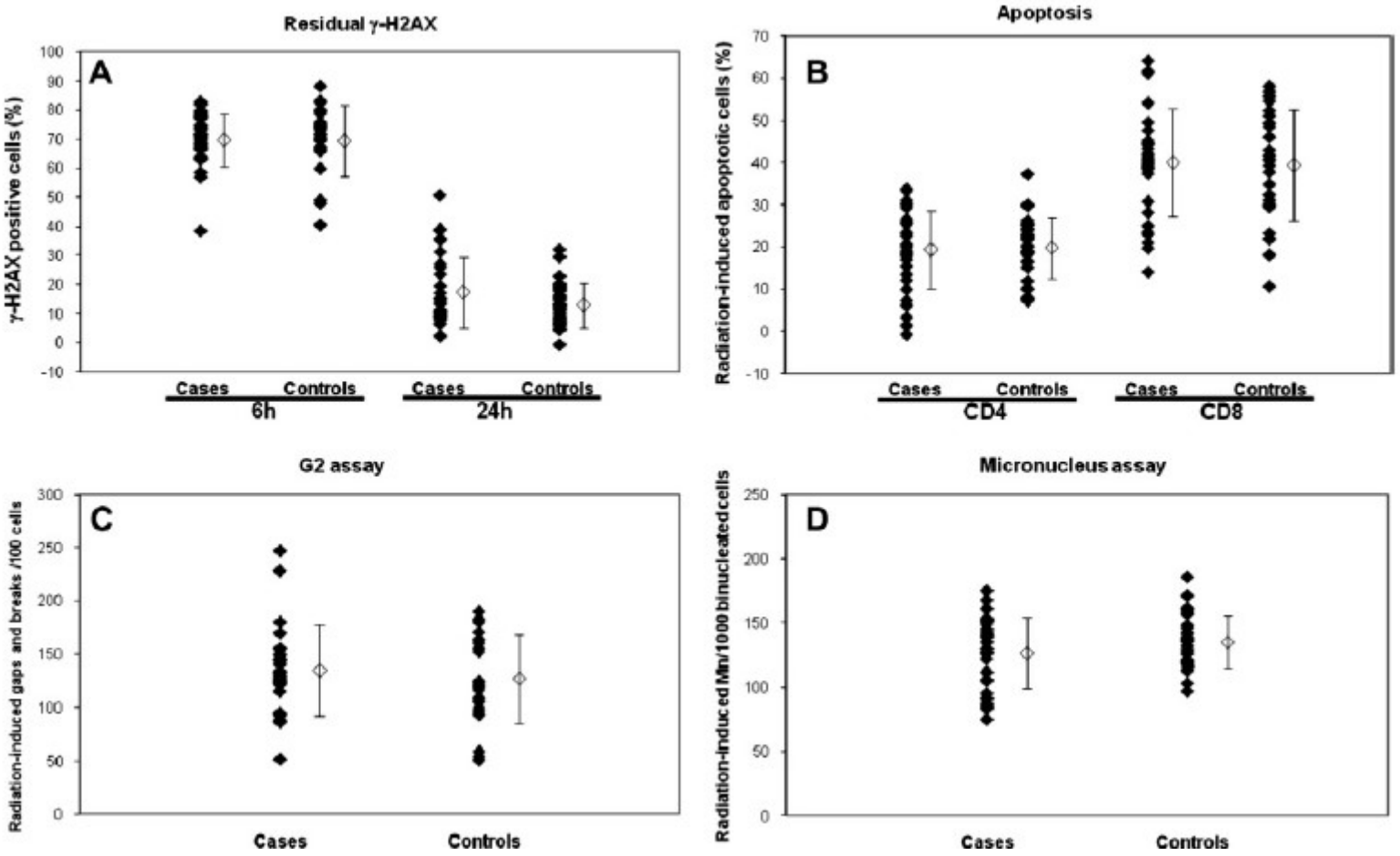
journal homepage: www.thegreenjournal.com

Original article

Correlation of *in vitro* lymphocyte radiosensitivity and gene expression with late normal tissue reactions following curative radiotherapy for breast cancer

Paul Finnon[a], Sylwia Kabacik[a], Alan MacKay[b], Claudine Raffy[a,1], Roger A'Hern[c], Roger Owen[d], Christophe Badie[a], John Yarnold[e], Simon Bouffler[a,*]

[a] Health Protection Agency, Didcot; [b] Breakthrough Breast Cancer Research Centre, London; [c] Institute of Cancer Research Clinical Trials and Statistics Unit (ICR-CTSU), Sutton; [d] Department of Oncology, Cheltenham; and [e] Division of Radiotherapy and Imaging, Royal Marsden NHS Trust, Sutton, UK



