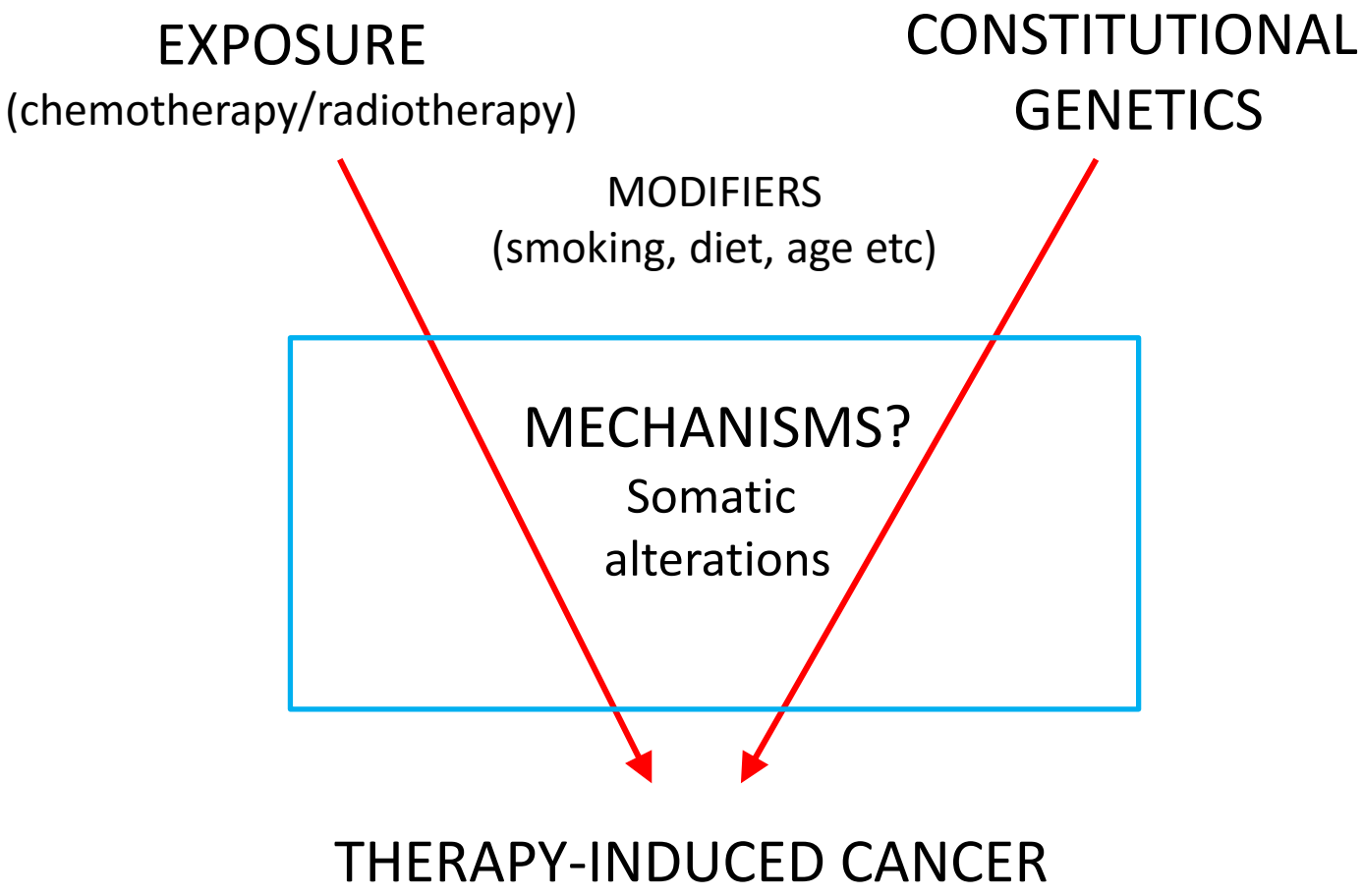
Molecular Mechanisms of Therapy-Induced Cancer

James M. Allan

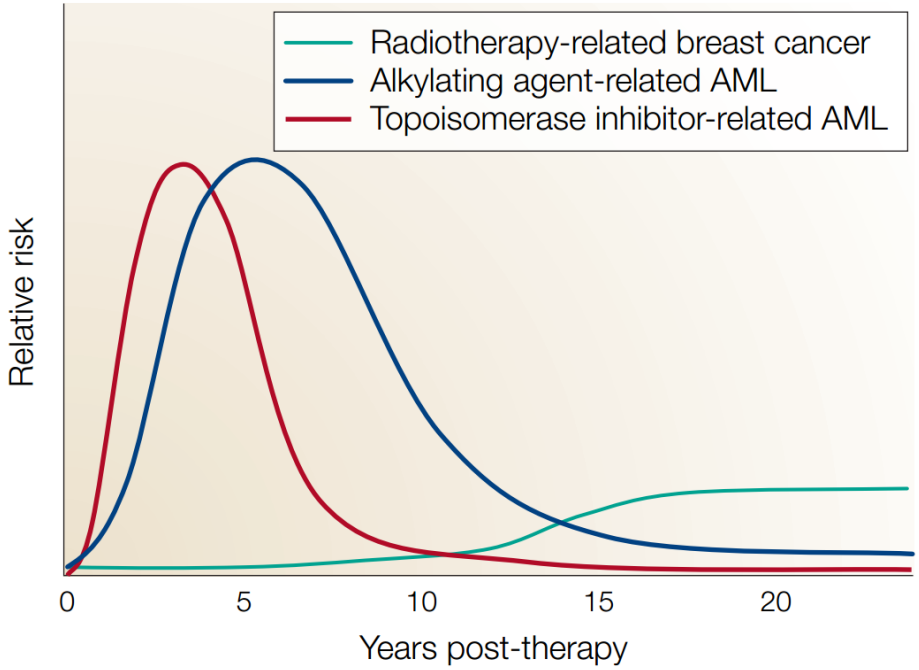
Mechanisms of therapy-induced transformation

Newcastle University
Northern Institute for Cancer Research



Chemotherapy-induced AML

Newcastle University
Northern Institute for Cancer Research



Relative risk of developing a therapy-associated cancer after Hodgkin lymphoma

ALKYLATING AGENTS
(procarbazine, dacarbazine, temozolomide, cyclophosphamide, carmustine etc)

- Risk increases 1-2 yr, peaks 5-10 yr, then decreases
- Preceding myelodysplastic syndrome (4-7 hits)
- Chromosomal deletions or monosomies (Chr. 5/7)
- DNA mismatch repair defective with high mutation rate (after methylating agents)

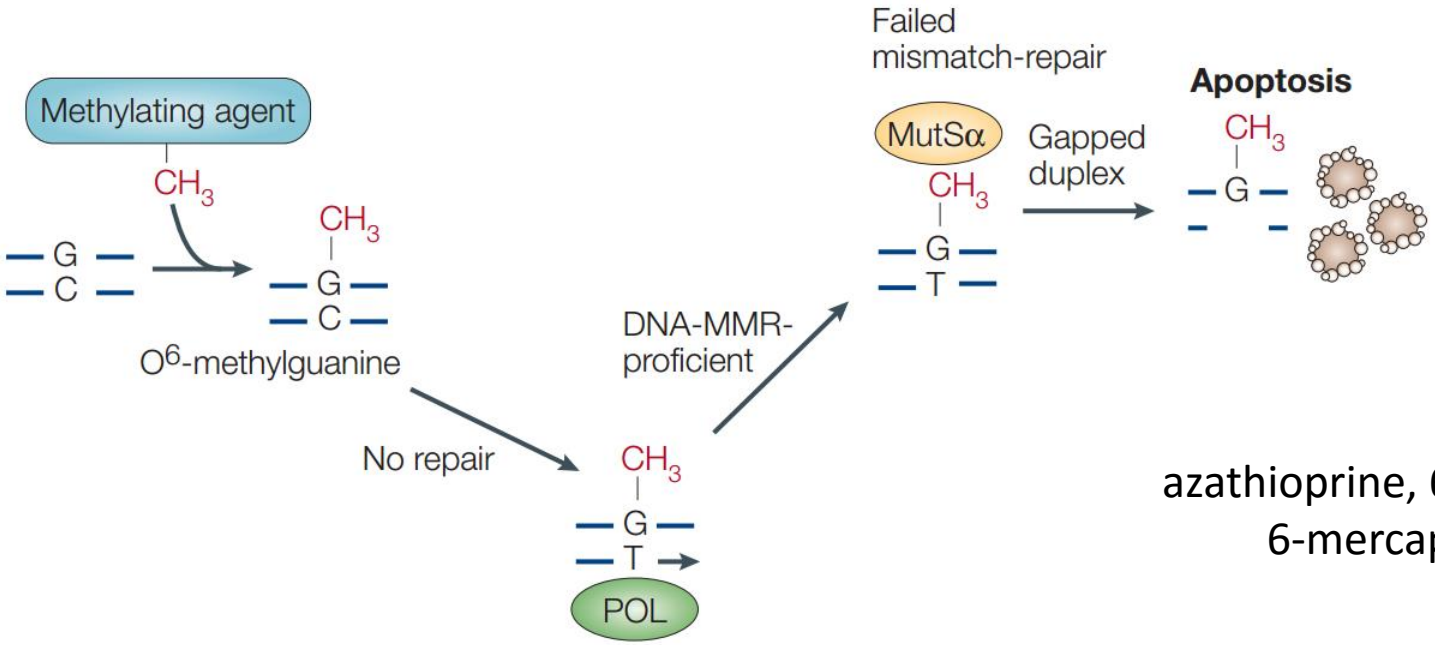
TOPOISOMERASE INHIBITORS
(etoposide, teniposide, doxorubicin, daunorubicin etc)

- Shorter induction period (median 2-3 yr)(1-2 hits)
- Balanced chromosomal translocations (*MLL* at 11q23)(MLL breaks map to topoisomerase binding sites)

Mechanisms of AML pathogenesis after methylating chemotherapy are well defined

Methylating agents select for DNA mismatch repair loss

Newcastle University
Northern Institute for Cancer Research



azathioprine, 6-thioguanine, 6-mercaptopurine

mutation rate

Molecular mechanisms of transformation

Newcastle University
Northern Institute for Cancer Research

DNA damage → Gene-specific effects → Altered phenotype

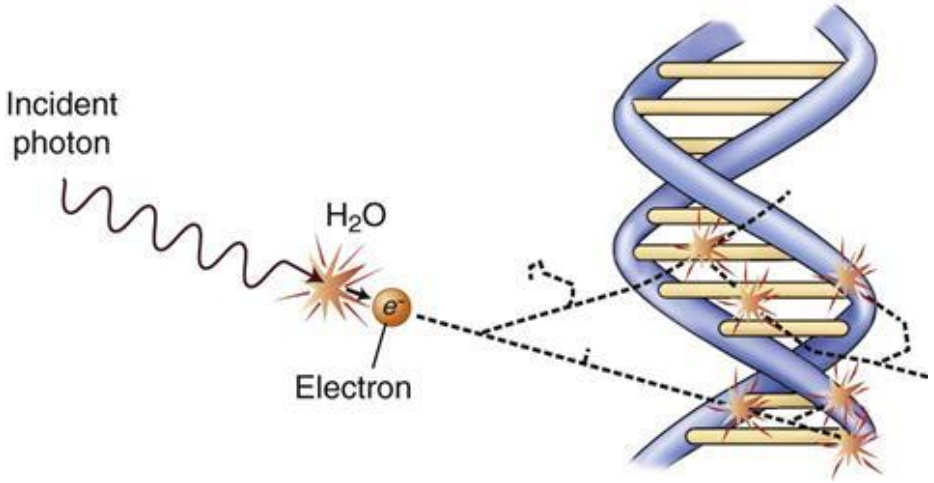
O^6-methylguanine/ 6-thiopurine → Loss of DNA MMR (MLH1, MSH2, MSH6, PMS1, PMS2 etc) → Mutation acquisition/ anti-apoptosis

DNA strand breaks at topoisomerase binding sites → *MLL* fusion gene → Dysregulated gene expression

IONISING RADIATION?

