

Northern Institute for Cancer Research

**A putative role for DNA-PK in the
regulation of NF- κ B activity in
Chronic Lymphocytic Leukaemia**

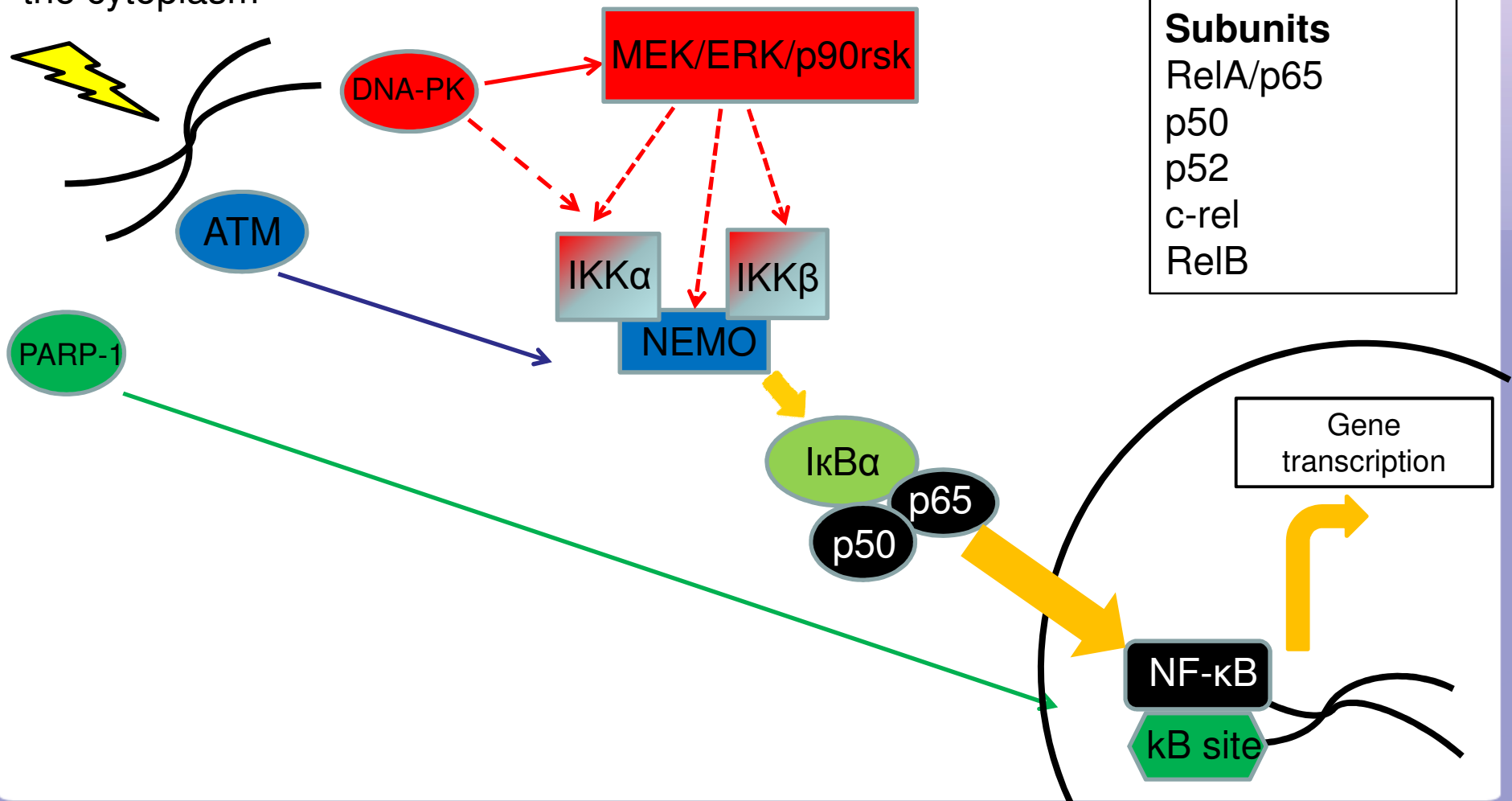
Evan Mulligan
Catovsky Prize Presentation
September 2010

