

# Targeting the homologous recombination repair defect in *ATM* null CLL

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## **Ataxia Telangiectasia Mutated (ATM)**

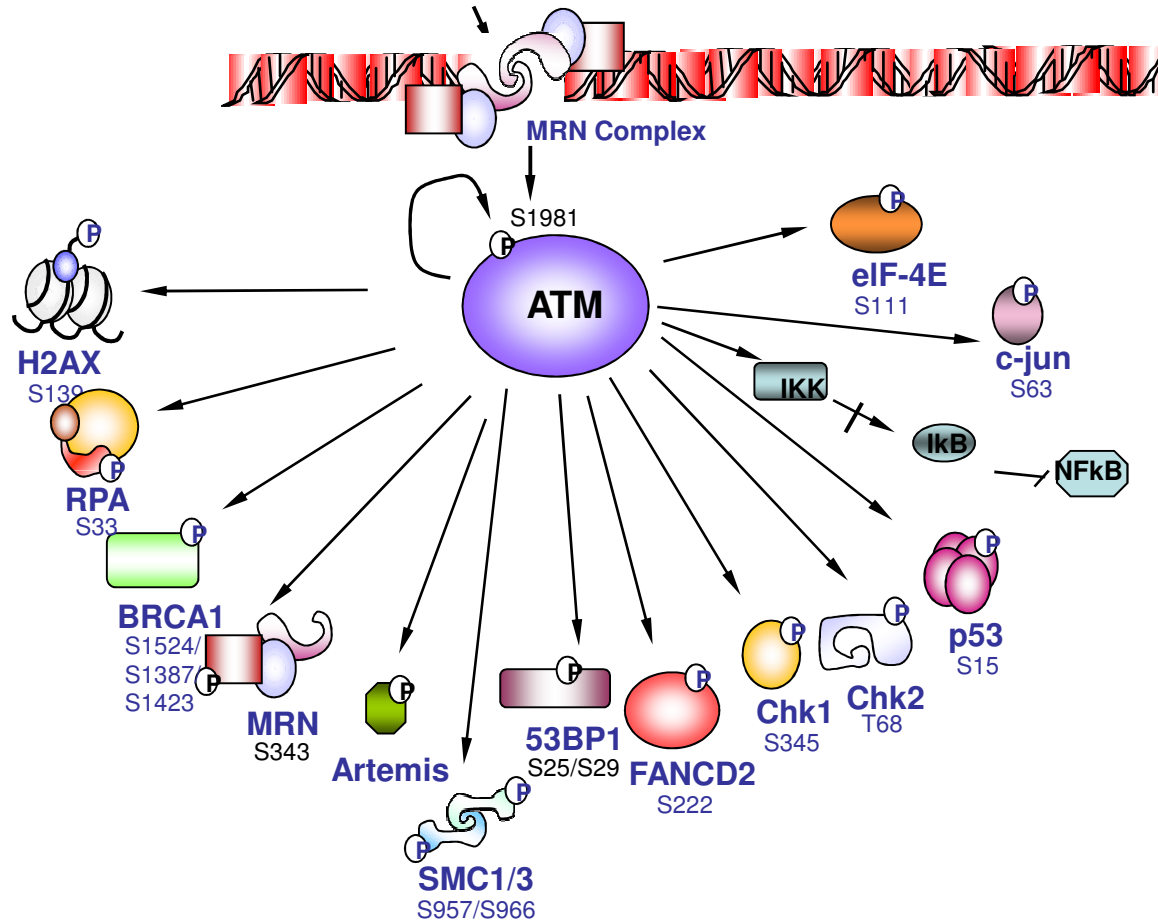
### *ATM* mutations

- Present in up to one third of CLL
- Enriched in 11q deleted progressive CLL subsets
- Frequent in rarer T-PLL and MCL

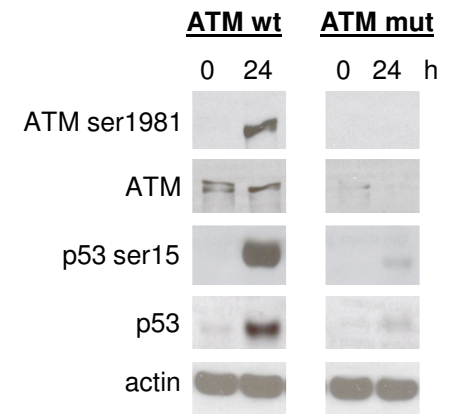
# ATM is a key DNA damage response protein kinase

## DNA DSB

(IR, chemotherapy, programmed immune system gene rearrangements...)