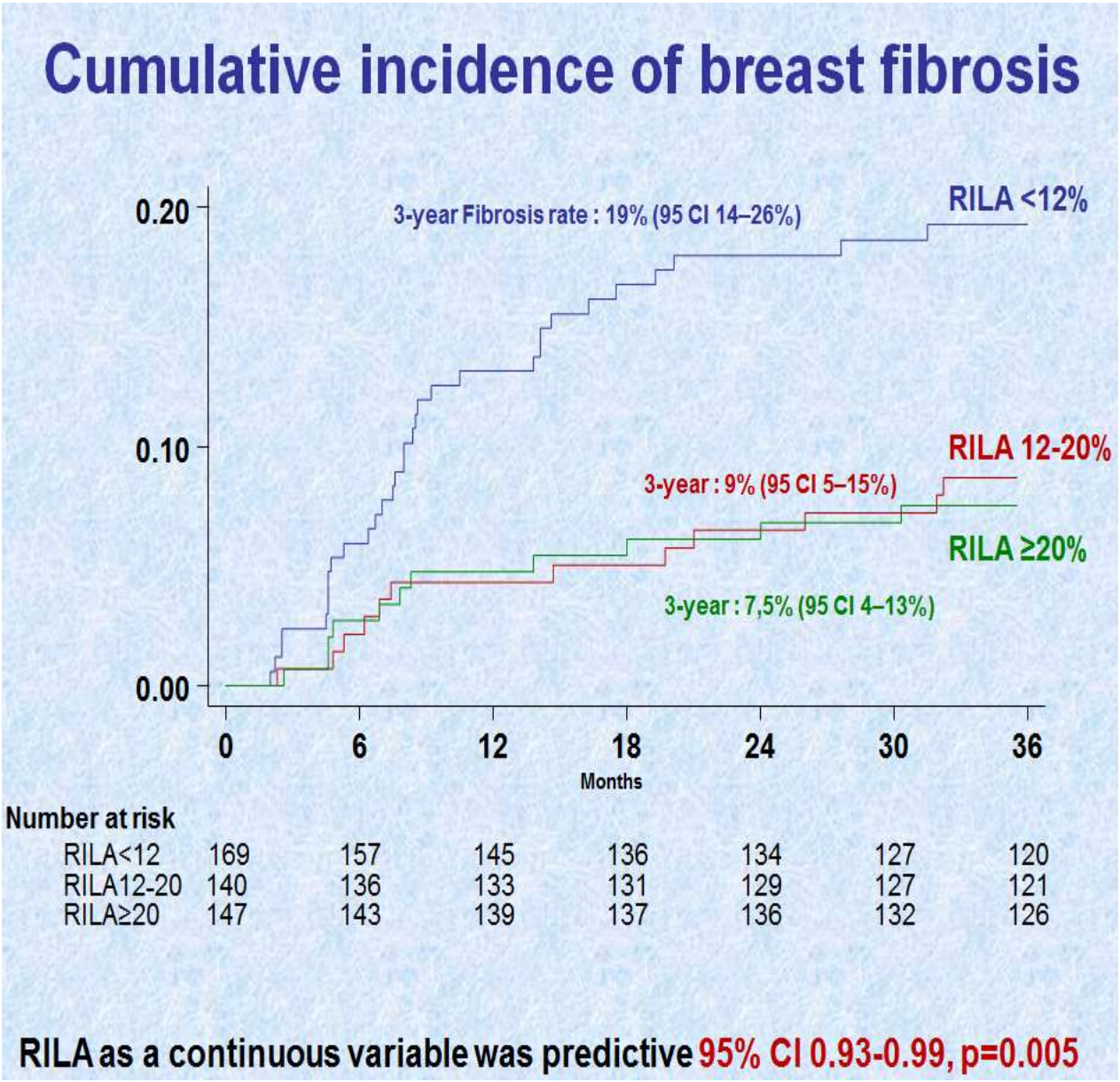
Variation in lymphocyte radiosensitivity does not necessarily correlate with normal tissue response to radiotherapy.

Radio-induced apoptosis of CD8 T-lymphocytes

RILA test

- Lymphocytes irradiated at 8 Gy
- Inverse correlation : The smaller the rate of apoptosis, the greater the Radiosensitivity.?
- Test predictive of late complications only after radiotherapy, e.g., breast fibrosis ≥ grade 2 for a level of apoptotic lymphocytes <12 %
- Mechanistic rationale ?
- Link between RILA and fibrosis ?

Azria et al, eBioMedecine 2015
Lapierre et al. Cancers 2022



The major assays used to quantify individual radiosensitivity (3/9)

3- Chromosome assays… also time consuming and no correlation with CTCAE grades !

- Staining assay (Giemsa) of chromosome breaks and aberrations
  - Correlation with radio-sensitivity and micronucleus frequency (+)
  - Requires metaphases and time necessary for DNA repair
- Premature chromosome condensation (PCC)
  - Correlation between unrepaired PCC fragments and radiosensitivity
- Fluorescence in situ hybridization (FISH)
  - Confirm that unrepaired chromosome breaks are good predictors of radiosensitivity
  - But it is not the case for chromosome aberrations rather linked to genomic instability
- Comparative genomic hybridization (CGH)
  - Provide the list of spontaneous chromosome breaks and aberrations
  - Does not predict radiosensitivity

Evans 1972, Carney 1999, Duker 2002, Grote et al 1981, Johnson and Rao 1970, Conforth and Bedford 1987, Joubert et al 2008, Darroudi et al 1998, Leona rd et al 2005, Brown and Kovacs 1993, Lucas and Sachs 1993, Ishkanian et al 2010, Tapio et al 2010

The genetic syndromes associated with radiosensitivity : an obvious link to DSB repair

But there are exceptions !

SYNDROMES	MUTATED GENE	SF2
Ataxia telangiectasia (classical homoz.)	ATM	1-5
Syndrome Ligase IV	LIG IV	2-6
Nijmegen syndrome	NBS1	5-9
➡ Progeria	Lamin A	8-19
Ataxia telangiectasia (variant homoz.)	ATM	10-15
➡ Usher 's syndrome	USH	15-20
Cockayne 's syndrome	CS	15-30
Xeroderma Pigmentosum	XP	15-30
AT-Like Disorder	MRE11	15-40
➡ Huntington Chorea	IT15	18-30
➡ Gardner 's syndrome	APC	20-30
Turcot 's syndrome	hMSH2	20-30
Fanconi anemia and BRCA2 mutations	FANC	20-40
BRCA1 mutations	BRCA1	20-40
Artemis mutations	Artemis	20-40

1 to 40 x

Deschavanne and Fertil, Int J Radiat Biol Oncol Phys, 1996, 34(1):251-66

The major assays used to quantify individual radiosensitivity (4/9)

4- DNA damage assays (1)

- DNA DSBs are linked to radiosensitivity
  - Micronuclei and unrepaired chromosome breaks
  - Observed in radiosensitive yeast, rodent mutants and human cells
  - Most genetic radio-sensitive syndromes associated with DSBs
  - Genetic syndromes with base damage or SSB repair defect are not necessarily radiosensitive
- Sucrose gradient sedimentation, neutral elution and pulse field electrophoresis (PFGE)
  - Discriminate DNA fragments on their size
  - Require tens of Gy : irrelevant for extrapolation to clinical exposures