THE *KAY KENDALL* LEUKAEMIA FUND
ESTABLISHED IN 1984 UNDER THE WILL OF THE LATE JAMES SAINSBURY CBE



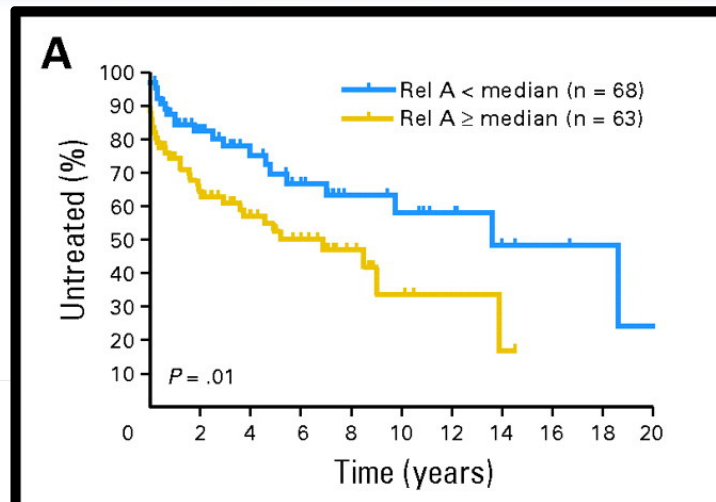
DNA damage activated NF- κ B

NF- κ B is a stress inducible transcription factor, made up of 5 subunits that dimerise in the cytoplasm

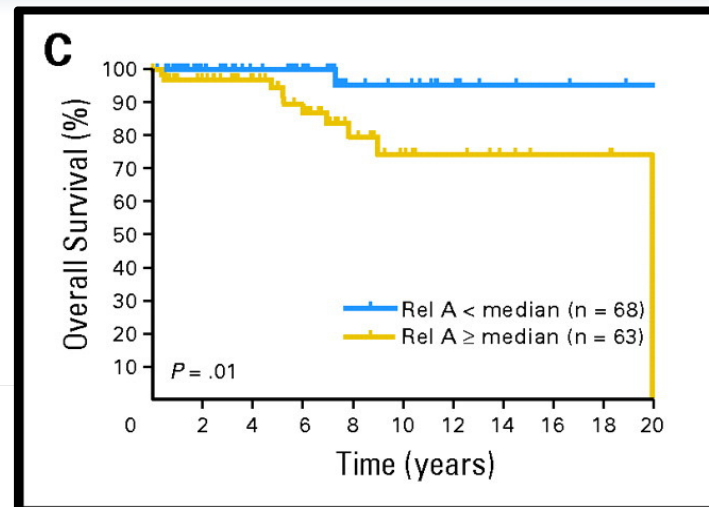


Known role of NF- κ B in CLL

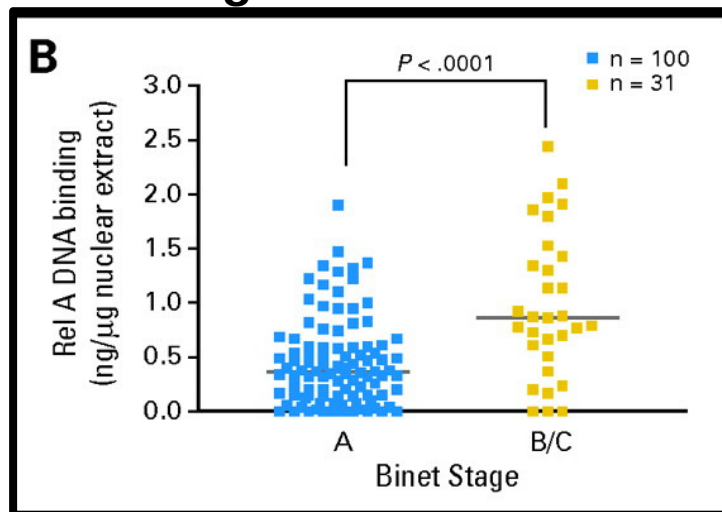
Time to first treatment



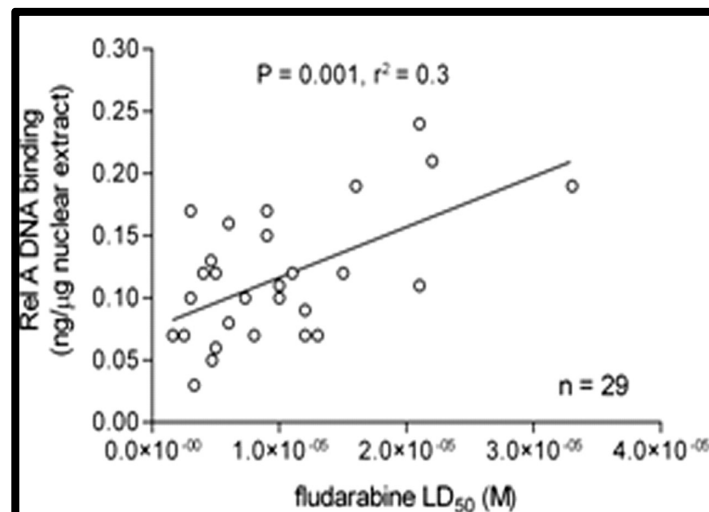
Overall Survival



Disease Progression



Chemoresistance



Aims and Hypothesis

- Constitutive activation of p65 subunit (RelA) confers poor survival
- Using NF- κ B-deficient cell lines, we demonstrated that sensitization to DNA damaging agents by ATM, DNA-PK and PARP-1 inhibitors was mediated by inhibition of NF- κ B*
- We showed that DNA-PK is overexpressed in poor prognosis CLL

