

Predicting response to therapy in chronic lymphocytic leukaemia

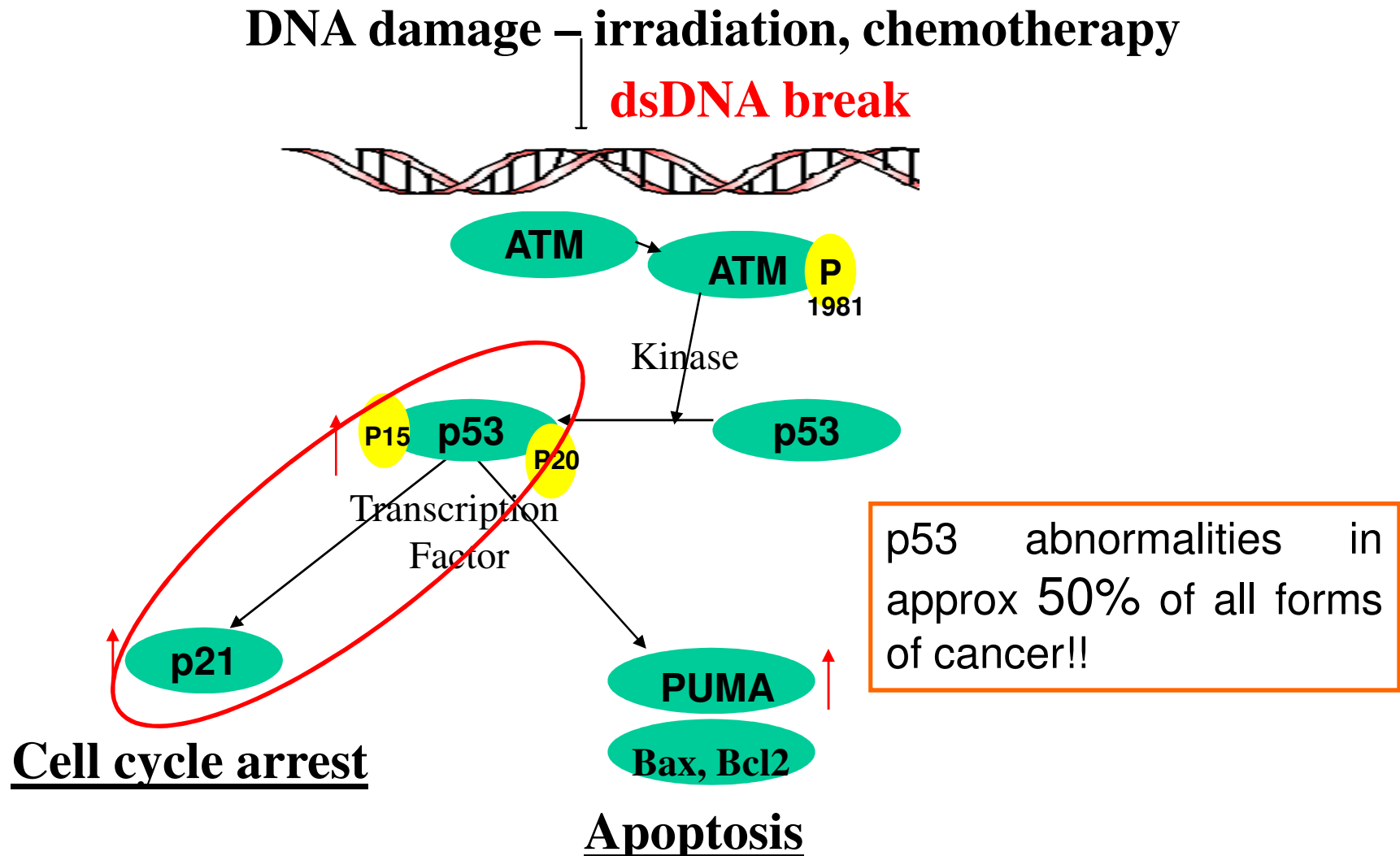
**Correlating high-risk genomic abnormalities
with functional assay responses**