Carney 1999, Duker 2002, Joubert et al 2008, Grote et al 1981, Iliakis 1991,

The major assays used to quantify individual radiosensitivity (5/9)

4- DNA damage assays (2)

- Halo assay and Comet assay (combines Halo assay and pulse field electrophoresis)
  - Mix chromatin de-condensation and DNA breaks
  - Difficult to interpret
- Cell free assays
  - Contributed to point out the predominance of end-joining DSB repair pathway in mammalian cells and the role of hyper-recombination in genomic instability
  - Too sophisticated technique for routine screening

Roti Roti and Wright 1987, Olive 2009, Thacker 1989, Bauman and West 1998

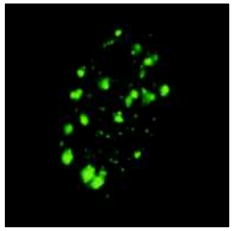
The major assays used to quantify individual radiosensitivity (6/9)

4- DNA damage assays (3)

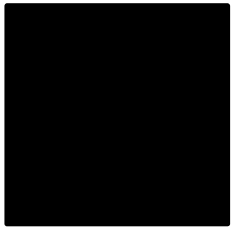
- γH2AX foci
  - Immunofluorescence technique
  - Identify each DSB into the nucleus
  - Lower limit of detection drastically decreased to 1 mGy
  - Not sufficient to predict moderate radio-sensitivity
- => a family of immunofluorescent biomarkers
  - Follow up of proteins in space and time into the cell
  - Visualize co-localizations by combination of different markers/colors
  - Very many data difficult to interpret
- Combination of assays : γH2AX foci, PFGE, SF2, plasmid assay
  - Proposal for a classification of radio-sensitivity in 3 groups: radioresistant; moderate and high radiosensitivity

Rothkamm and Lobrich 2003, Renier et al 2007, Joubert et al 2008

Yield of unrepaired DSB are not sufficient to predict all the human radiosensitivity

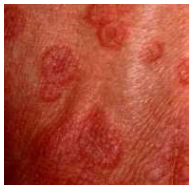


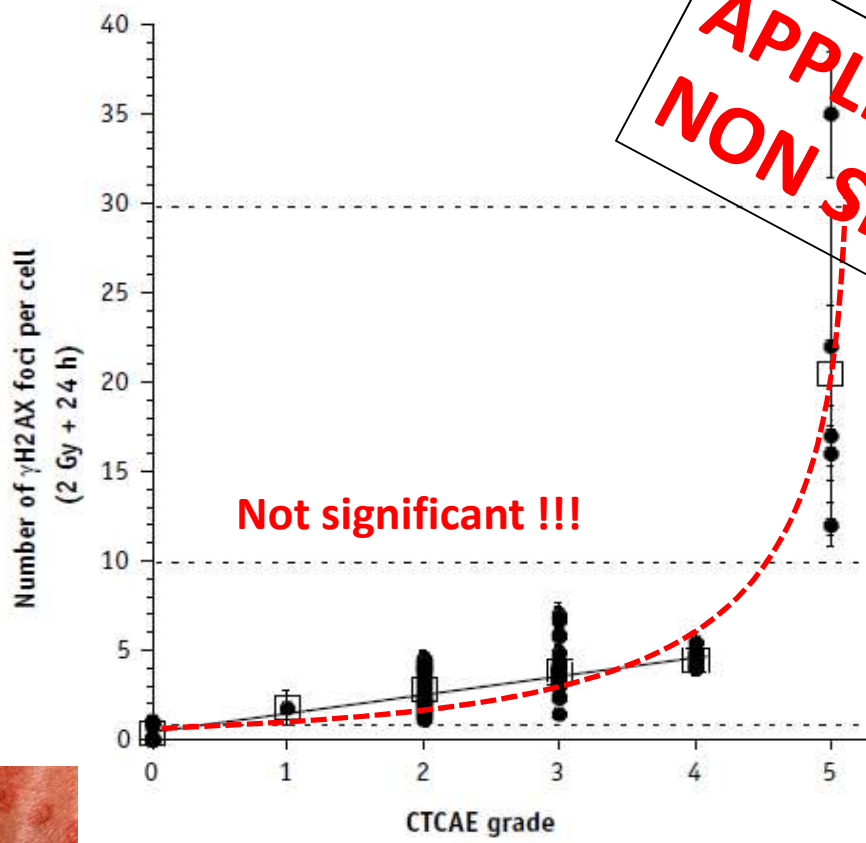
γH2AX



γH2AX

Cellular radiosensitivity





Vogin et al. IJROBP 2018
Pereira et al. IJROBP, 2018
Granzotto et al., IJROBP, 2016

APPLICABLE BUT NON SIGNIFICANT

Clinical radiosensitivity

The promising DSB approach do not predict intermediate radiosensitivity

The major assays used to quantify individual radiosensitivity 7/9

5- Genomic approaches (1)