## 15μM fludarabine



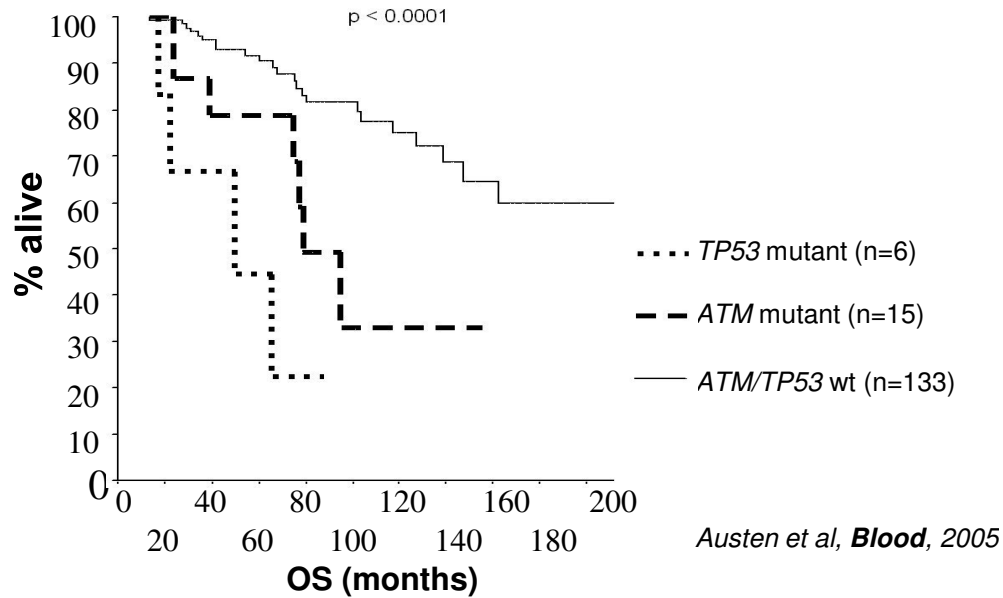
DNA repair

S-phase Checkpoint

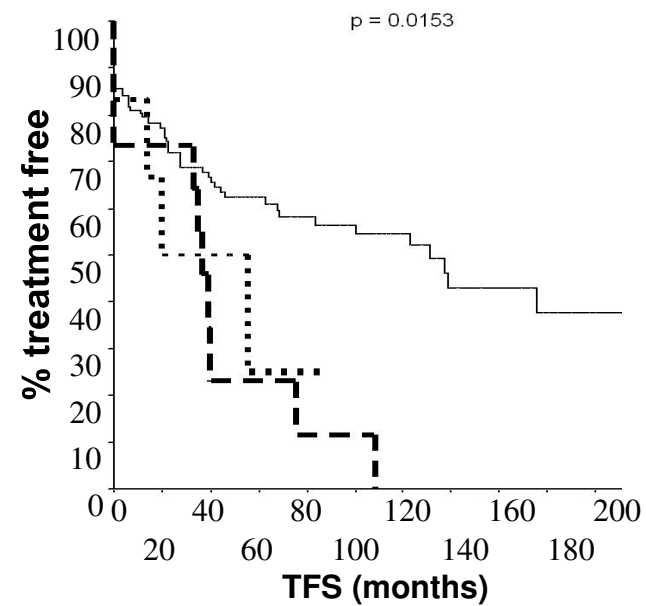
Cell cycle arrest/Apoptosis

# Loss of ATM function significantly affects clinical