Hypothesis:

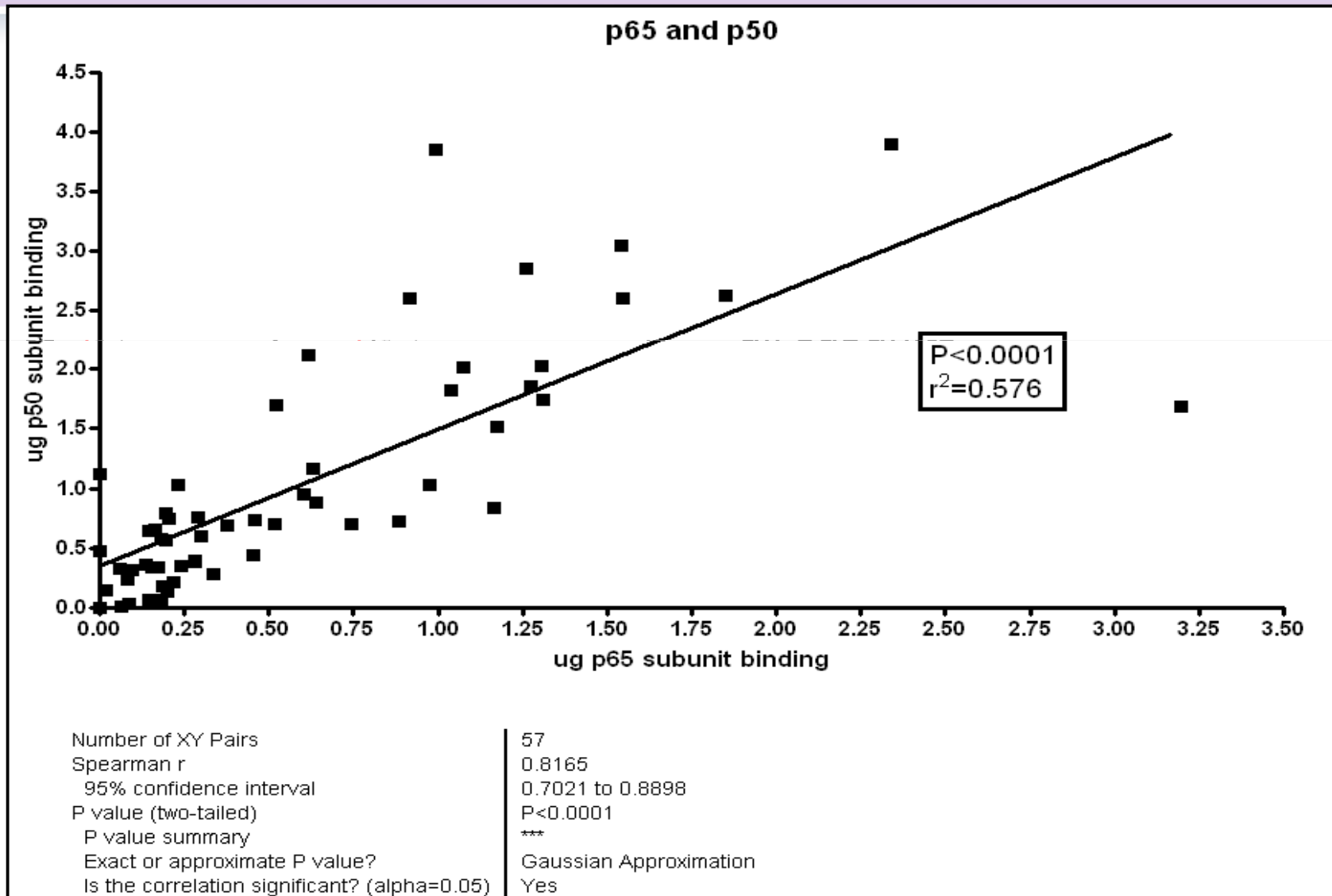
Chemo-sensitisation of DNA damaging agents following DNA-PK inhibition is regulated *via* NF- κ B

- Used ELISA kit (TransAM) to measure DNA binding (and hence activation) of NF- κ B subunits in nuclear extracts of CLL cells
- Determine link between DNA-PK and NF- κ B