Radiation induced cellular damage

Newcastle University
Northern Institute for Cancer Research



- Strand break induction
- Hydrolysis of water

Radiation exposure induces DNA double-strand breaks and DNA base
– cytotoxic AND mutagenic –

Clustered DNA damage difficult to repair and can be converted to DNA DSBs.

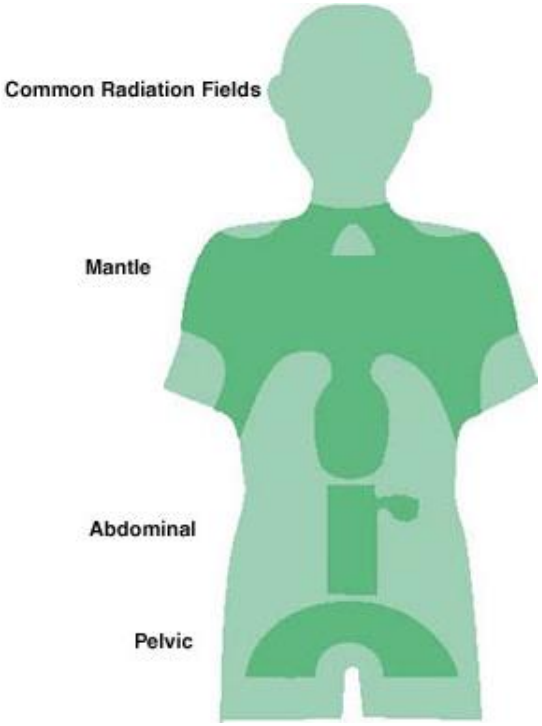
DNA DSBs difficult to repair with high fidelity – translocations, loss (deletion) and gain (amplification) of genetic material.

How do we identify genetic lesions induced by radiation?

MODELS !

Radiogenic cancer in Hodgkin lymphoma survivors

Newcastle University
Northern Institute for Cancer Research



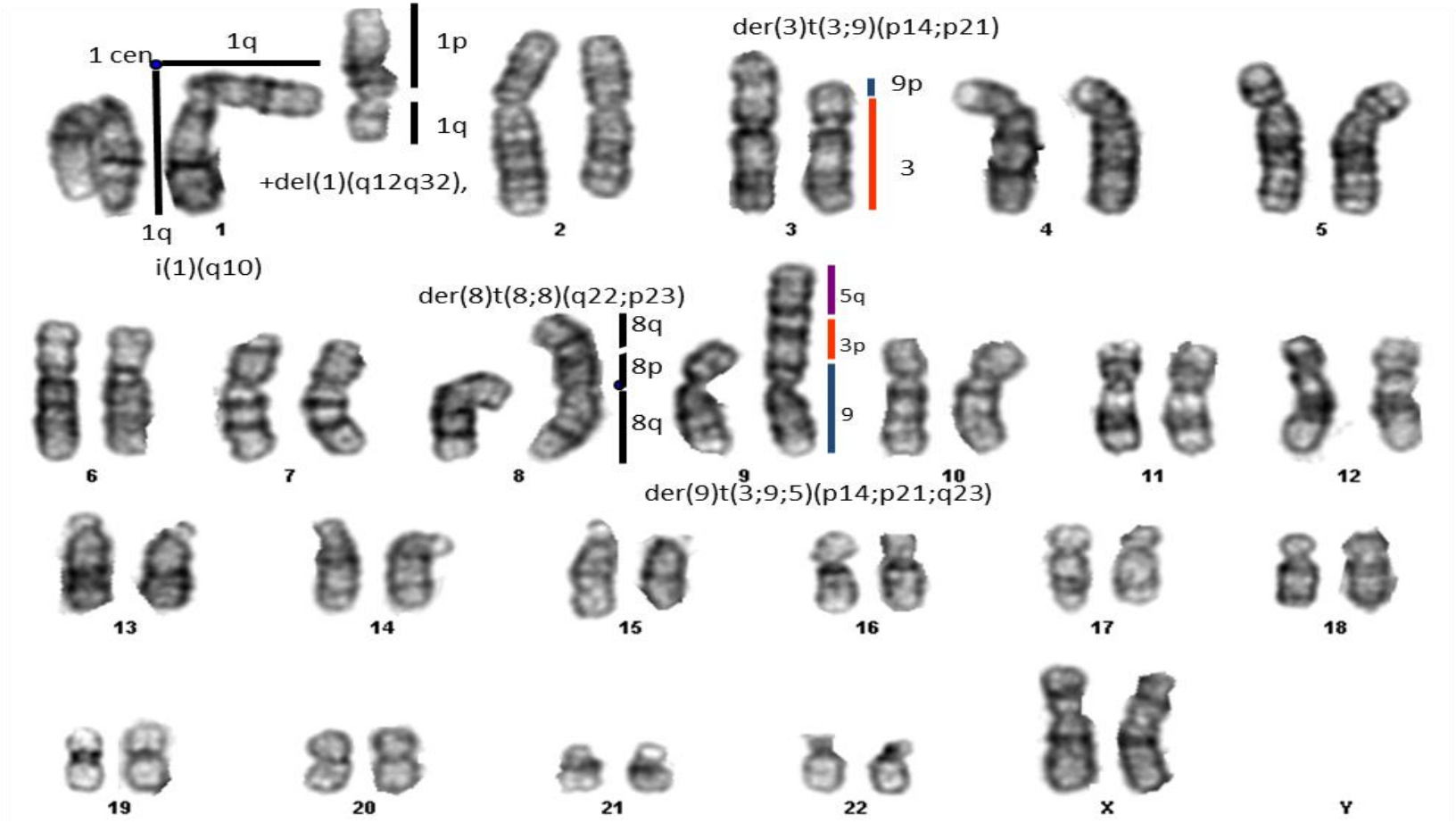
- Risk of second cancer associated with radiation exposure
  - Positively correlated with dose
  - Inversely correlated with age
- Most common second cancers
  - Breast
  - Thyroid
- Contribution to risk of chemotherapy is unclear (site-specific?)
- Responsible for 20% of the mortality in HL survivors

In vitro model system based on the MCF10A breast epithelial cell line

MCF10A

47, XX, i(1)(q10),+del(1)(q12q32), der(3)t(3;9)(p14;p21), der(8)t(8;8)(q22;p23), der(9)t(3;9;5)(p14;p21;q23).

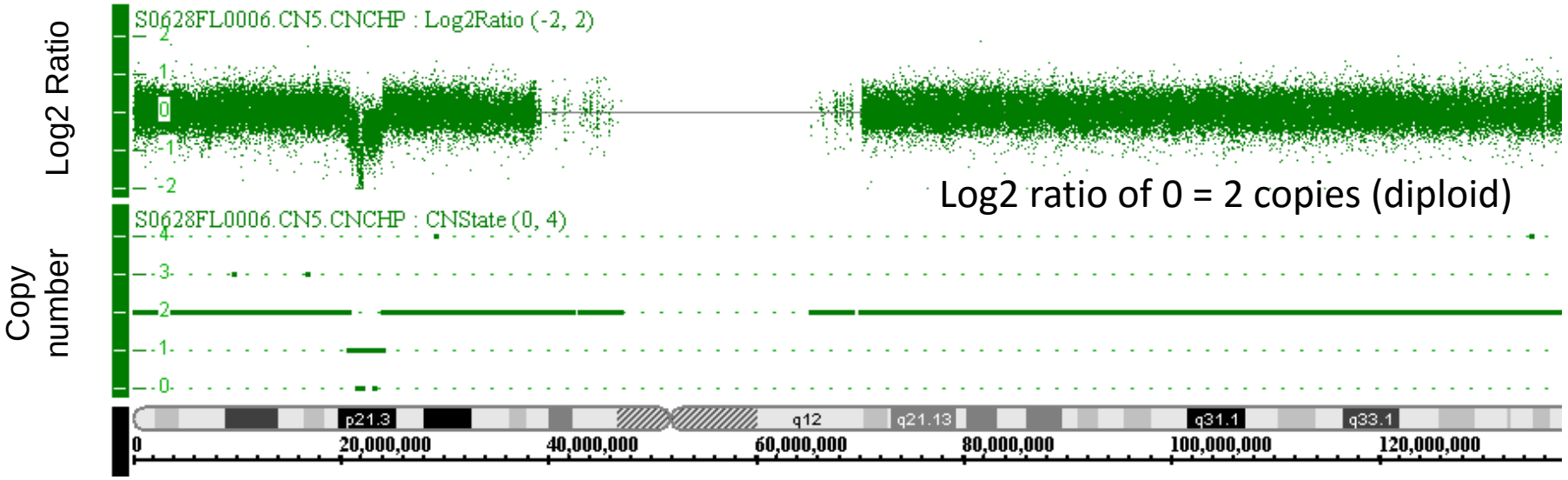
Newcastle University
Northern Institute for Cancer Research



- reciprocal translocation between chromosomes 3 and 9 (deletion of *CDKN2A/2B*)
- non-reciprocal duplication of the end of the q arm of chromosome 5 to the derivative chromosome 9
- Isochromosome 1 and 8q duplication