outcome

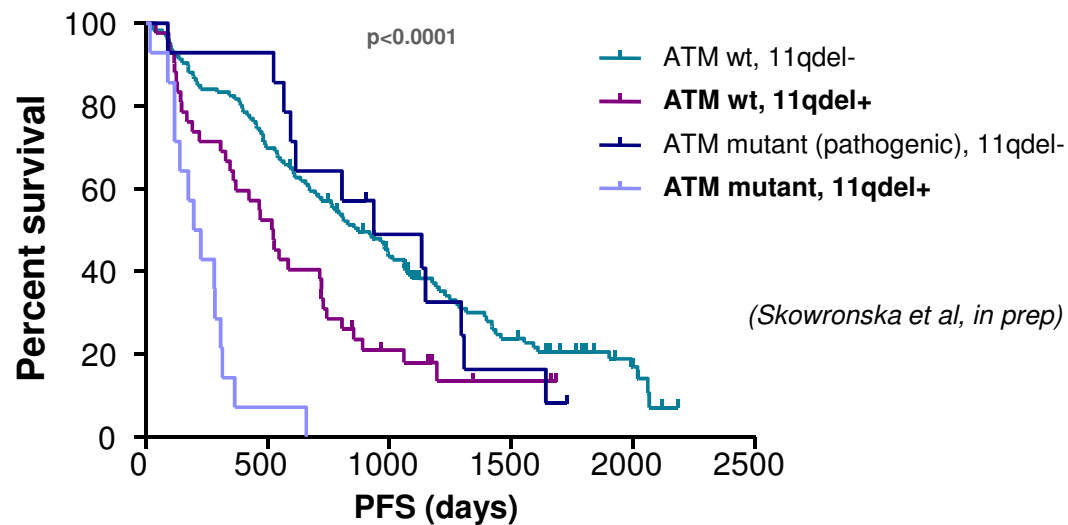
Overall survival (unselected cohort):



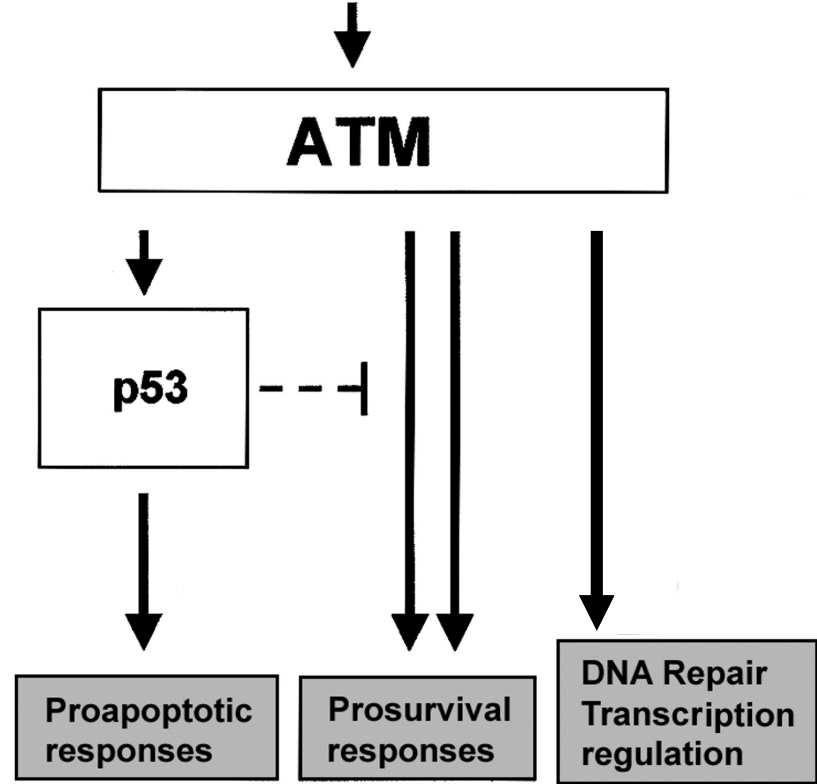
Treatment free survival (unselected cohort):



Progression free survival (CLL4 cohort):