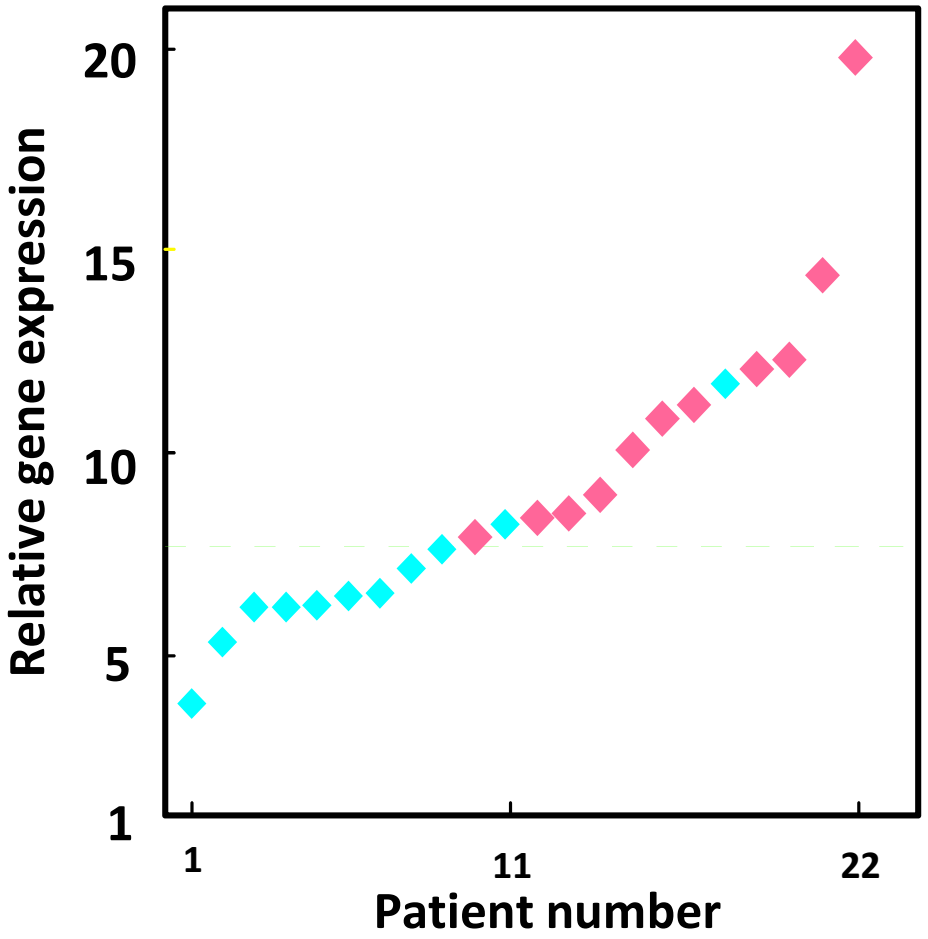
- Hypothesis that a given gene is able to predict human radiosensitivity = the expression of the gene must change with dose IR
- Micro-array techniques
  - The expression of the most radio-responsive genes is not linked with radiation toxicity in prostate cancer patients
  - The basal and post-irradiation expression of CDKN1A in T cells from breast cancer patients predicts SF2
- Single nucleotide polymorphisms (SNPs)
  - Some SNPs of ATM, XRCC1, XRCC3, RAD21, TGF-B1 and PARP identified and associated with abnormal IR response
  - No general correlation between a large number of SNPs and radiosensitivity
  - Genome editing techniques to identify if a SNP is indeed involved in radiosensitivity
  - => SNPs as aggravating factors of IR response

Svenson et al 2006, Badie et al 2008, Azria et al 2008, De Ruyck et al 2006, Willems et al 2008, Matsuura 2015, Carlotta Massi et al 2020

UK Health Security Agency

QRT-PCR analysis of T-lymphocytes from breast cancer patients Irradiated T-lymphocytes (2Gy, 2h)

CDKN1A as a marker of severe early radiation toxicity



Normalized to HPRT relative to sham

◆ **severe reactor**
◆ **normal reactor**

Prediction of reaction sensitivity>90%

Badie et al 2006. Br J Cancer
Finnon et al 2012. Radiother Oncol

The major assays used to quantify individual radiosensitivity 8/9

5- Genomic approaches (2)

– Genome wide association studies (GWAS)

- RAPPER study identified common genetic variants associated with late radiotherapy toxicity
- Associations are tumor site specific
- Do not allow so far an individual assessment of radiosensitivity
- New era of big data in radiogenomics : promising !

Barnett et al 2009, Rosenstein et al 2014, Kerns et al 2014, Barnett et al 2014

The genetic syndromes associated with radiosensitivity : an obvious link to DSB repair

But there are exceptions : degenerative diseases !

SYNDROMES	MUTATED GENE	SF2
Ataxia telangiectasia (classical homoz.)	ATM	1-5
Syndrome Ligase IV	LIG IV	2-6
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Artemis mutations	Artemis	20-40

1 to 40 x

Deschavanne and Fertil, Int J Radiat Biol Oncol Phys, 1996, 34(1):251-66

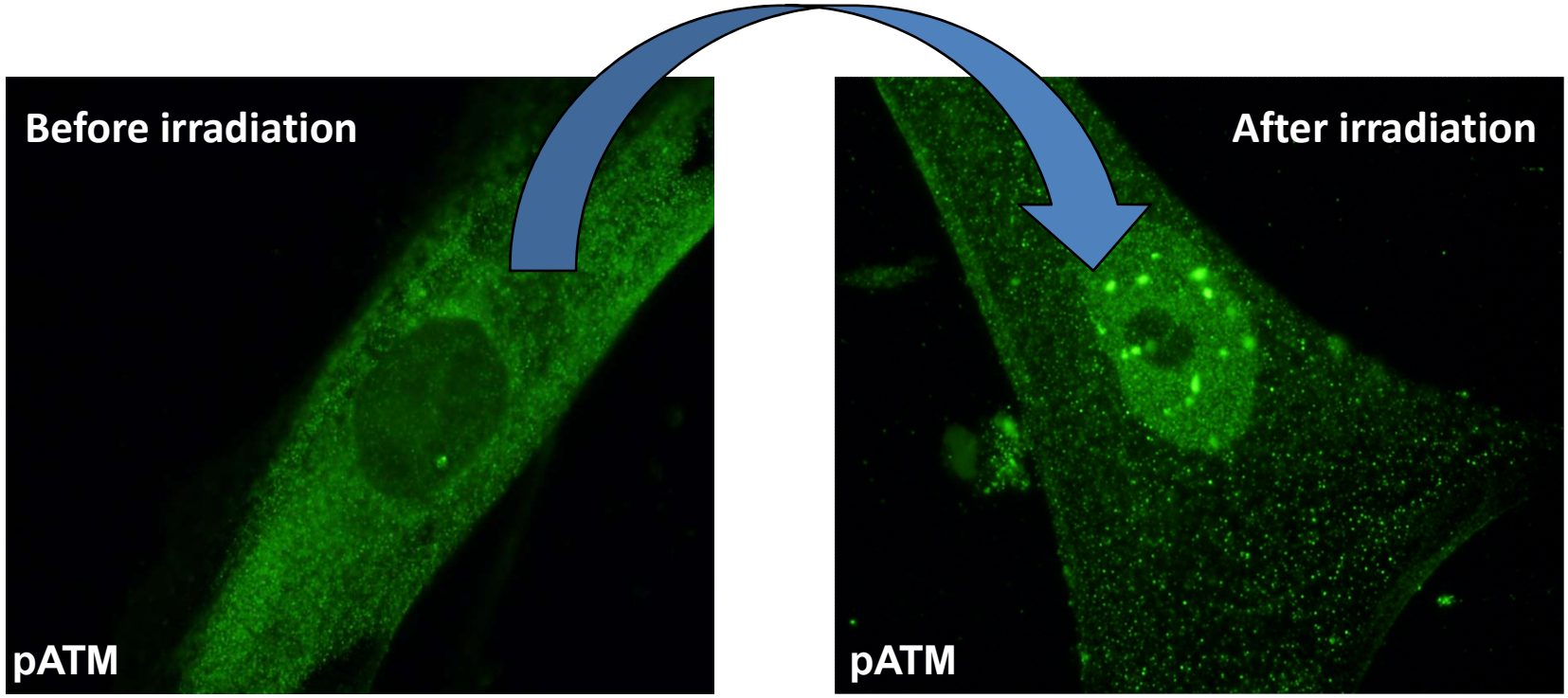
The major assays used to quantify individual radiosensitivity (9/9)