SNP array karyotyping

Newcastle University
Northern Institute for Cancer Research



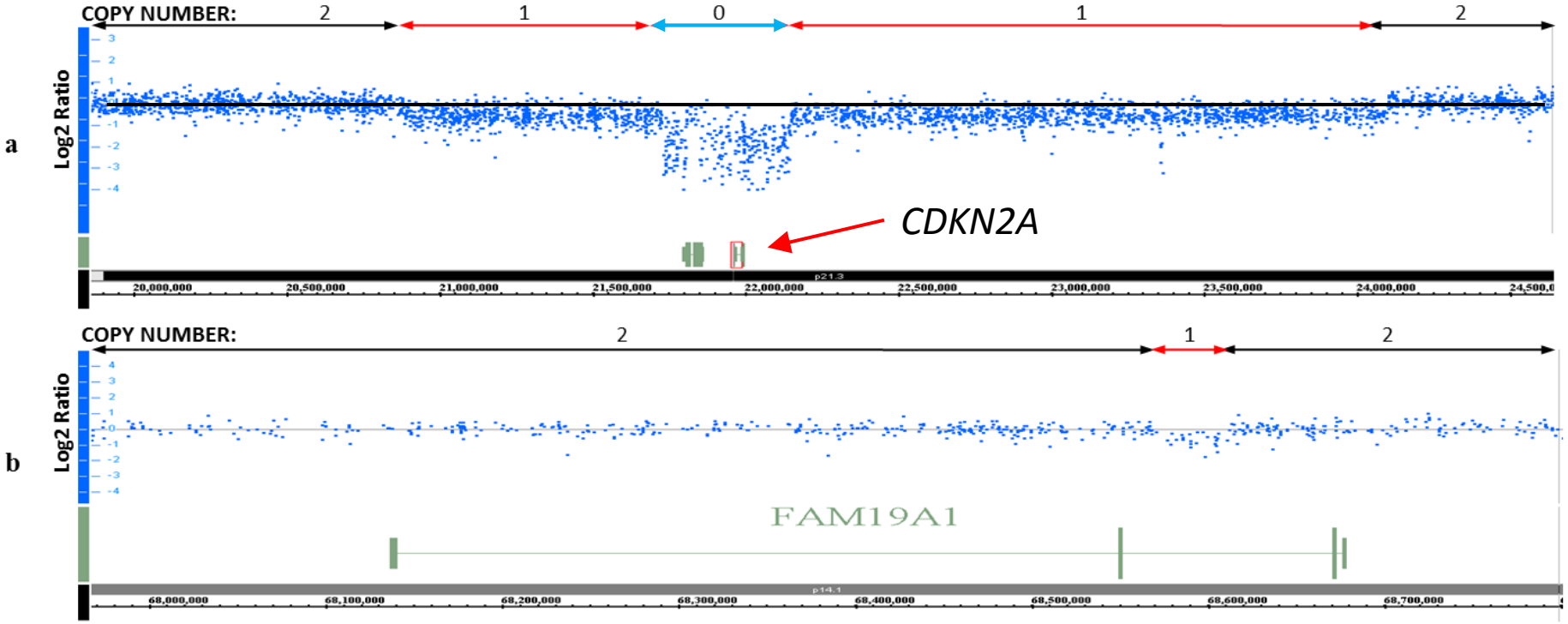
Log2 ratio of 0 = 2 copies (diploid)

Chromosome 8

Immortality conferred by *CDKN2A* deletion

Newcastle University
Northern Institute for Cancer Research

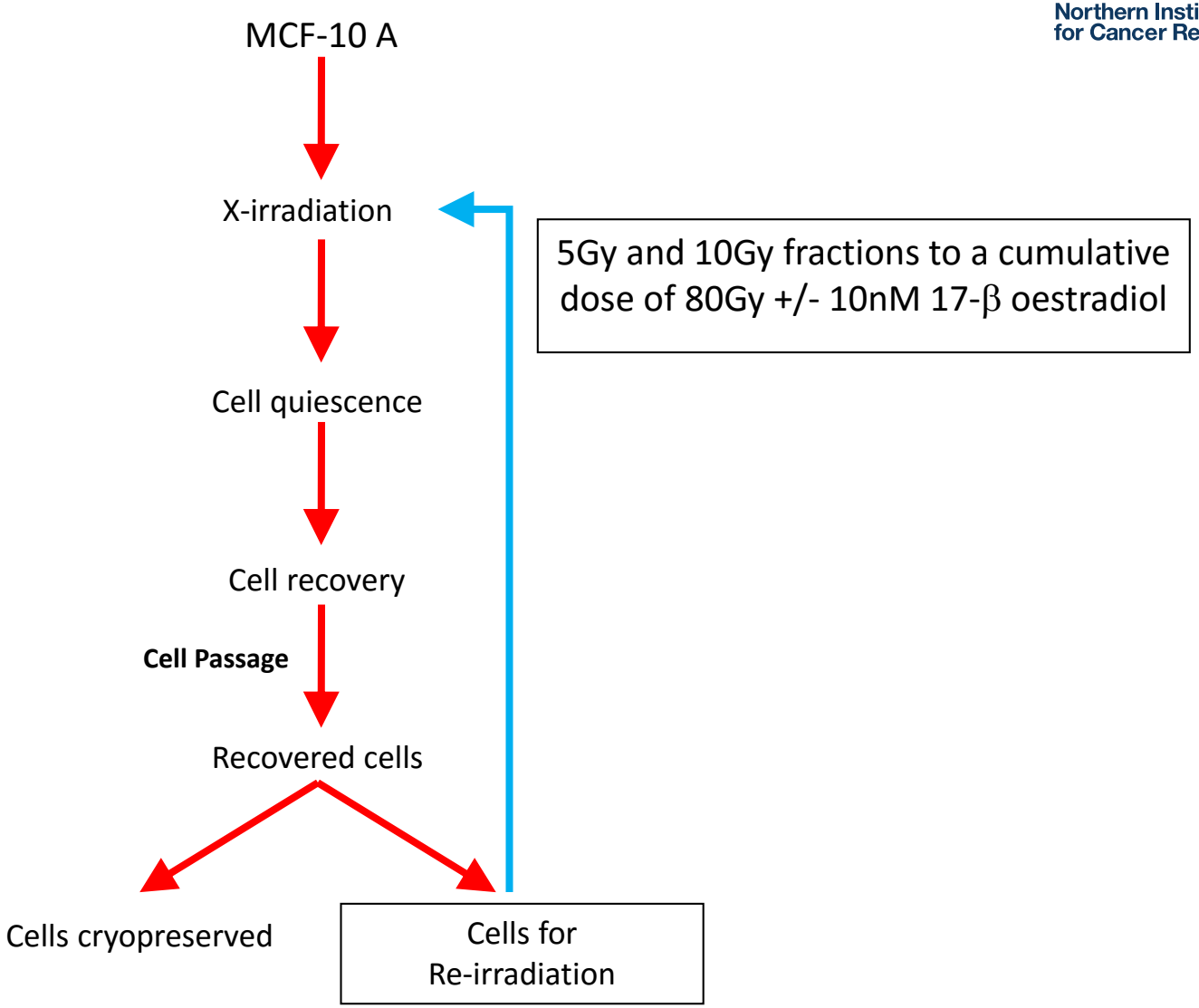
Chromosome 9 breakpoint



Chromosome 3 breakpoint

Irradiation protocol

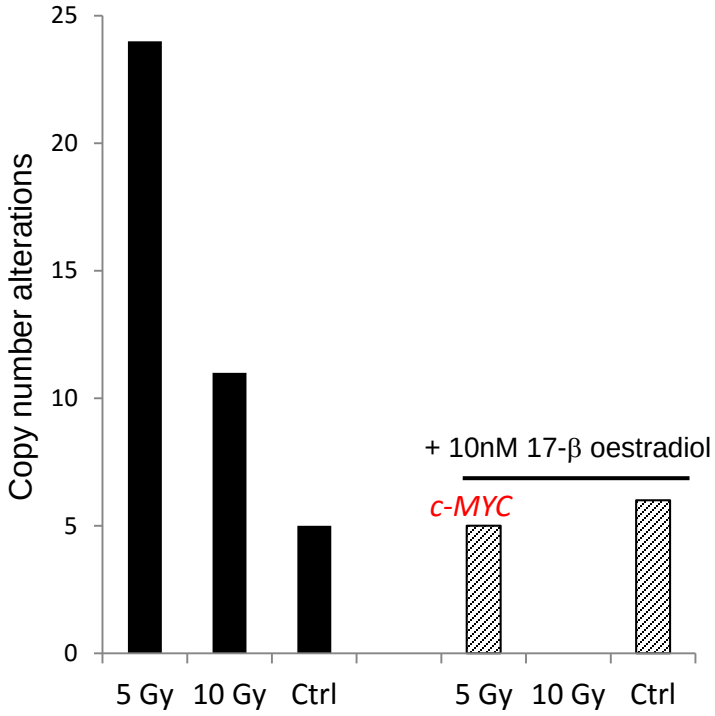
Newcastle University
Northern Institute for Cancer Research