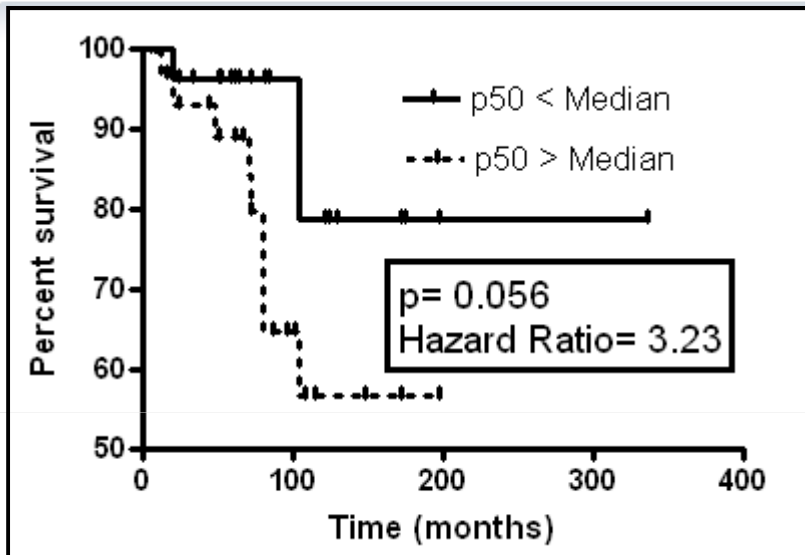
*Veuger et al, *Oncogene* 2009

*Hunter et al, *Oncogene* (submitted)

Subunit Correlations

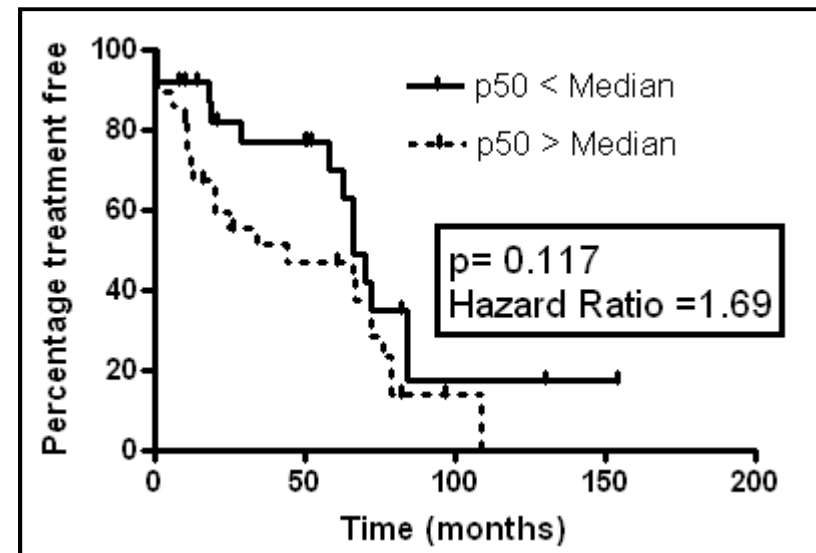


Survival and Time to first treatment (TTFT)

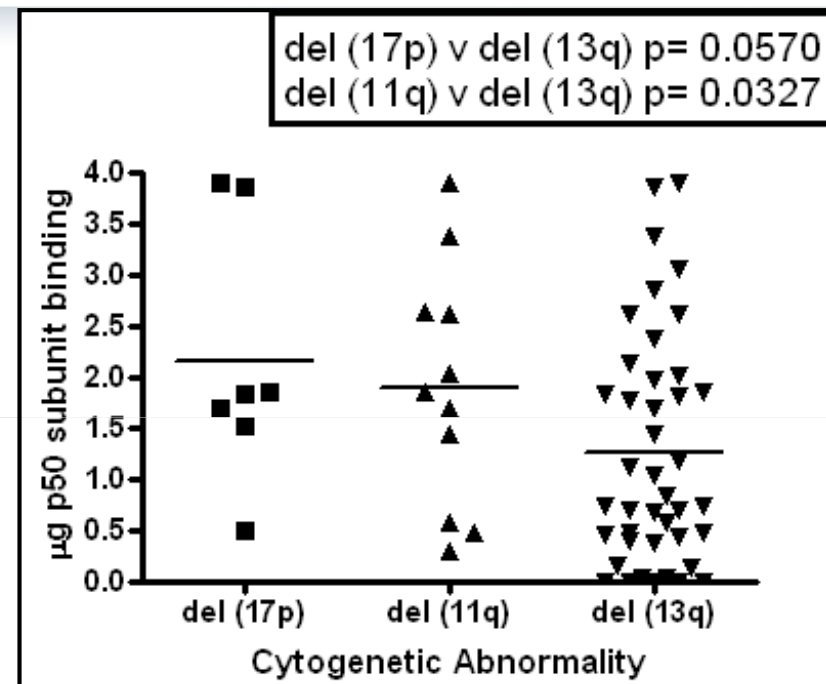
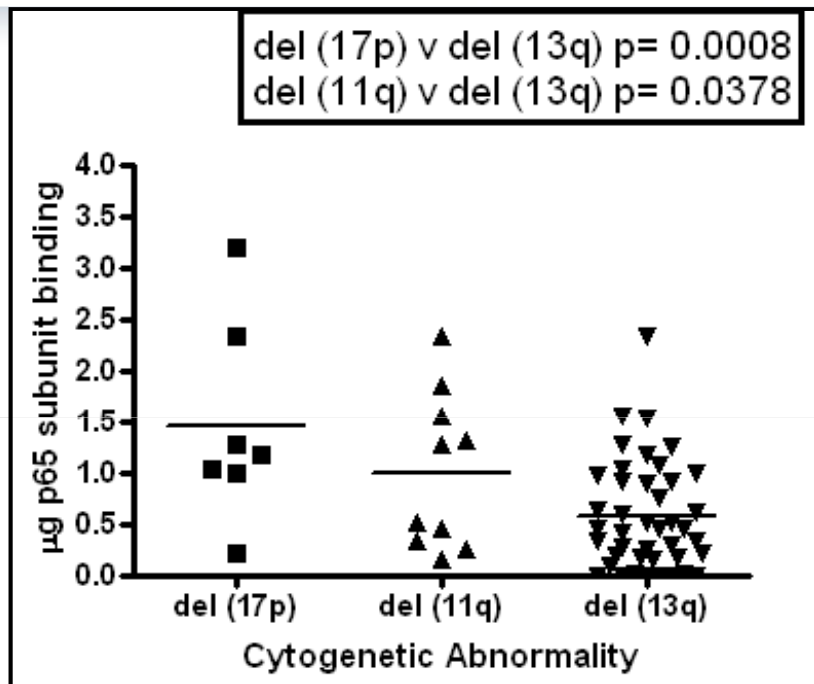