6- Immunofluorescent ATM nucleo shuttling

- Key protein for DSB repair by non-homologous end-joining (NHEJ) and inhibition of nuclease activity of MRE11 and genomic instability
- Present as inactive dimer into the cytoplasm
- Activated and phosphorylated into ATM monomers after irradiation
- Normal fast nucleo-shuttling from cytoplasm to nucleus
- Delayed nucleo-shuttling in progeroid syndromes, neurofibromatosis, Huntington's chorea, Bruton's disease … those syndromes for which mutated protein is not directly involved in DSB repair
- Delayed nucleo-shuttling due to ATM sequestration into the cytoplasm by the mutated proteins
- Good quantitative correlation between the delay of nucleo-shuttling and radiosensitivity evaluated by CTCAE grade

Ferlazzo et al 2014, Bodgi et al 2013, Granzotto et al 2016

ATM, believed to be exclusively nuclear from1995 is also cytoplasmic !!!!!



The radiation-induced nucleo-shuttling of ATM is systematically delayed in the radiosensitive patients!!!!!

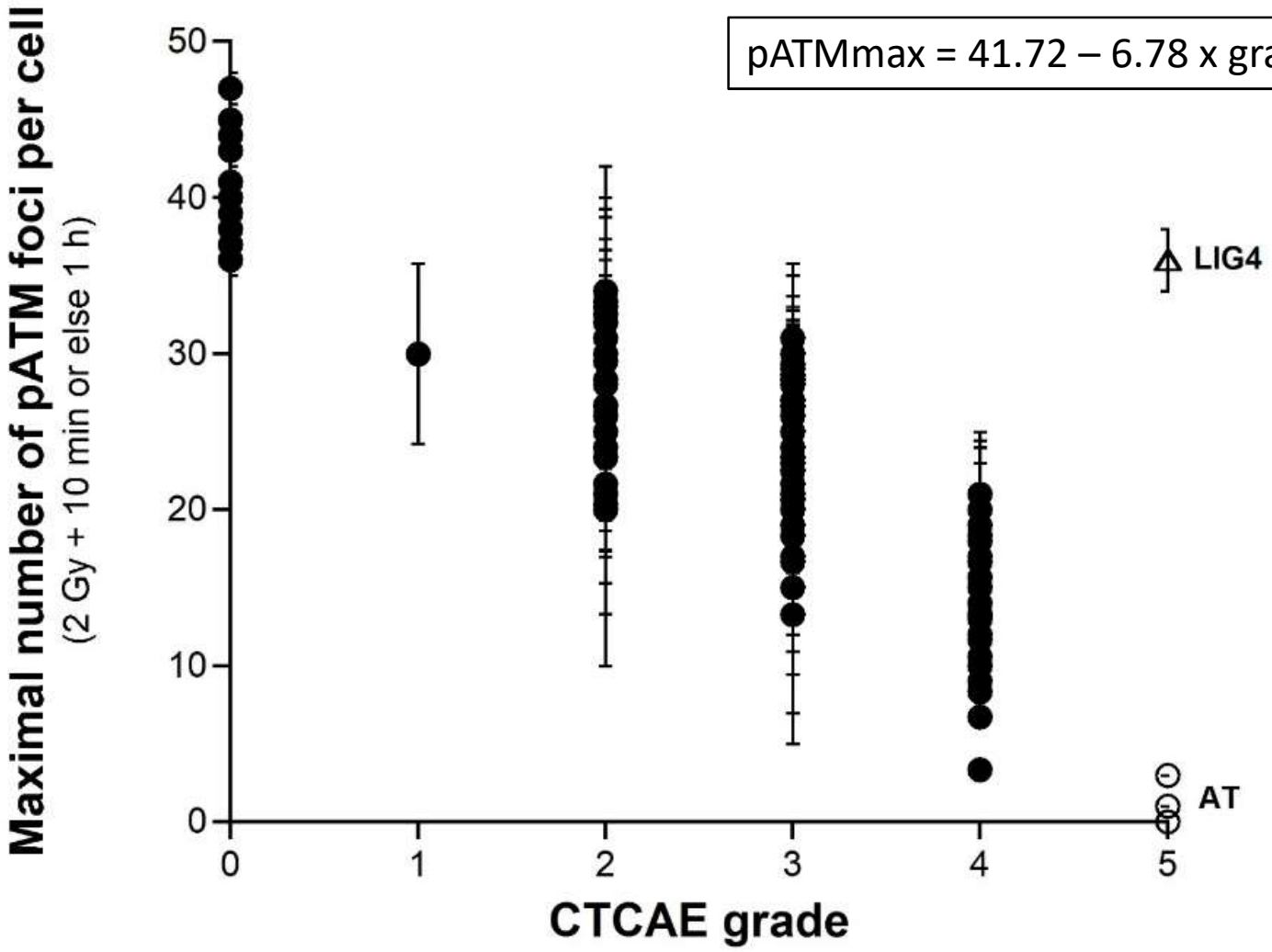
Observed by immunofluorescence, western blot, mass spectrometry