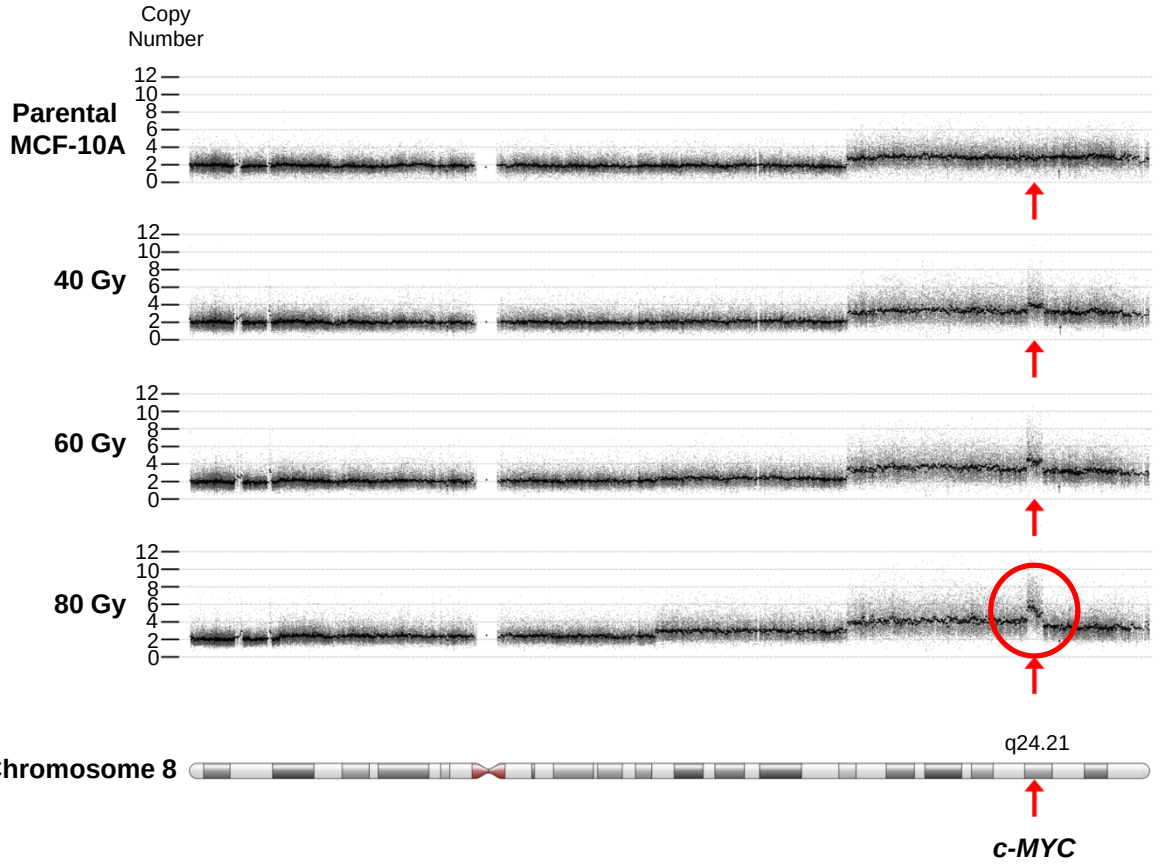


Radiogenic copy number alterations

Newcastle University
Northern Institute for Cancer Research

>50kb discernible



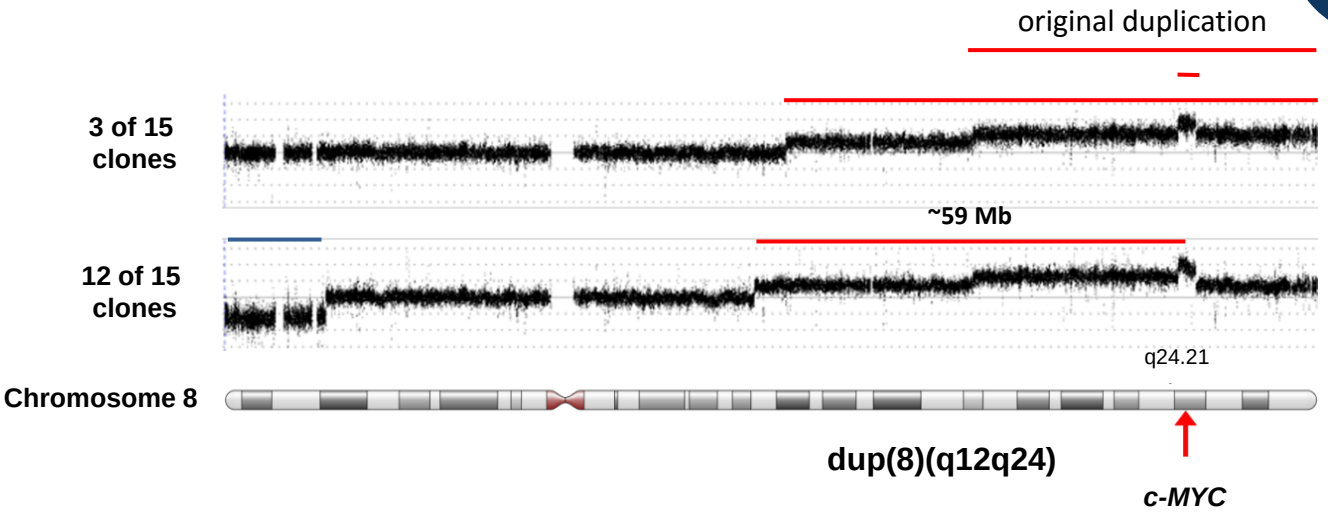


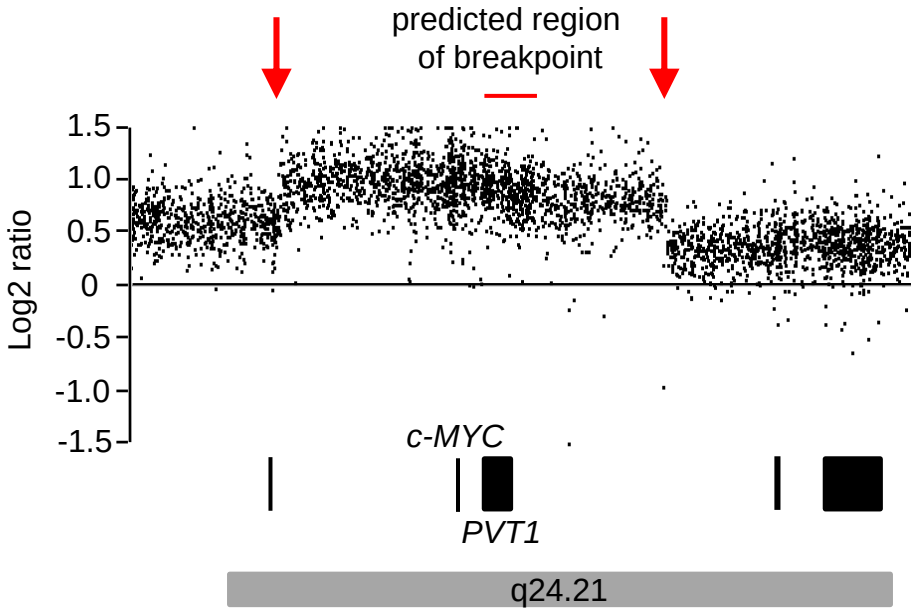
c-MYC……..

- has a pivotal function in growth control, differentiation and apoptosis.
- over-expression has potent oncogenic activity.
- dysregulation is a hallmark of many cancer types.

Evolution of the *c-MYC* locus in irradiated cells

Newcastle University
Northern Institute for Cancer Research

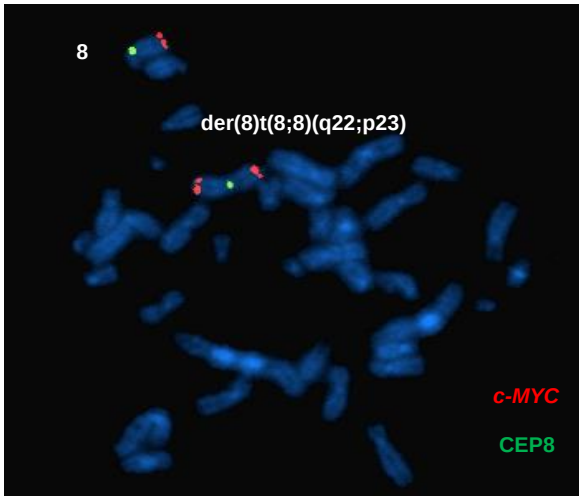




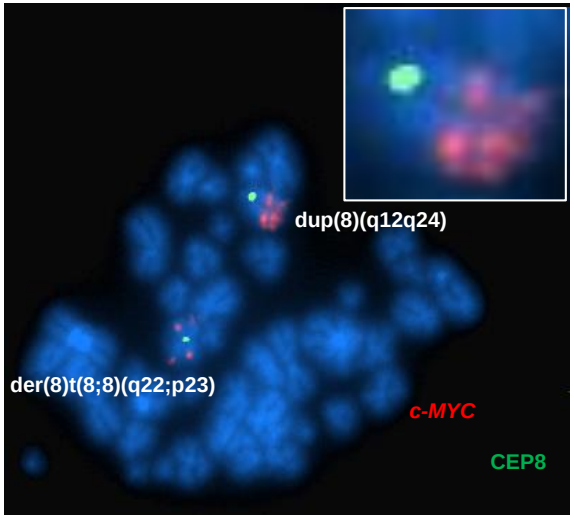
Evolution of the *c-MYC* locus in irradiated cells

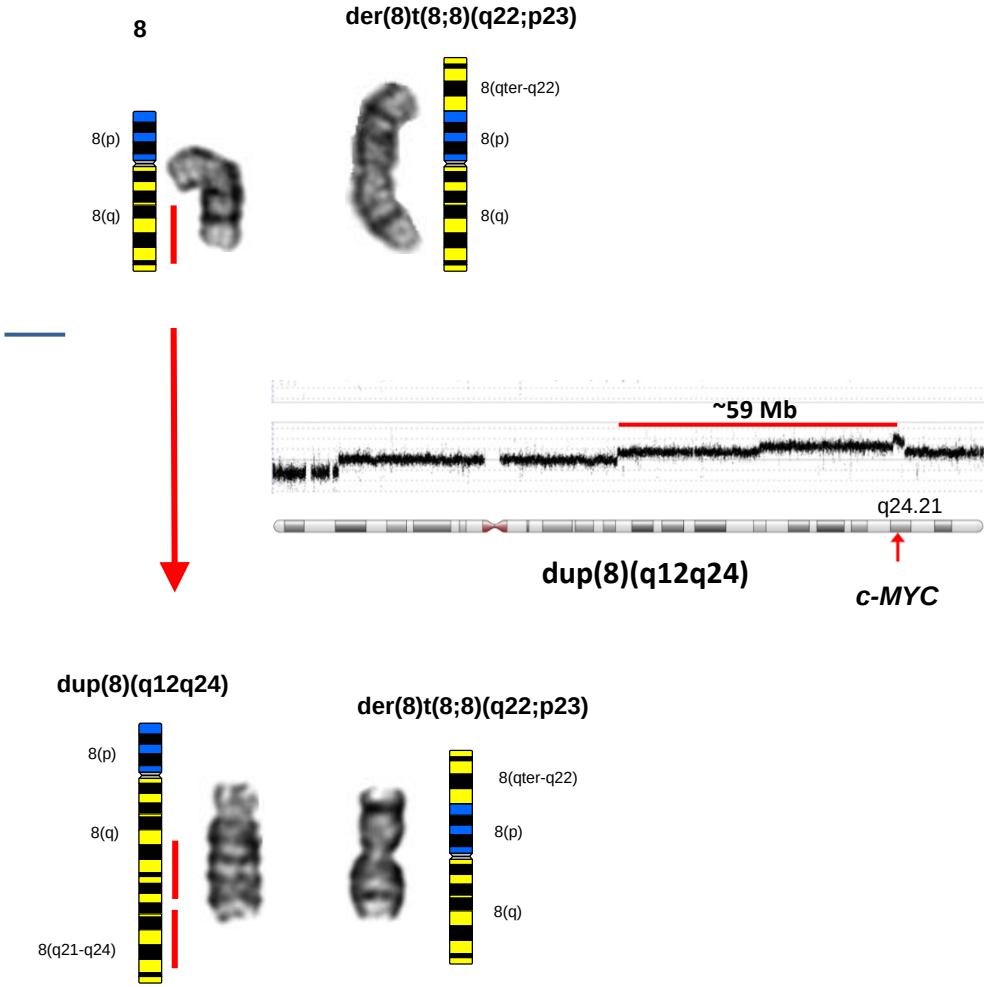
Newcastle University
Northern Institute for Cancer Research