# DNA Double Strand Breaks



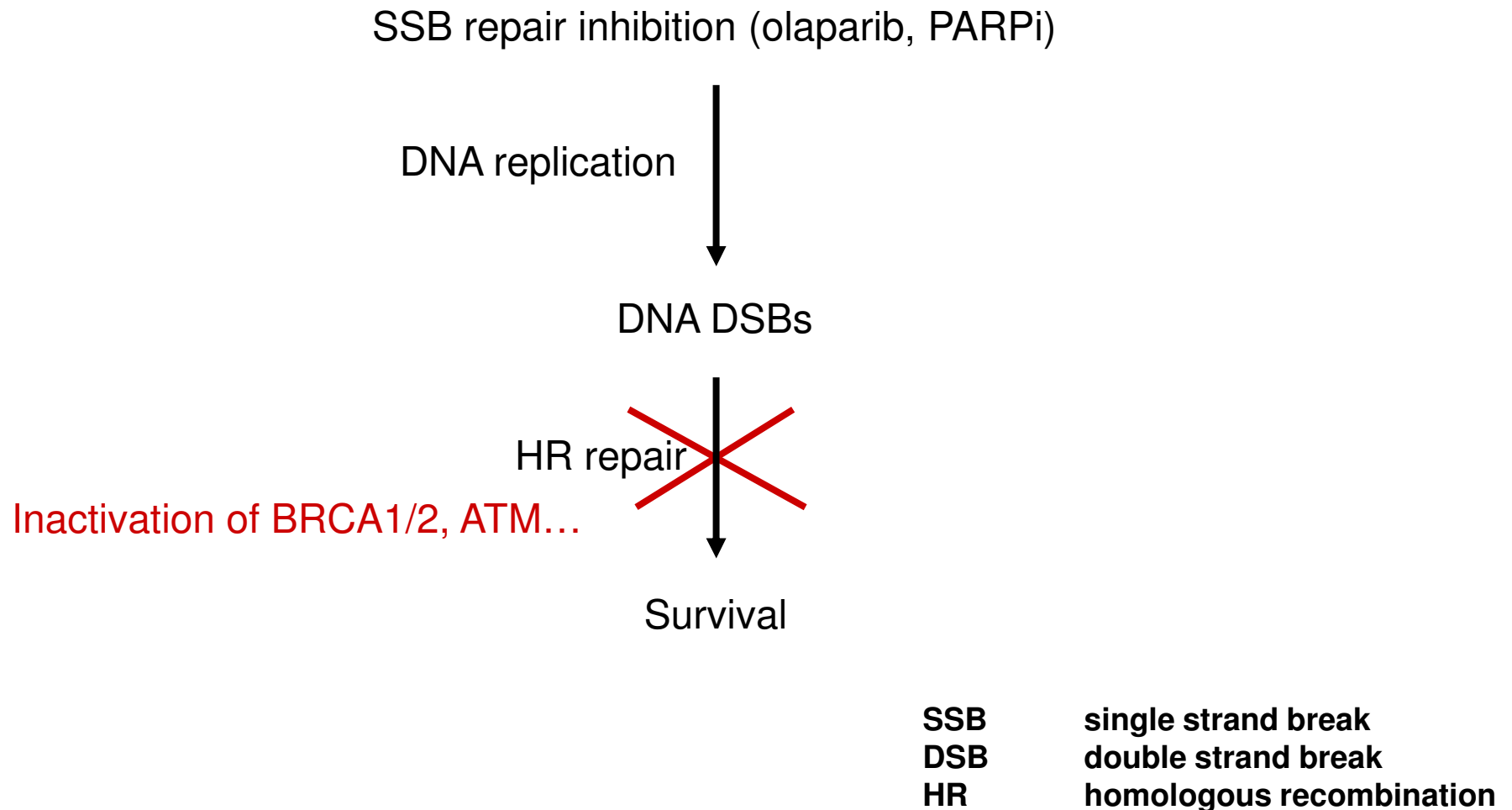
CLL 3 h after IR with 5Gy IR

Stankovic *et al*, **Blood**, 2004  
Stankovic *et al*, **Blood**, 2002

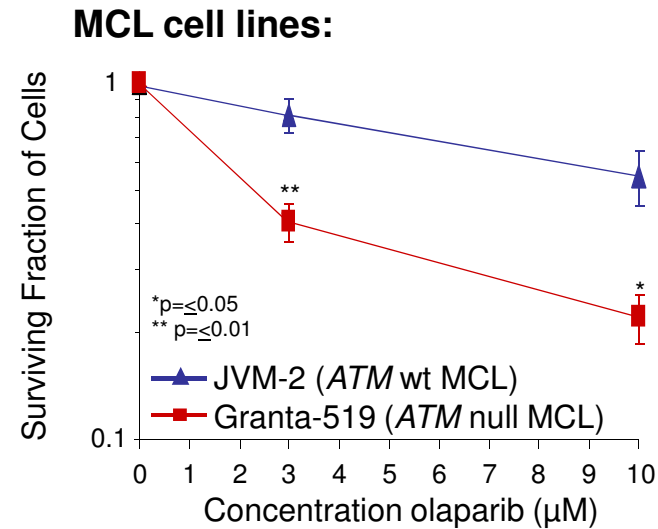
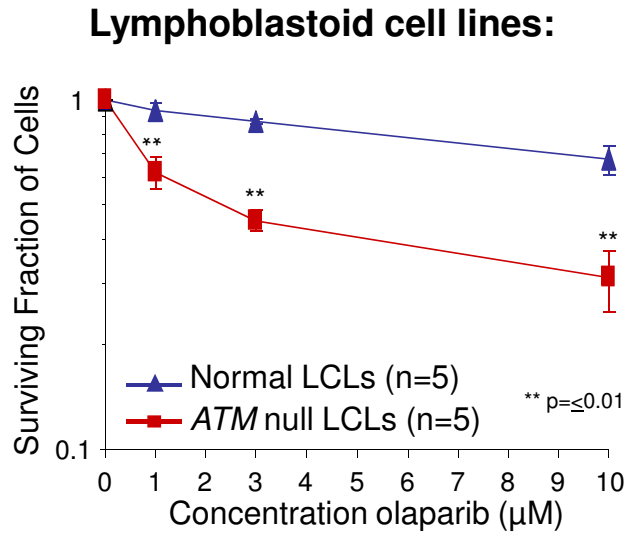
# Targeting defective repair in *ATM* mutant CLL: PARP inhibition (PARPi)