Granzotto et al., Int J Radiat Biol Oncol Phys, 2016
Bodgi and Foray, Int J Radiat Biol, 2016

Max pATM foci (10-60 min) vs CTCAE grades

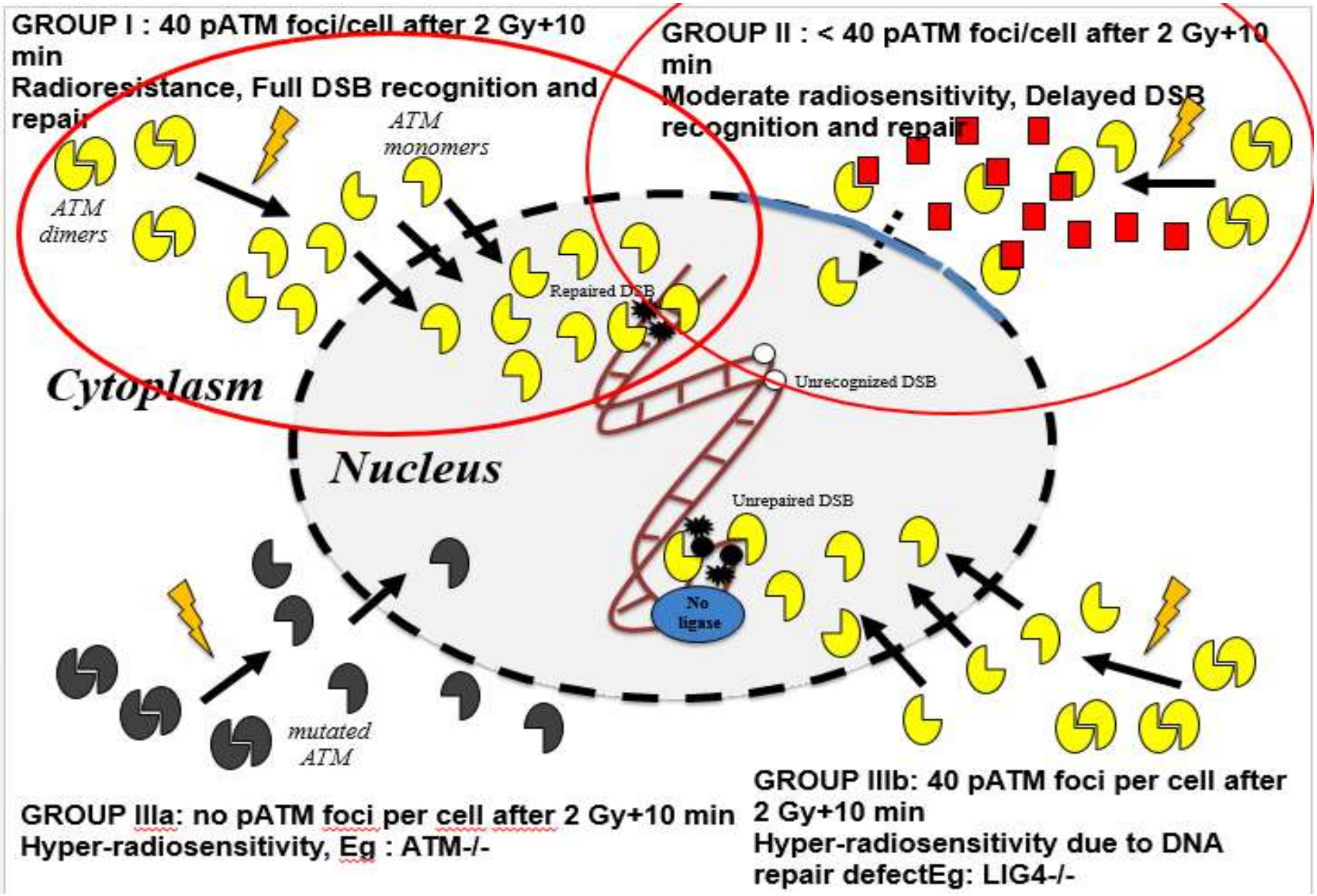
200 Copernic patients

pATMmax = 41.72 – 6.78 x grade (r^2 = 0.74)



UMR 1296

Radiation induced nucleo-shuttling of ATM (RIANS)