Parental MCF-10A



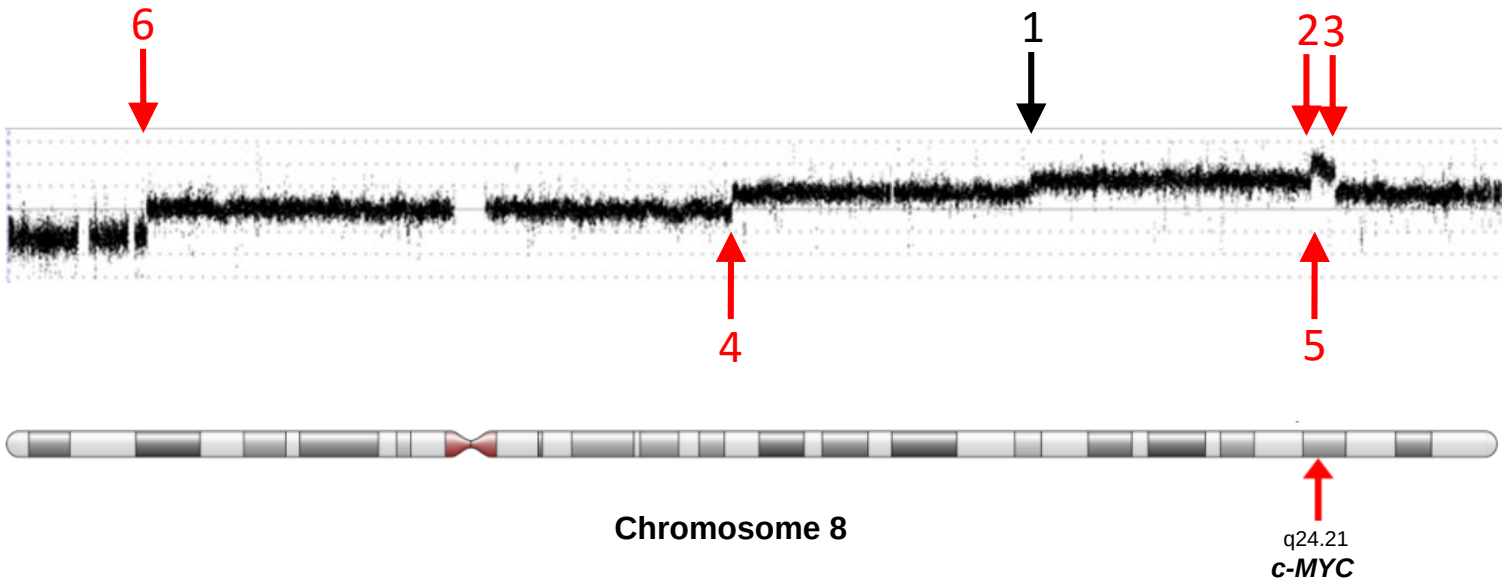
80Gy Population





Evolution of the *c-MYC* locus in irradiated cells

Newcastle University
Northern Institute for Cancer Research

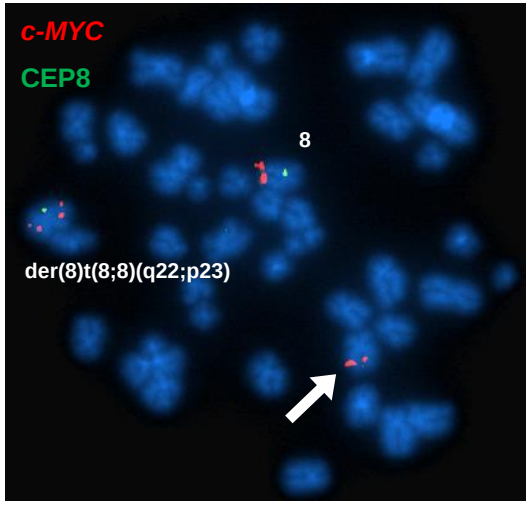


↑ Radiation-induced

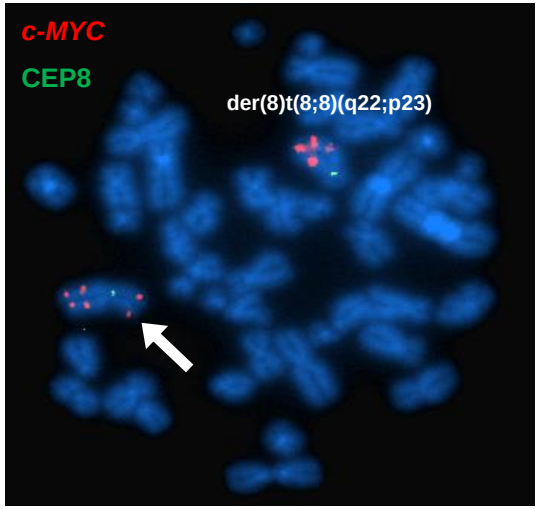
c-MYC is prone to alteration in irradiated cells

Newcastle University
Northern Institute for Cancer Research

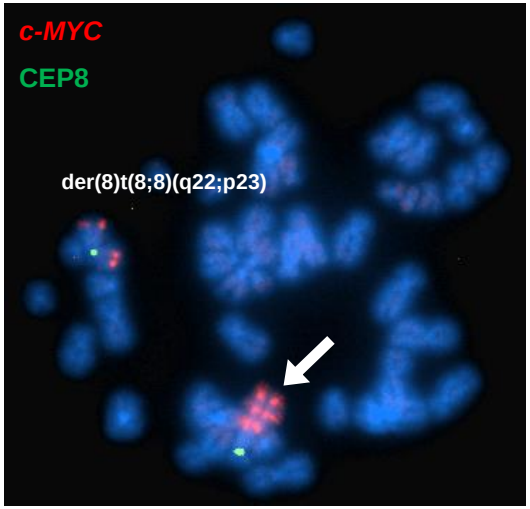
Translocation



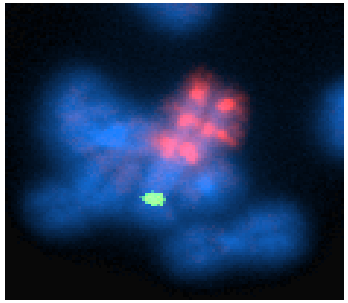
Duplication



Triplication



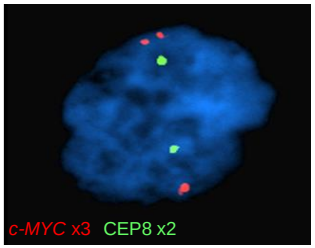
Rare events – not part of the major clone

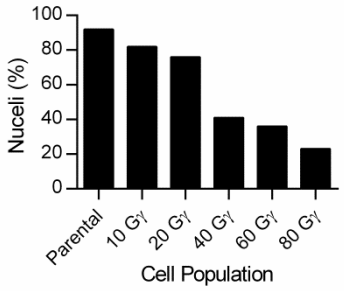


Radiation selects for *c-MYC* amplified cells

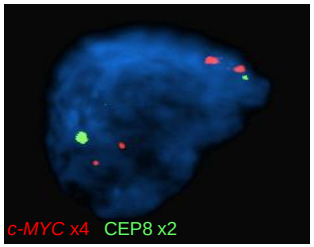
Newcastle University
Northern Institute for Cancer Research

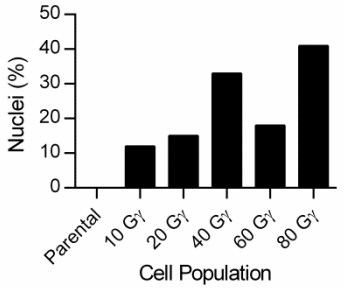
3 *c-MYC* copies (MCF-10A)



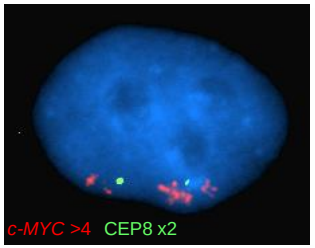


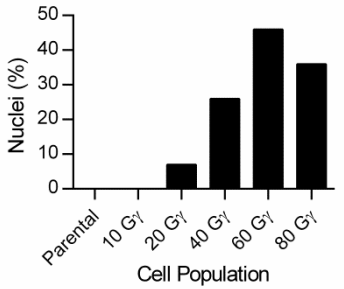
4 *c-MYC* copies (MCF-10A)





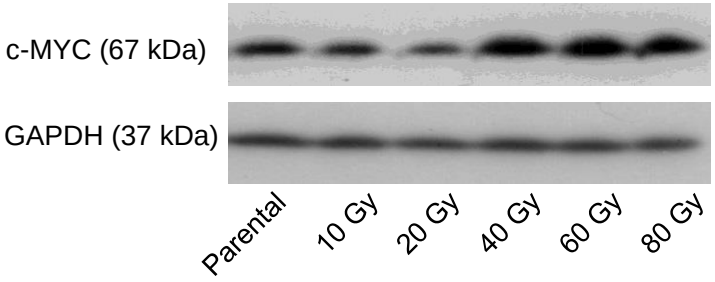
>4 *c-MYC* copies (MCF-10A)





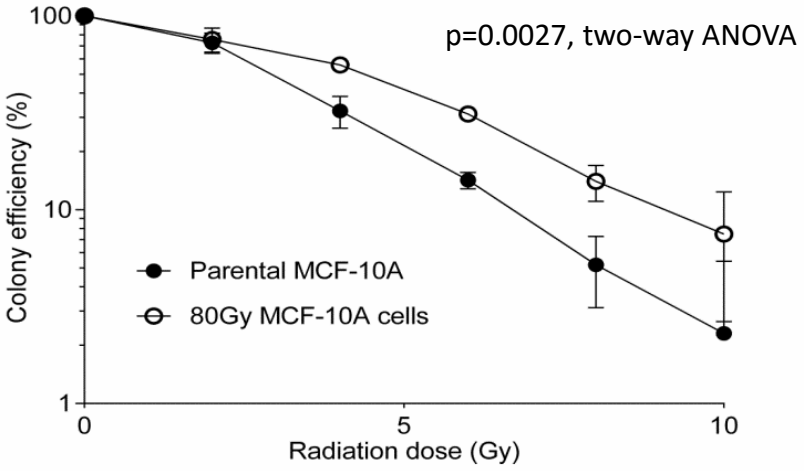
Multiple copies (<10) of *c-MYC*

Amplification correlated with expression



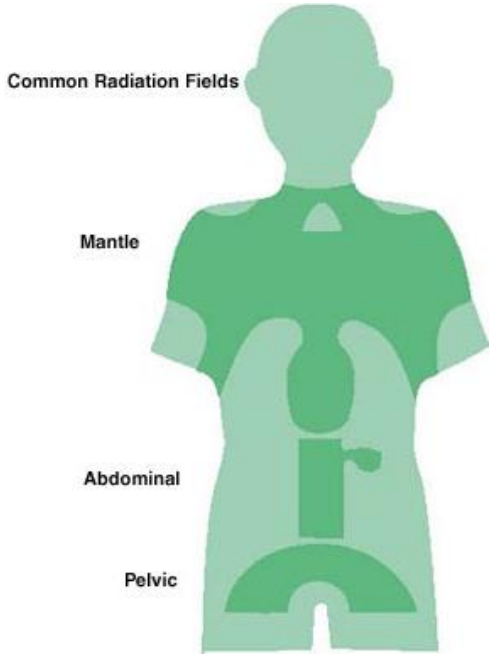
MCF-10A Cell population

Amplified cells are radioresistant



Breast cancer (BC) in HL survivors

Newcastle University
Northern Institute for Cancer Research



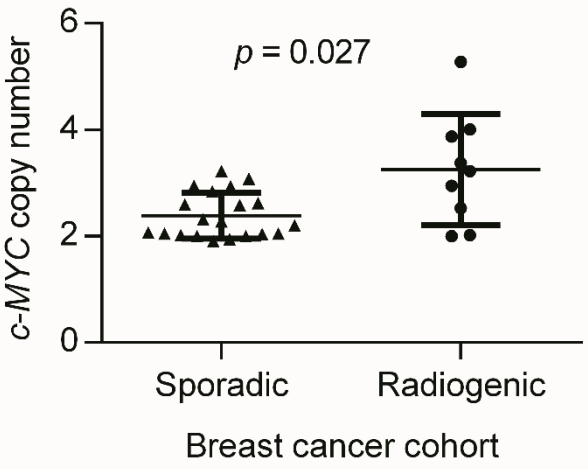
- Breast cancer after radiotherapy for Hodgkin lymphoma
  - N=18
  - Mean age at BC diagnosis 37 years, range 28-47 years
  - Mean age at HL diagnosis 21 years, range 16-29
  - Mean latency between HL and BC 15 years, range 7-24 years
  - Mean cumulative RT dose 19.9 Gy, range 1.2-42.7