## Synthetic Lethality

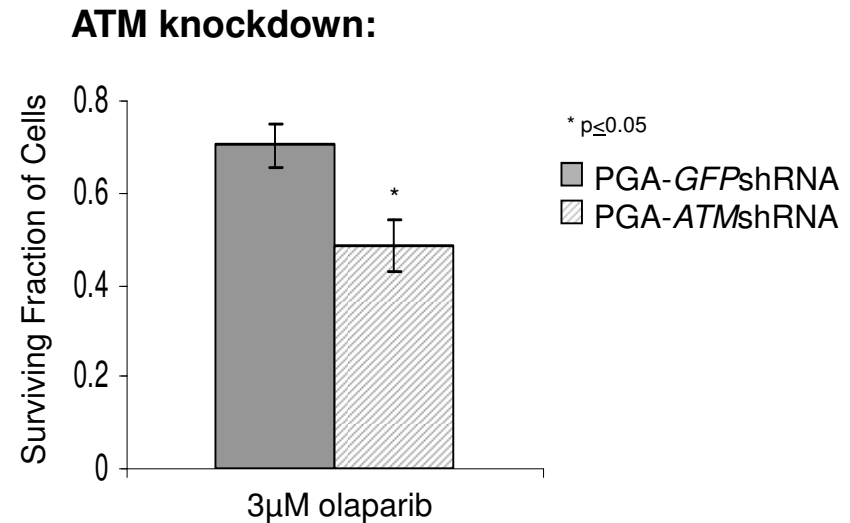
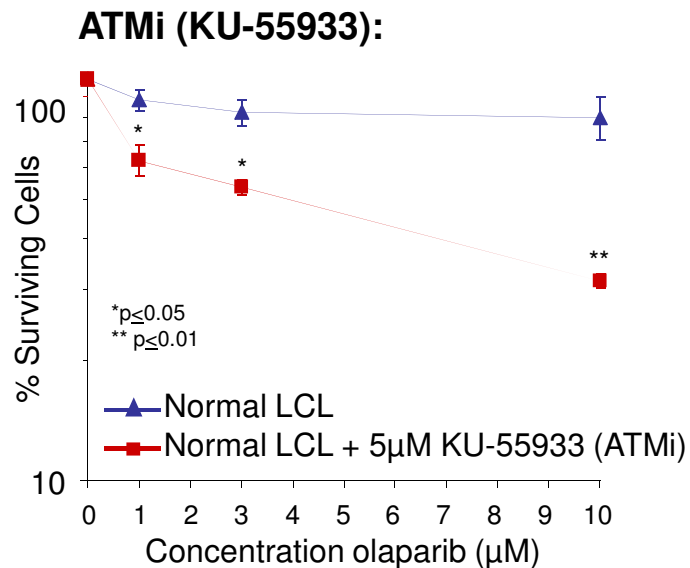
Two mutations which can exist individually in a cell but together cause loss of viability