International Journal of *Molecular Sciences*

Int. J. Mol. Sci. 2022, 23, 10434.
https://doi.org/10.3390/ijms231810434

MDPI

Article

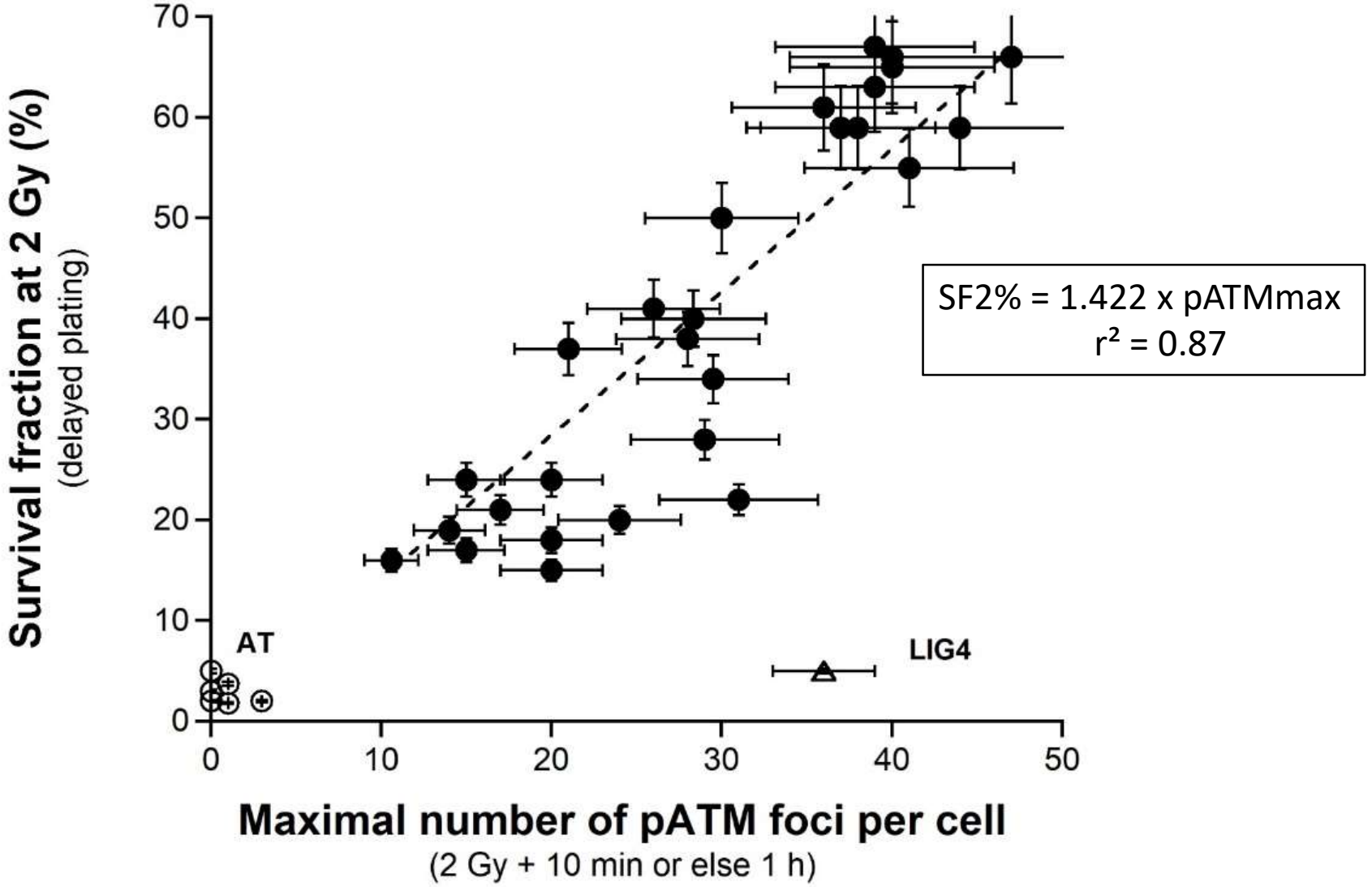
Quantitative correlations between radiosensitivity biomarkers show that the ATM protein kinase is strongly involved in the radiotoxicities observed after radiotherapy

Eymeric Le Reun[1†], Larry Bodgi[1,2,3†], Adeline Granzotto[1], Laurène Sonzogni[1], Mélanie L. Ferlazzo[1], Joëlle Al-Choboq[1], Laura El-Nachef[1], Juliette Restier-Verlet[1], Elise Berthel[1], Clément Devic[1], Audrey Bouchet[1], Michel Bourguignon[1,4], and Nicolas Foray[1,*]

A synthesis of the research on all functional tests of radiosensitivity and on radiation induced ATM nucleo-shuttling after irradiation (RIANS test) in our laboratory (INSERM UMR 1296)
COPERNIC cohort of radiosensitive Patients, xx Controls
Studies on untransformed skin fibroblasts
At least 3 independent triplicates
Irradiation at 2 Gy, Observations at t=0, 20 min, 1h, 6h, 24h

SF2 vs max pATM foci relationship

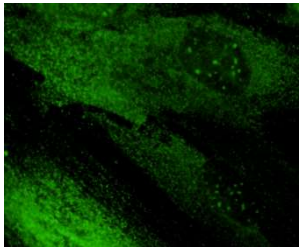
36 Copernic patients

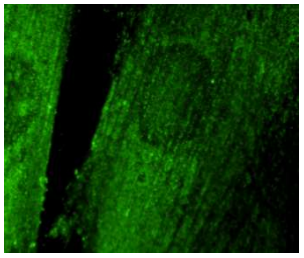


SF2% = 1.422 x pATMmax

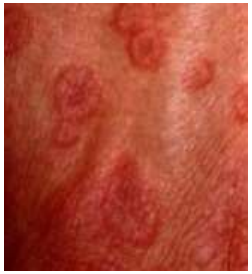
$r^2 = 0.87$

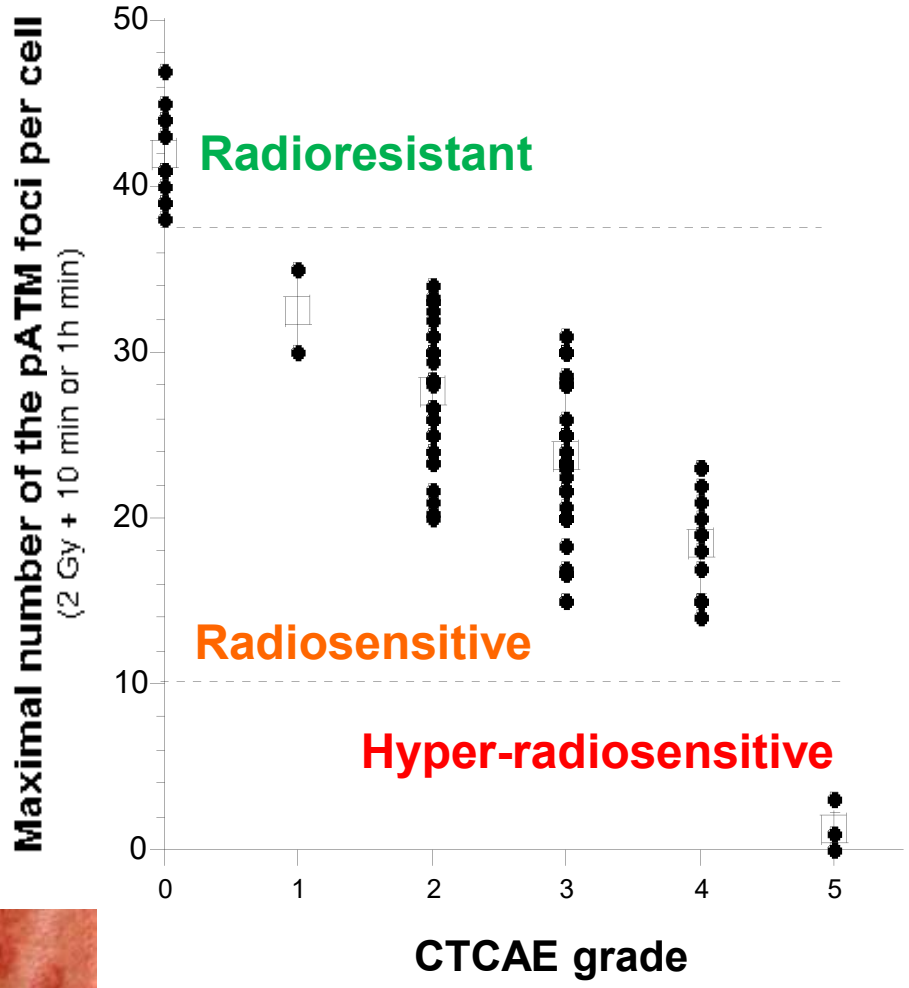
Correlation between ATM kinase activity and CTCAE scale severity grade