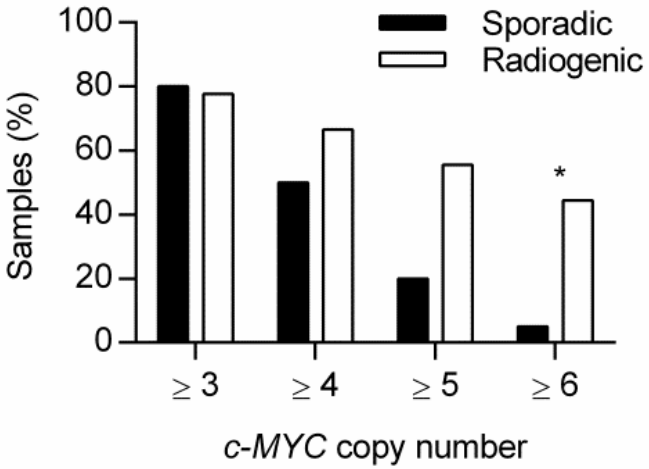
- Breast cancer without a radiation aetiology
  - N=33
  - Mean age at BC diagnosis 40 years, range 32-49 years.

c-MYC amplification in BC

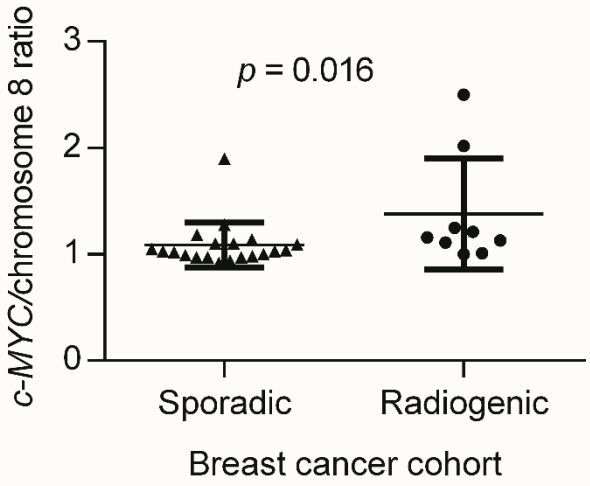
Newcastle University
Northern Institute for Cancer Research

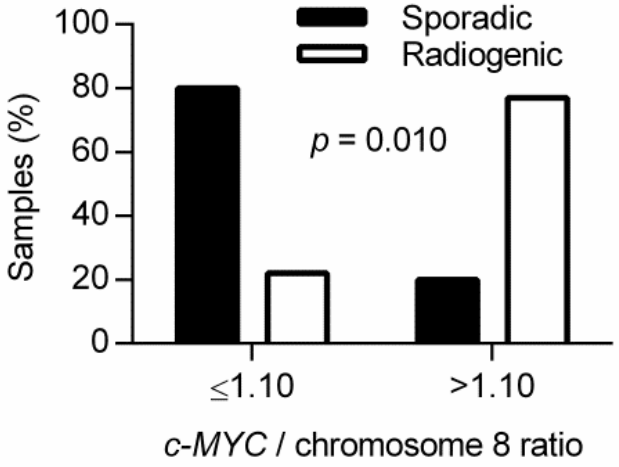
Mean *c-MYC* copy number





Mean *c-MYC/centromere 8* ratio

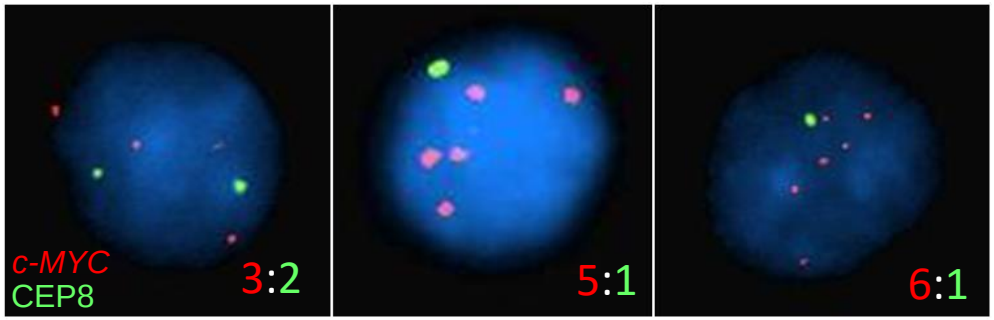




Heterogeneous *c-MYC* amplification

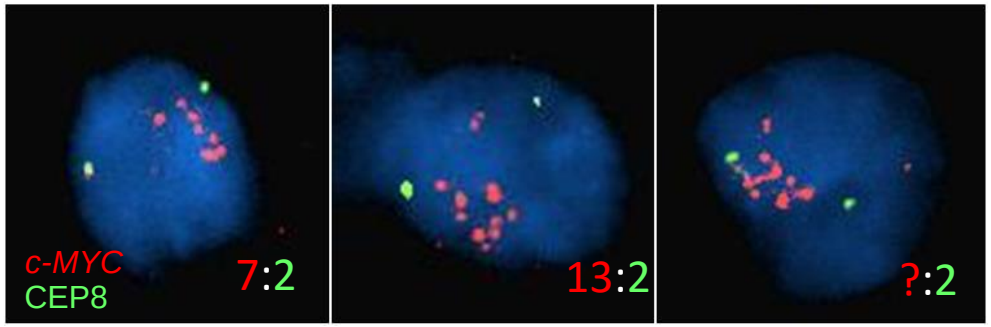
Newcastle University
Northern Institute for Cancer Research

a SPO2



	Copy number of probe[A]													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
SPO2	5	53	8	6	21	5	2							
	38	62												

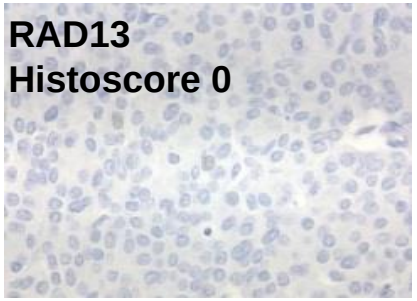
d RAD10

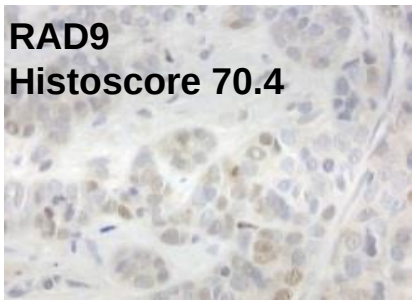


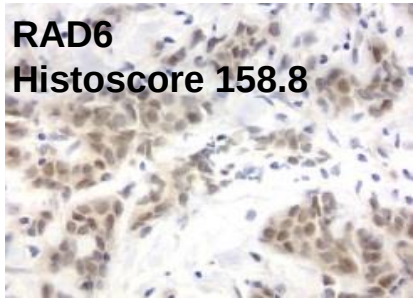
	Copy number of probe[A]													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
RAD10		53			3	5	1	8	13	6	5	5		1
	11	74	9	5	1									

Radiogenic cancer has high c-MYC expression

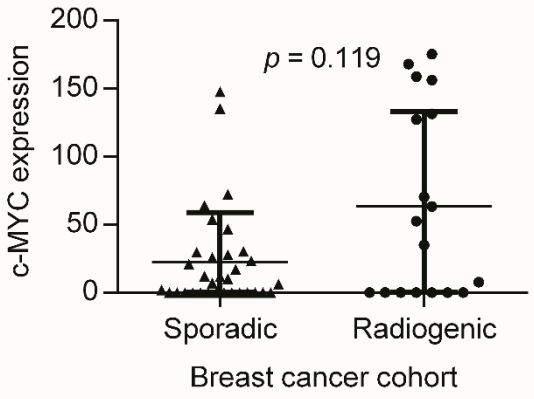
Newcastle University
Northern Institute for Cancer Research