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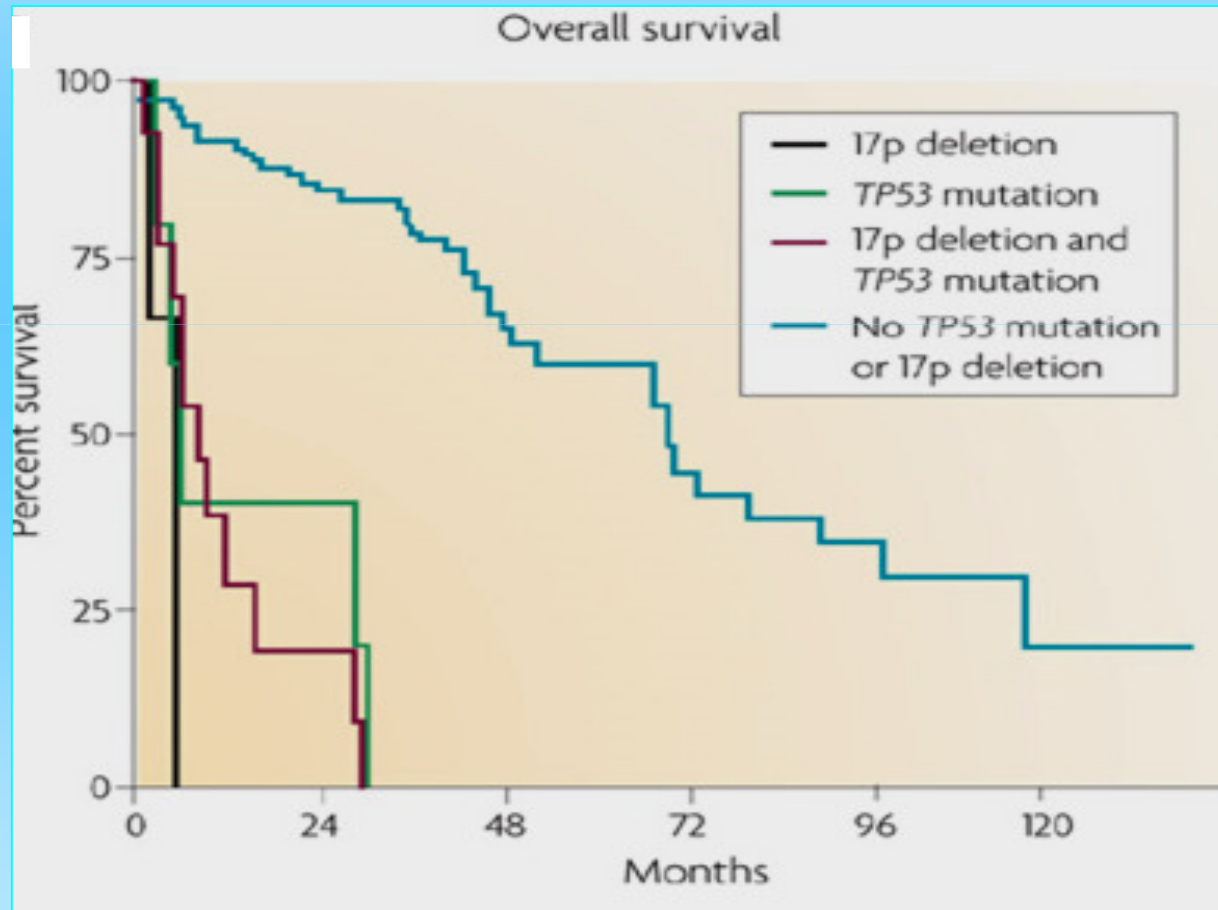
Chronic Lymphocytic Leukaemia (CLL)

- Approximately 2,000 new CLL diagnosis per year in the UK
- Mature B-lymphocyte disorder
- Median age: 65-70yrs

p53: necessary for chemo-sensitivity and genomic stability



Aberrations of p53 predict poorest overall survival in CLL

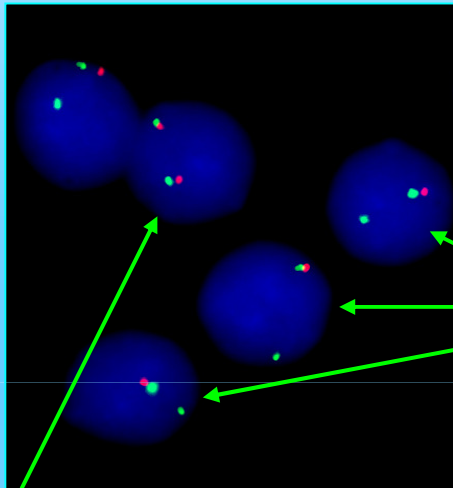


p53 abnormalities in CLL

- Mutation of the p53 coding sequence
- Deletion of one copy of the *TP53* gene
- Abnormalities in:
 - 6% of all CLL patients (UK CLL4 trial)
 - 44% of Fludarabine-refractory patients (CLL2H trial)