# ATM mutant lymphoid cell lines are differentially sensitive to Olaparib

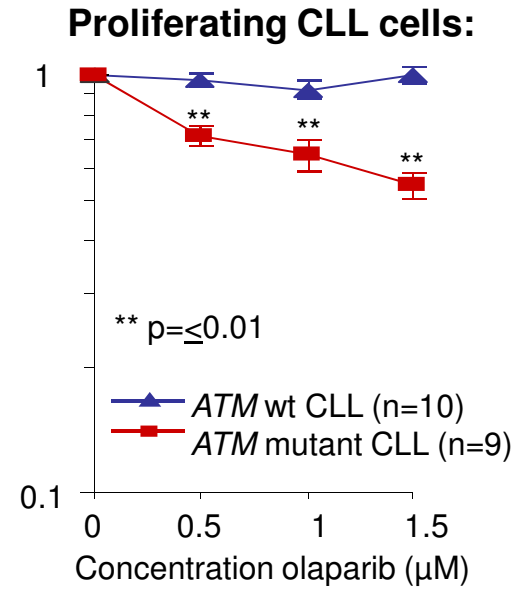
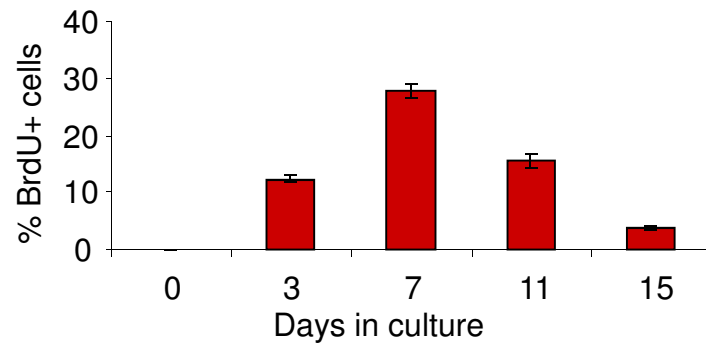