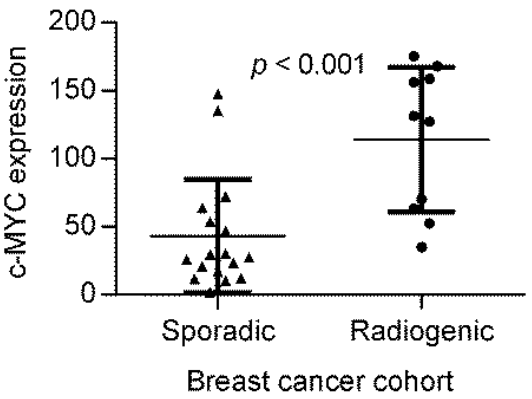


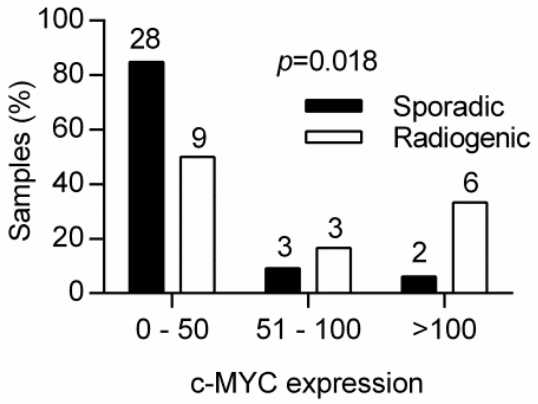


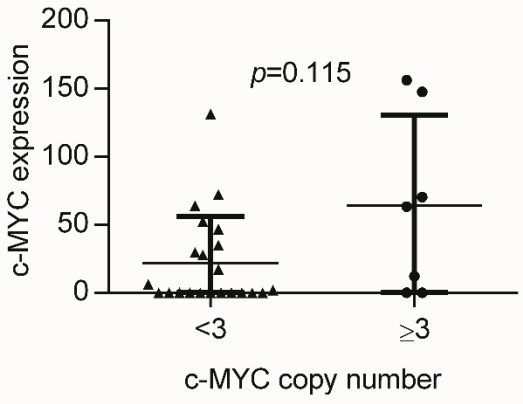
All cases



+ve for c-MYC expression



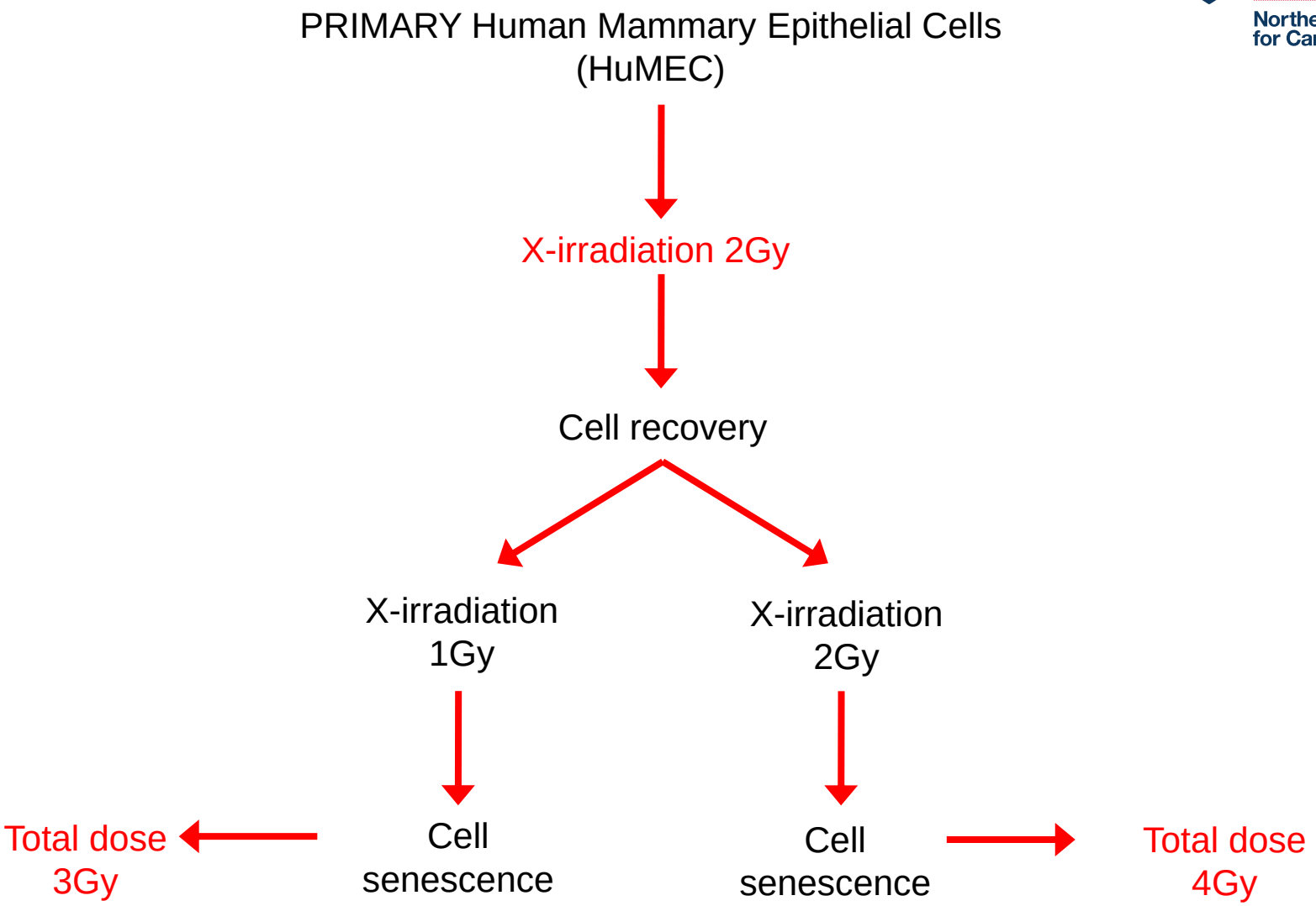




Does radiation induce *c-MYC* amplification in non-immortalised primary cells?

Primary human mammary epithelial cells

Newcastle University
Northern Institute for Cancer Research

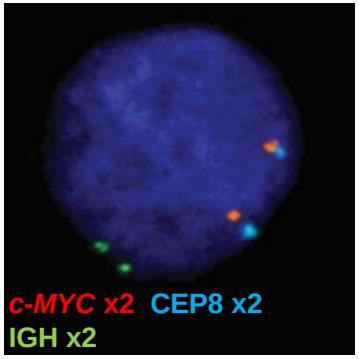


Primary human mammary epithelial cells

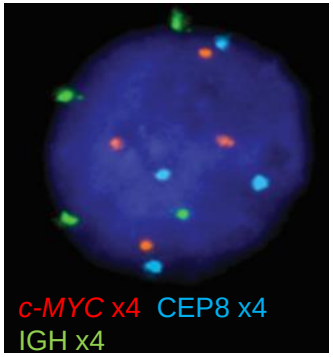
Newcastle University
Northern Institute for Cancer Research

HuMEC FISH

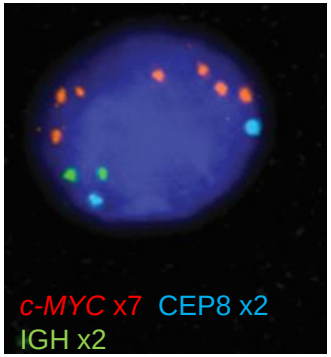
Non-amplified Diploid



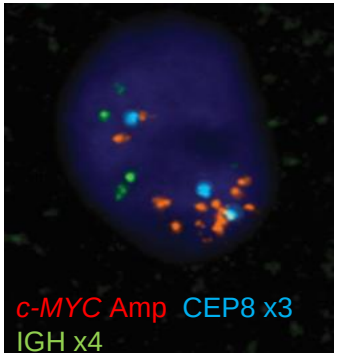
Tetraploid

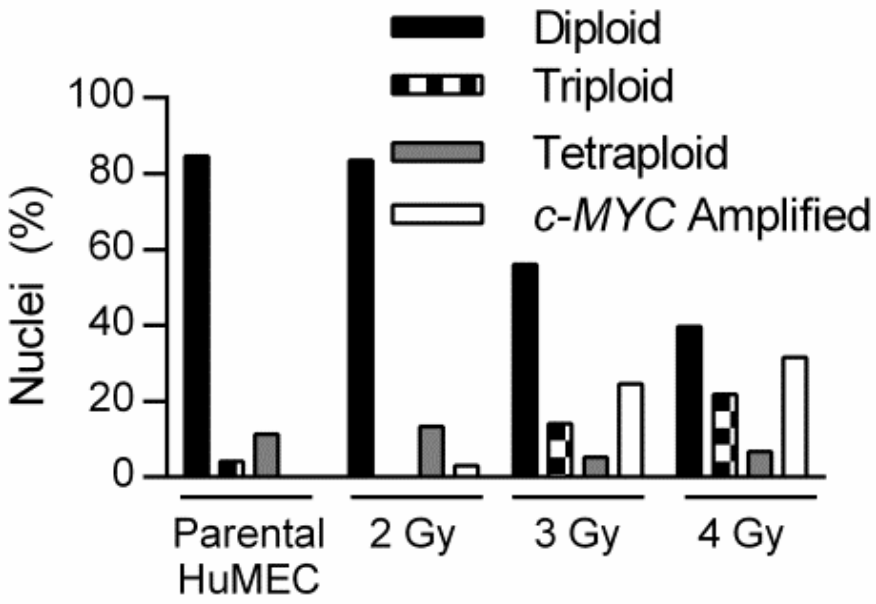


Diploid Amplified



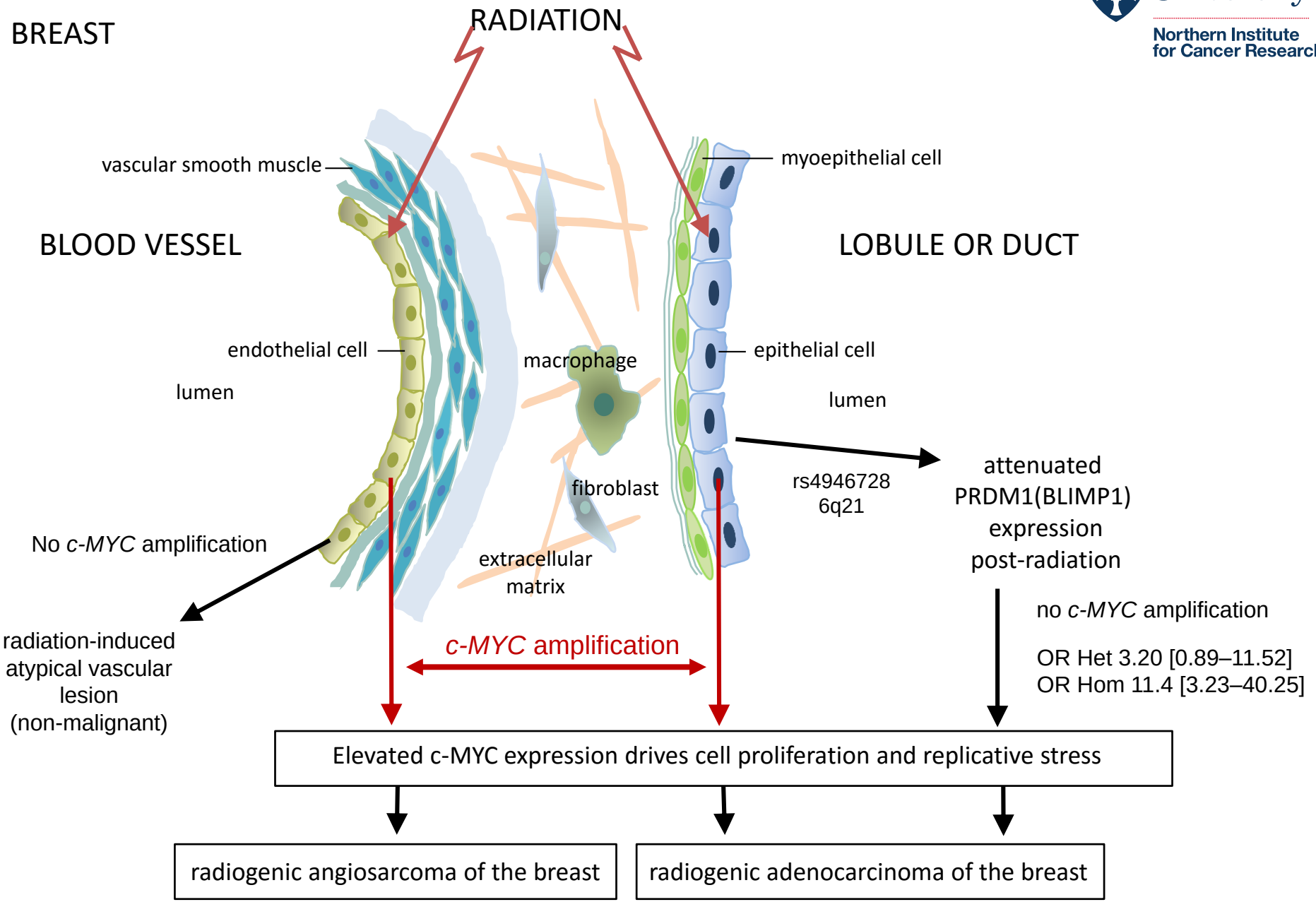
Polyploid Amplified





Model for radiogenic breast cancer

Newcastle University
Northern Institute for Cancer Research



Molecular mechanisms of transformation

Newcastle University
Northern Institute for Cancer Research

DNA damage → Gene-specific effects → Altered phenotype

O^6-methylguanine/ 6-thiopurine → Loss of DNA MMR (MLH1, MSH2, MSH6, PMS1, PMS2 etc) → Mutation acquisition

DNA strand breaks → *MLL* fusion gene → Dysregulated gene expression

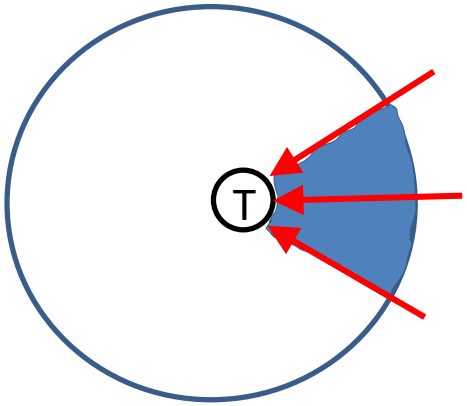
IONISING RADIATION?