Overall Survival significantly reduced in patients with high p50 levels.

TTFT reduced in patients with high p50 or p65

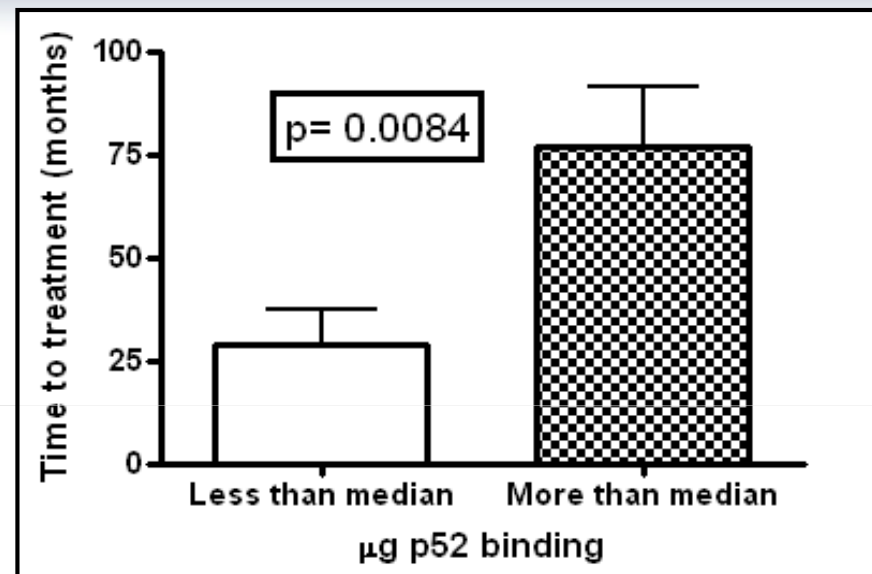
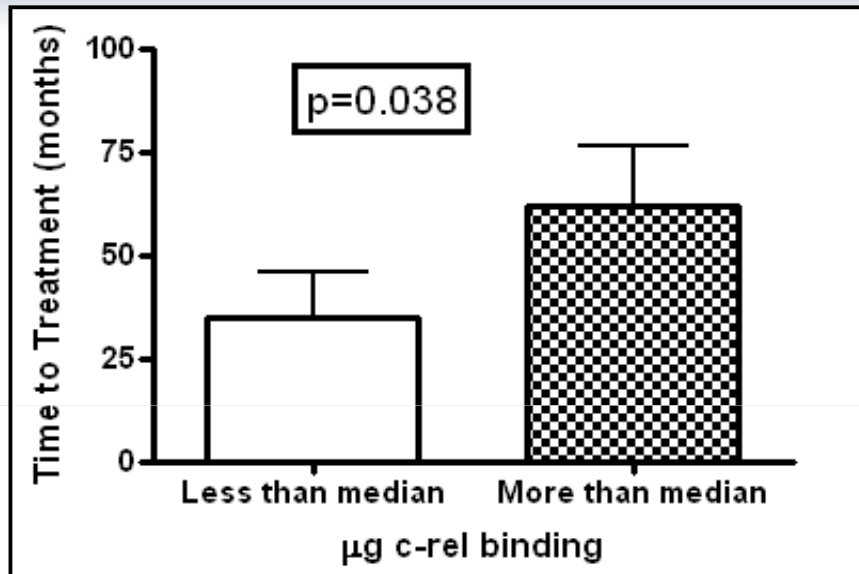


del(17p) patients have higher p65 and p50 subunit activation



- del(17p) cases: disease progression
- First evidence that NF-κB subunit activation differs among groups
- Patients with most aggressive disease have higher NF-κB activity