Cellular radiosensitivity





Clinical radiosensitivity

Significant correlation relevant for:

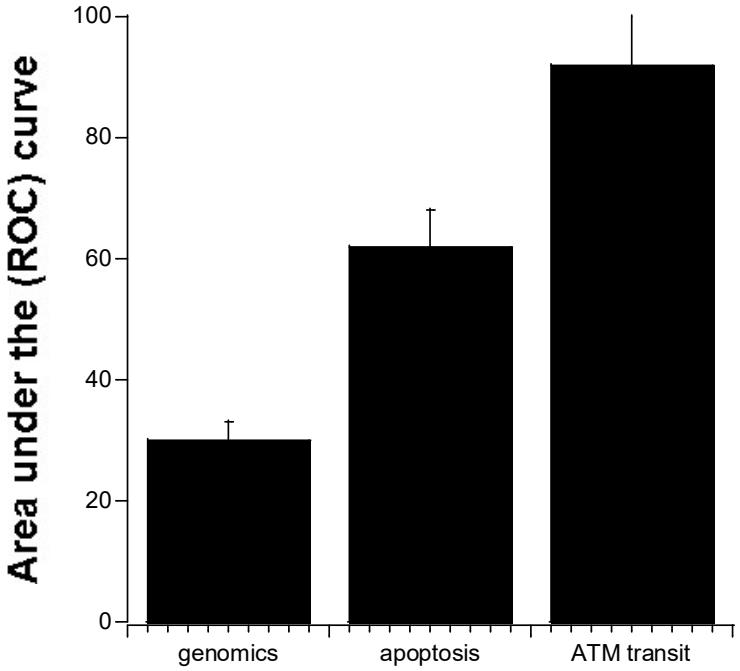
- **Any tumour localization**
- **Any type of reaction**

Concordance p=0.86

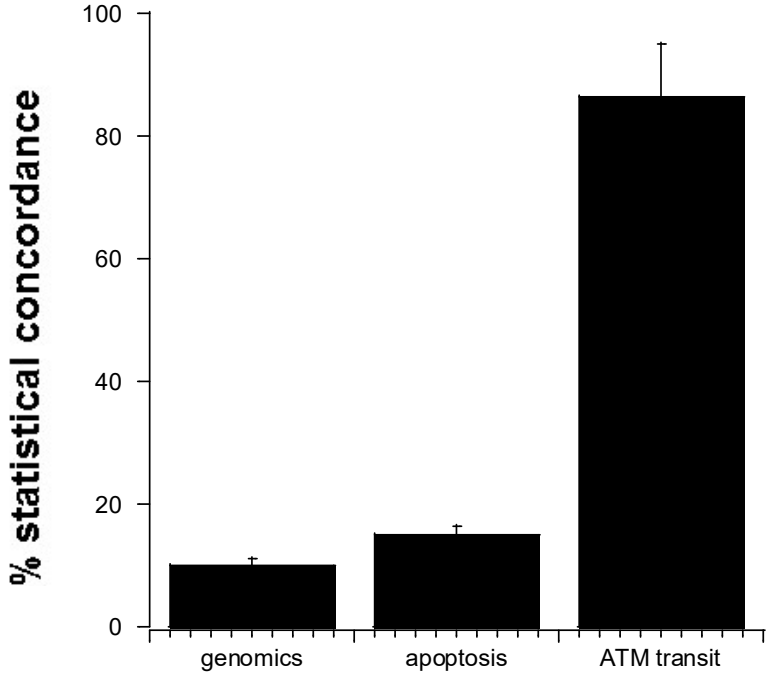
Granzotto et al., IJROBP, 2016
Belkacemi et al., IJROBP, 2016
Pereira et al. IJROBP 2018

Intercomparisons with some other predictive assays

« all-or-none » radiosensitivity
radiosensitivity/radioresistance



« Radiosensitivity as a continuum »
differents grades of radiosensitivity



It is time to compare predictive assays performances !

Granzotto et al., IJROBP 2016
Pereira et al. IJROBP 2018
Vogin et al. IJROBP 2018

Conclusion

Clinical radiosensitivity after radiation therapy

- Exists in a significant number of patients (up to 20%)
- Continuous phenomenon between normal and highly abnormal
- Has intrinsic individual component

Predictive functional assays :

- Radio-oncologists require a predictive functional assay applicable in routine
- SF2 provides the best correlation with CTCAE grades but is not clinically applicable
- Max pATM is the only other functional test, based on a significant mechanism and providing a correlation with CTCAE grades and explaining the quadratic model (*Bodgi et al, 2016 IJRB)*

Thank you for your attention