base damage/ strand breaks (clustered?) → *c-MYC* amplification → Transcriptional reprogramming

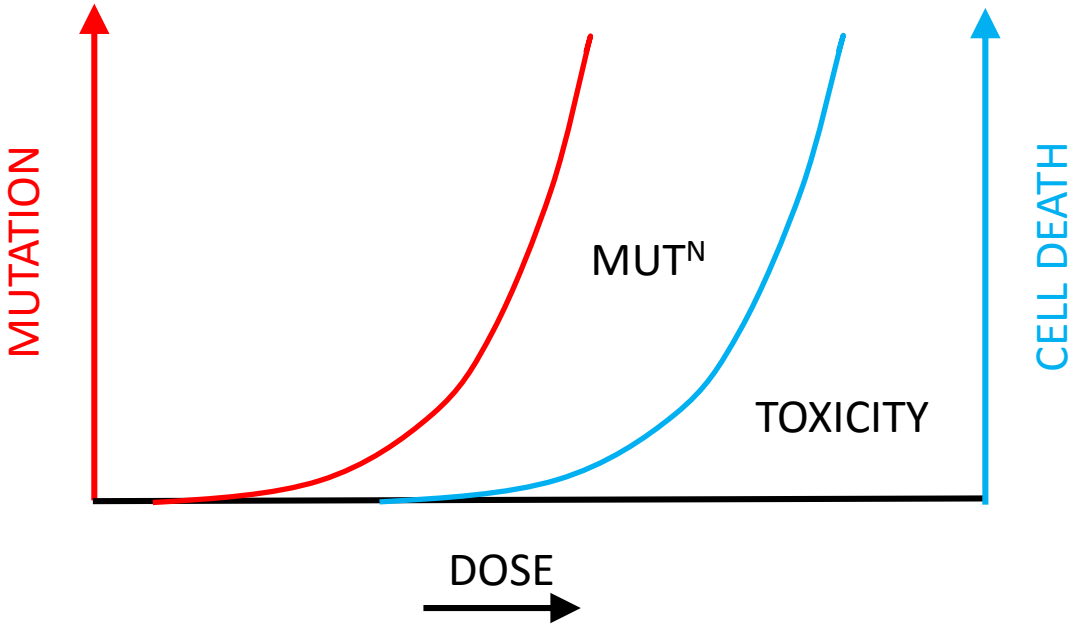
Risk modifiers?

Newcastle University
Northern Institute for Cancer Research

Critical to minimise healthy tissue exposure



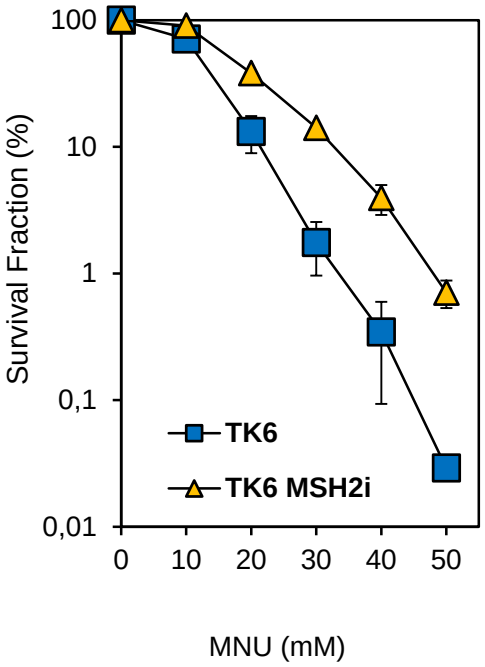
MUTATION CELL DEATH

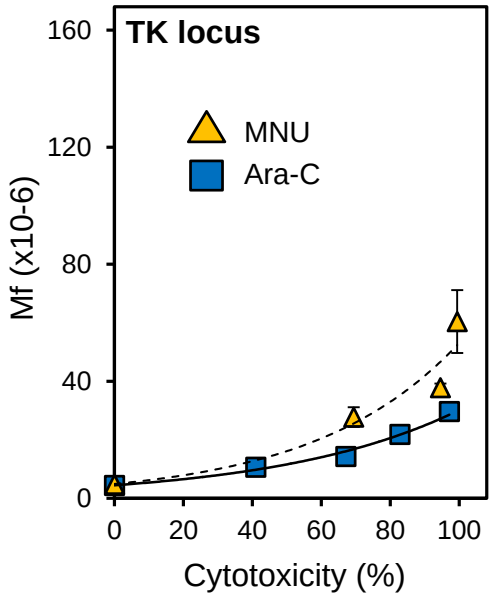


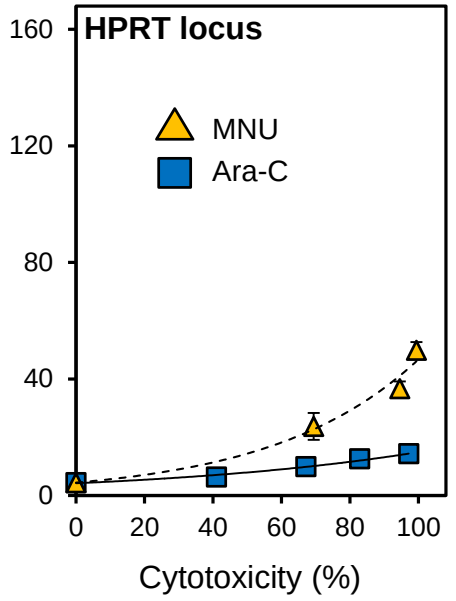
Identify modifiers that change the relationship between mutation and cell death

Chemotherapy-induced mutation

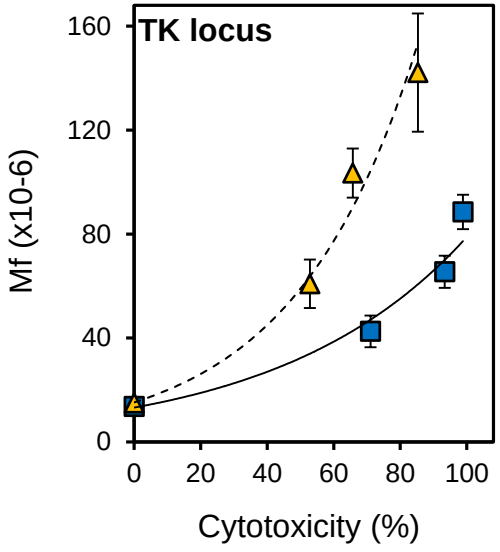
Newcastle University
Northern Institute for Cancer Research

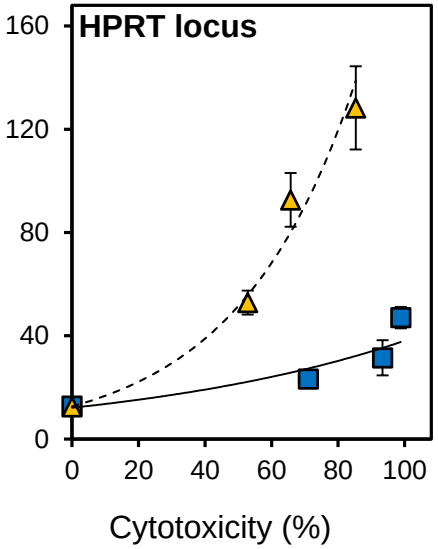






WT

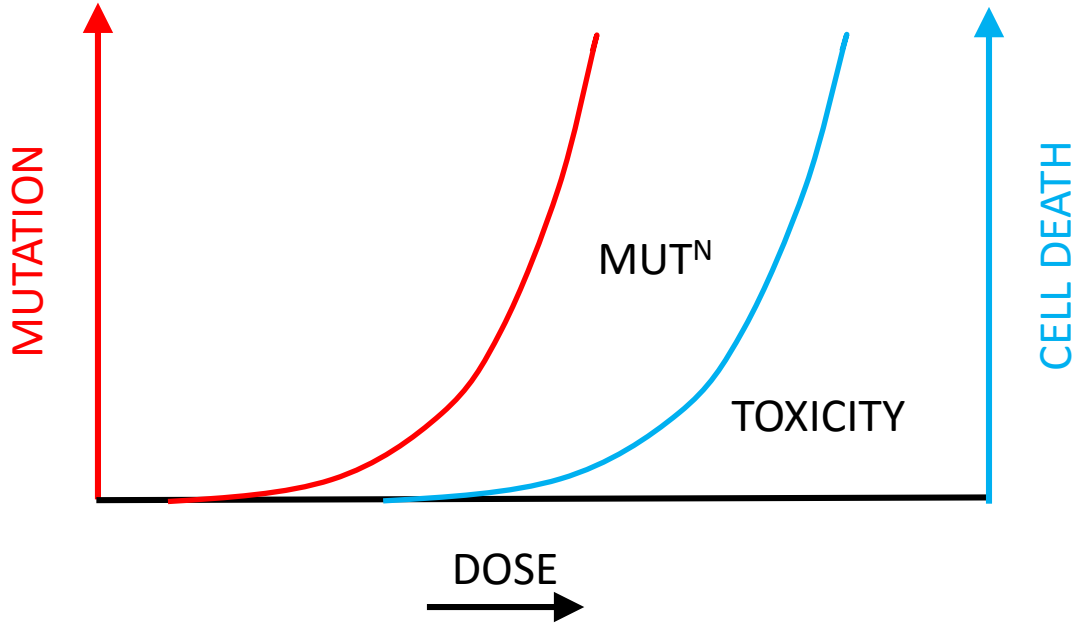




MSH2 deficient

Loss of MSH2 increases the mutagenic window

Newcastle University
Northern Institute for Cancer Research



Identifying radiation risk modifiers?

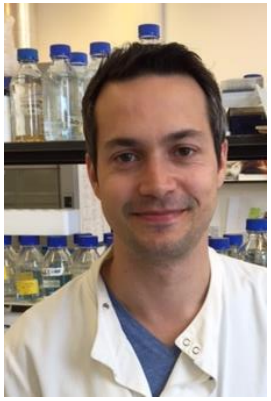
- Secondary particles, bystander effects (likely mutagenic, are they also cytotoxic?)
- Proliferative index of the exposed healthy tissue (dividing cells fix more DNA to mutation)
- Volume of healthy tissue exposed (probability of SMN = mutations per cell X no. cells)
- Constitutional genetics (eg. *PRDM1* MAF 0.22; *RB1* MAF 10^{-5}, *TP53* rare)
- Acquired somatic alterations (DNA repair loss or apoptotic signalling dysfunction)
- Dose fractionation

Newcastle University

Northern Institute for Cancer Research

Sarah Fordham

Mark Wade



Newcastle
Sarah Fordham
Victoria Forster
Nicola Sunter
Helen Marr
Mohammed Nahari
Mark Wade
Felicity May

Chicago
Ken Onel

Indiana
Lois Travis

Newcastle University

Bloodwise
Beating blood cancer since 1960

MRC Medical Research Council

bright red
Fighting blood cancer for a brighter future.

The Newcastle upon Tyne Hospitals NHS
NHS Foundation Trust