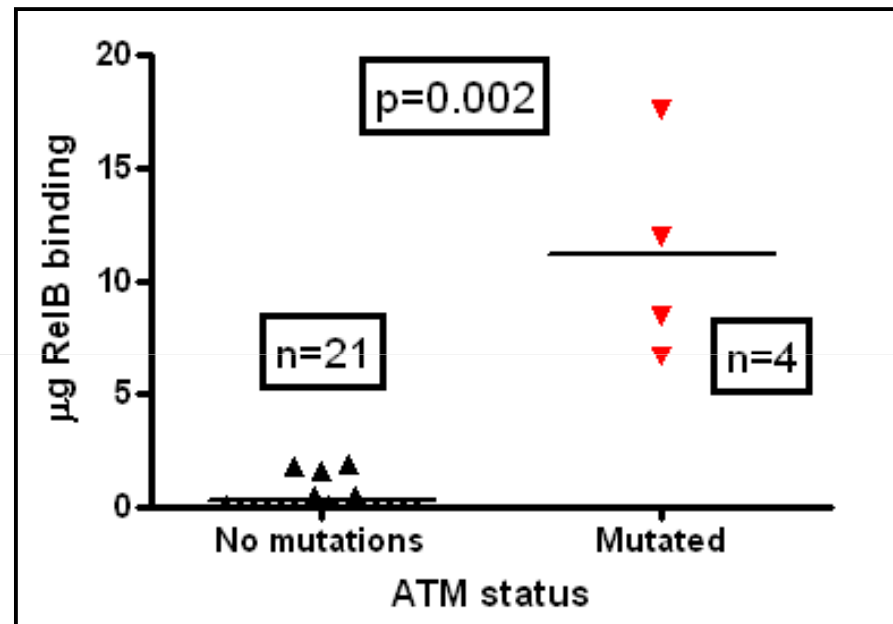
Patients with high p52 and c-rel have better outcome



- In contrast to p65 and p50, patients with high p52 and c-rel have longer TTFT.
- Interplay between activation of the subunits/pathways of activation that determines disease progression? Or a c-rel switch.