Therefore a need to detect p53 abnormalities pre-treatment

Detection of p53 abnormalities



Normal 2x signal

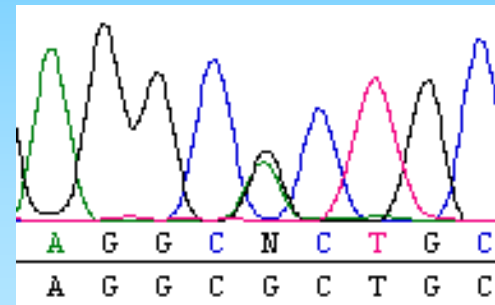
- Fluorescent *in-situ* hybridisation (FISH)

Abnormal 1x signal

RED=TP53 locus

GREEN= 17p centromere

- Mutation detection
 - Sequencing
 - Screening



PhD project

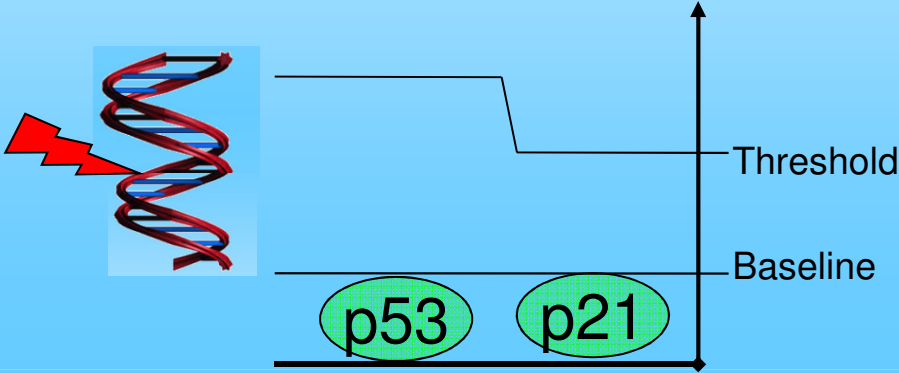
- Evaluating a functional assay
 - *In-vitro* using patient tumour cells
 - Assess the integrity of the DNA damage response
- Detects clinically important genomic abnormalities in CLL

p53 Function Assay

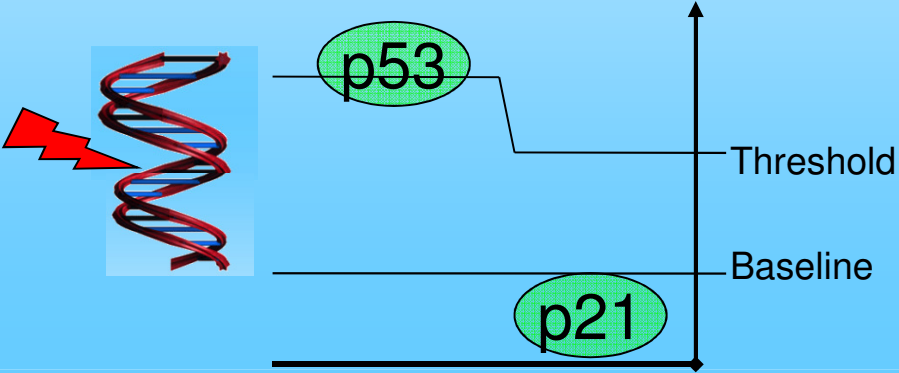
- DNA damage induced by etoposide
- Flow cytometric analysis of protein levels of p53 and its target, p21
- Measured before and after DNA damage by etoposide
- %age change used to determine different response groups