ATM mutation status and RelB

ATM mutation analysis kindly carried out by Dr Tanja Stankovic's group

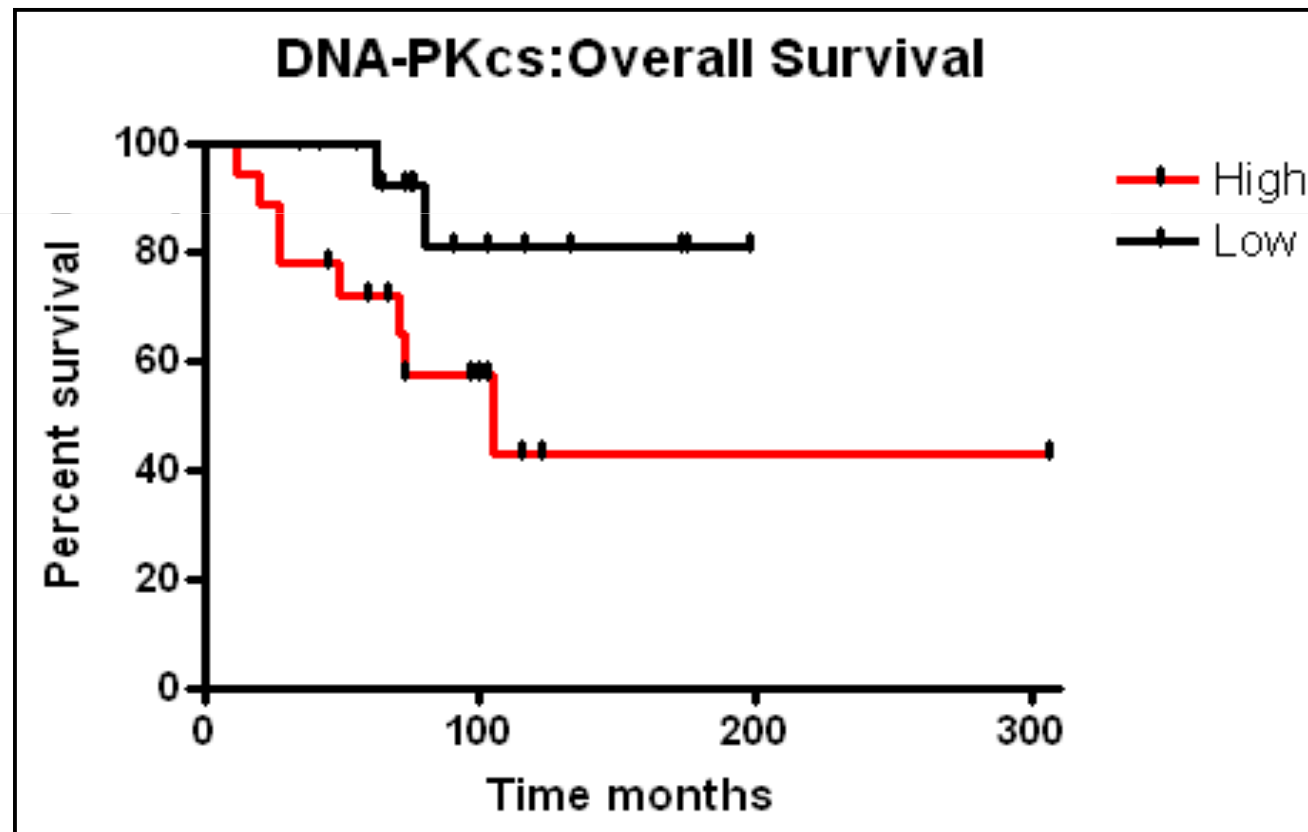


Majority of tested CLL cases have undetectable RelB activation

Of four cases with ATM mutations, all showed significantly raised RelB activation.

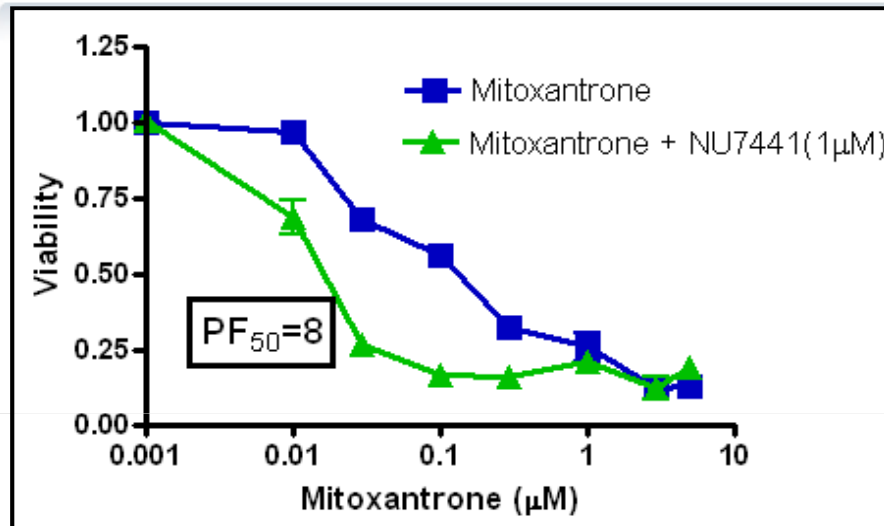
DNA-PK and NF- κ B

Our previous work showed that the DNA damage sensor DNA-protein kinase, DNA-dependent protein kinase, is over expressed in poor prognosis CLL*



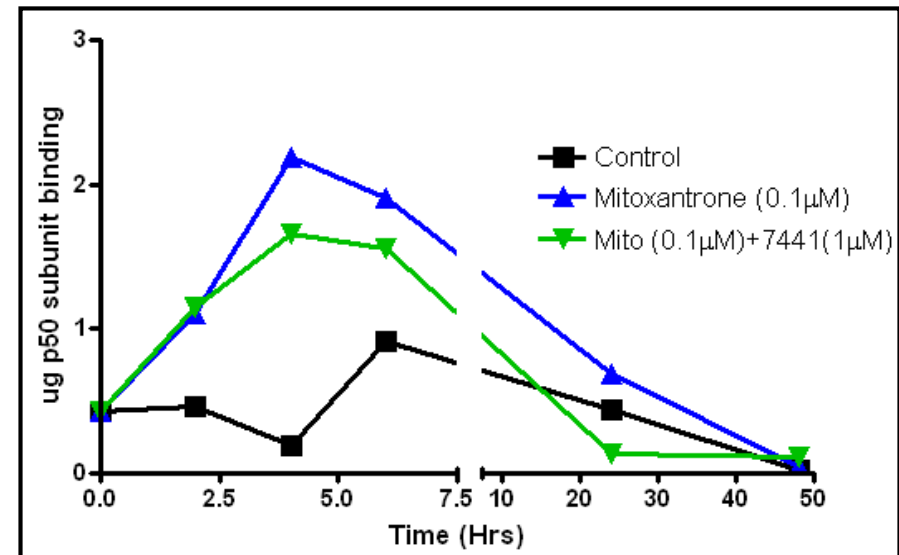
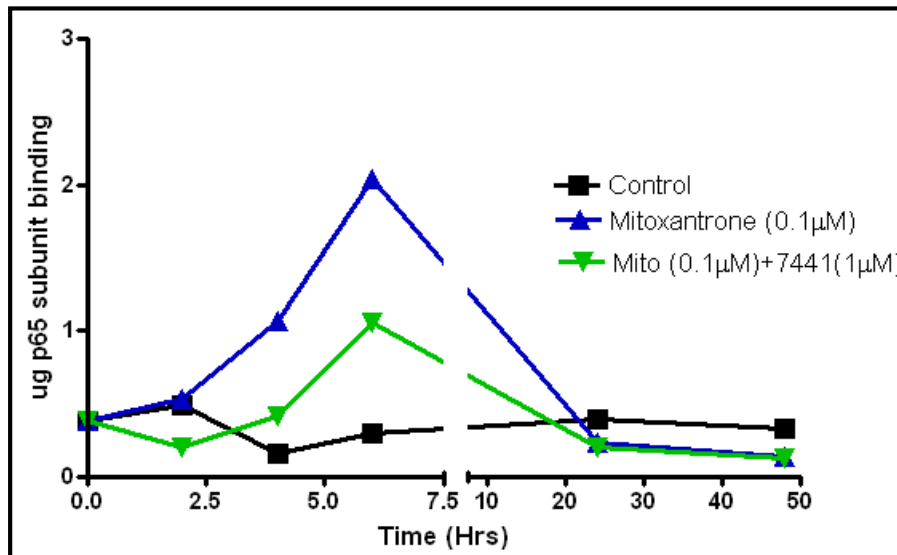
*Elliott et al BJJ, in press

Correlation of subunit activation with *ex vivo* treatment



Complex relationship between the DNA repair proteins and NF- κ B

Here we link chemosensitisation to a reduction in p65 and p50 subunit activation.

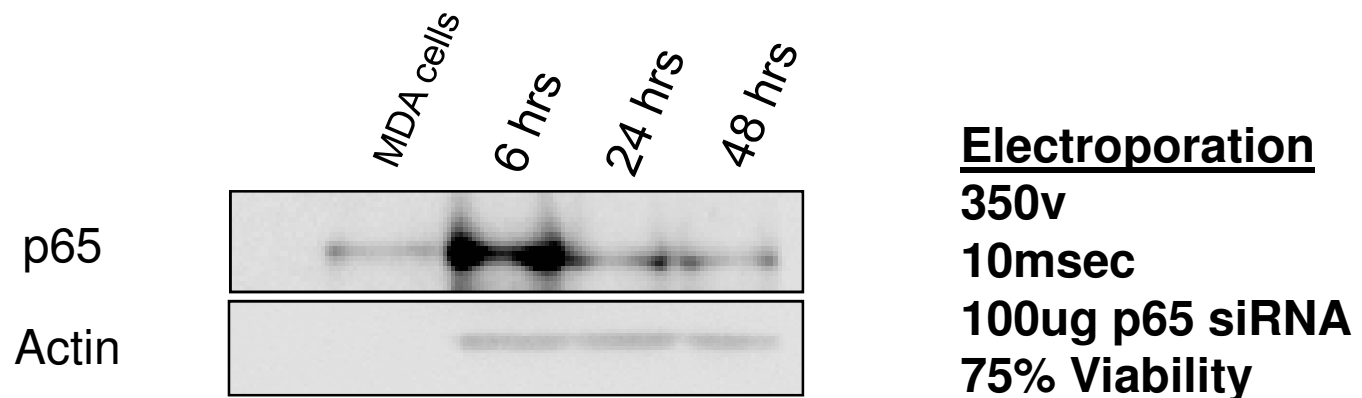


ChIP and siRNA Investigations

Already undertaking chromatin immunoprecipitation (ChIP) experiments to determine the specific direct interaction of the five subunits with the promoters of the PARP, ATM and DNA-PK.

Key link to ChIP experiments and *ex vivo* chemosensitivity would be siRNA knockdown of each of the NF- κ B subunits.

Patient #0111- No known cytogenetic abnormalities, Binet stage A, pre treatment.



Repeat experiments with this patient and others have failed to show significant knockdown compared to non specific siRNA.

Conclusions

- Each of the NF- κ B subunits plays a role in CLL, the outcome is a result of the balanced activation of the five subunits.
- Patients with ATM mutations appear to have a particular reliance on the RelB subunit.
- Patients with raised DNA-PKcs have greater than average p65 and p50 levels.
- p65 and p50 are activated in the response of CLL to DNA damage, this response is mitigated by the use of a DNA-PK inhibitor.
- Suggesting a direct link between DNA-PK and NF- κ B

Thanks to:

**All CLL Patients
Clinical Team**

**Elaine Willmore
Jill Hunter
DDI lab members
Barbara Durkacz**

THE *KAY K* RENDALL LEUKAEMIA FUND
ESTABLISHED IN 1984 UNDER THE WILL OF THE LATE JAMES SAINSBURY CBE