Functional responses

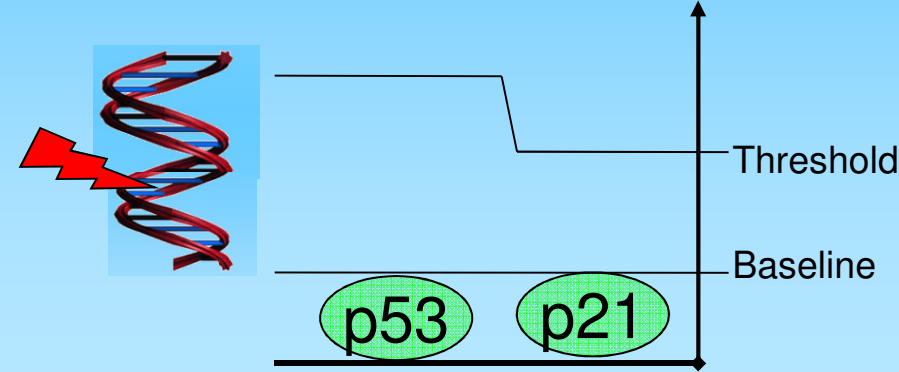
Normal



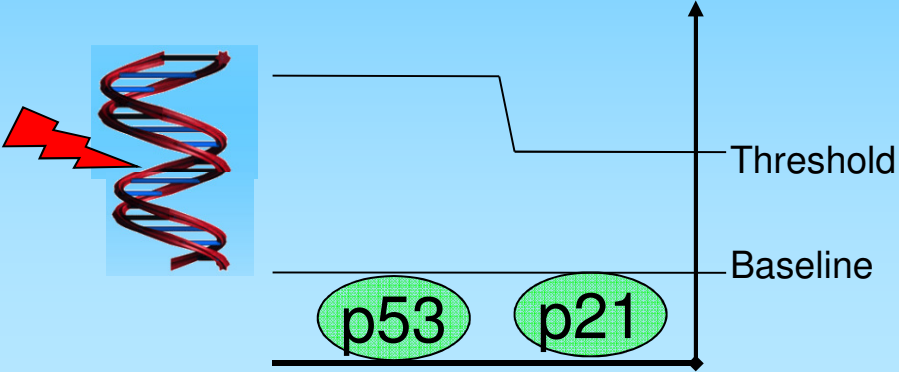
Type-A



Type-B



Type-C



Type-C: p21 gene?

- Our series
 - 10/105 CLLs = Type C
 - 25/500 CLLs = Type C
 - Radiation resistance
 - In study of 180 selected cases
 - Type C not associated with variation of p21 gene (p=0.5368)
 - Type C associated with genetic variations of p21 gene (p=0.0002)

Another cause?

Type C samples have abnormalities of *TP53* gene

15/25 (60%) Type-C have detectable p53 abnormality

	Incidence
p53 deletion	7
p53 mutation	4
p53 mut & del	4
No p53 aberration	10

Association of TP53	mutation	17p loss
Type C vs Normal	p<0.0001	p<0.0001
Type C vs Type A	p=0.0768	p=1
Type C vs Type B	p=1	p=0.2606

TP53 mutation and p53 deletion are independently associated with the type C response.

Problem?

- Why do these cases with p53 abnormalities not present a Type-A or B response?
- Due to small sub-population of leukaemic cells with a p53 abnormality
- Evidence?
 - Experimental and clinical

Mixing normal and type-B cells mimics the type-C response

Pt1: Type-B
Deletion in 68% of cells
Mutation of p53

Pt2: Normal
No p53 abnormality

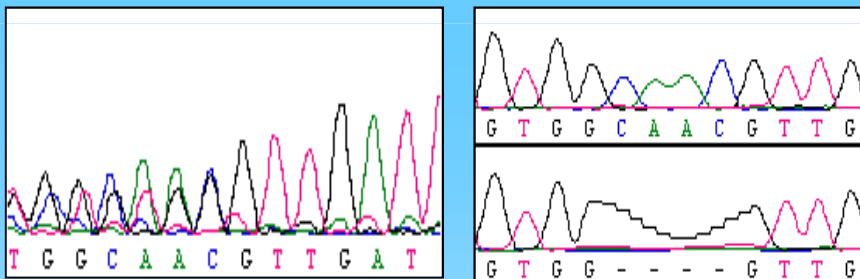
Mix Experiment 1			
% Type-B cells	% p53	% p21	Function
0	65	20	Normal
4	49	9	Type-C
5	45	5	Type-C
10	31	2	Type-C
26	21	2	Type-B
30	17	0	Type-B

Evolution from type-C to type-B

Example 1: 7 month interval

09/2006 → 04/2007
Type C response → Type B response

P53 abnormal 45% → P53 abnormal 68%

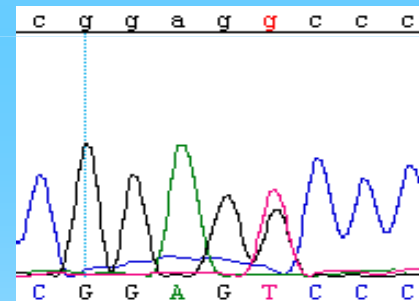


P53 mutation detected by cloning and sequencing

Example 2: 15 month interval

10/2007 → 01/2009
Type C response → Type B response

P53 abnormal 71% → P53 abnormal 90%



P53 mutation detected by sequencing was not detectable in earlier sample

Type C response due to low level p53 abnormal clone?

Conclusions

- Type-C response may represent an early indicator of an emerging p53 abnormality
- Relationship between Type-C cases and overall survival and treatment response currently being assessed
- Not all cases explained by p53 or p21 abnormalities



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