## Sensitivity to Olaparib is mediated by ATM loss

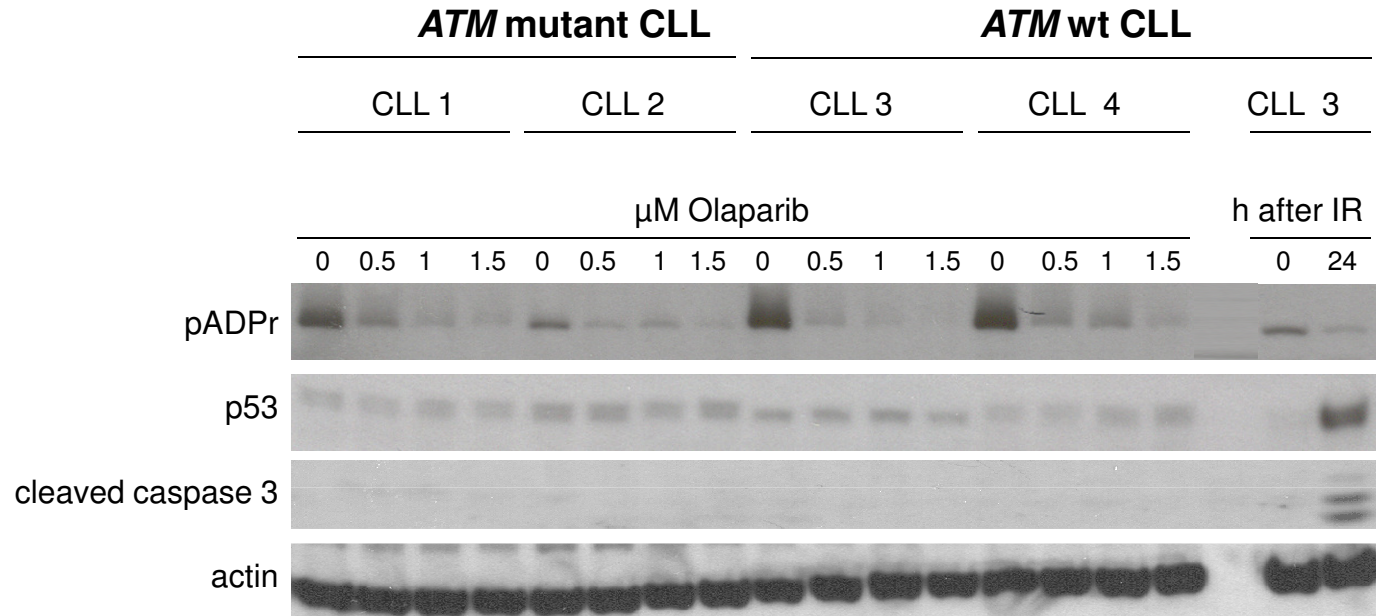


# Proliferating *ATM* mutant CLL are sensitive to Olaparib *in vitro*

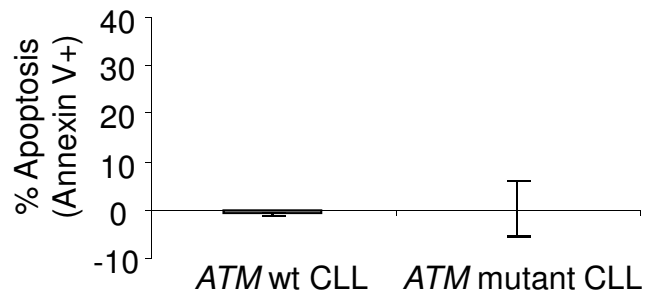
(CLL + CD40L/IL4 culture)  
BrdU Incorporation:



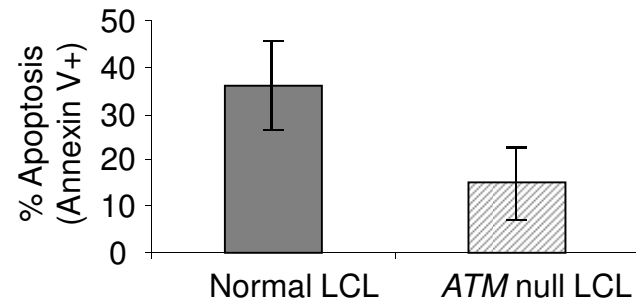
# Olaparib does not induce p53-dependent apoptosis in *ATM* mutant CLL cells



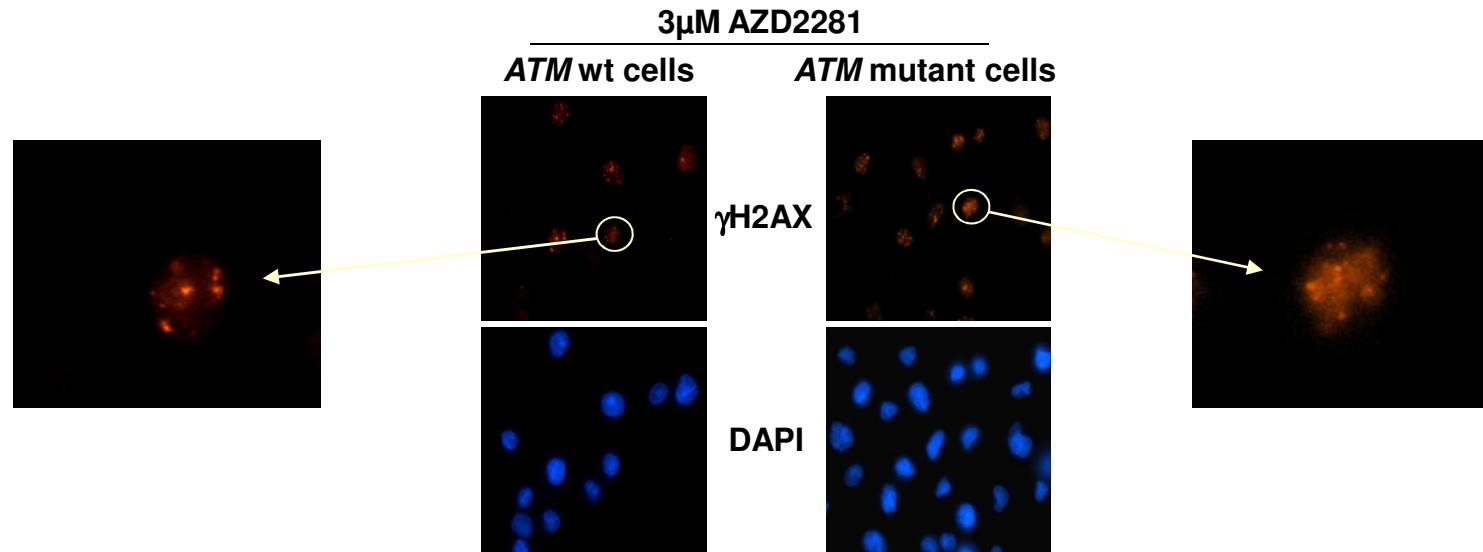
3 $\mu\text{M}$  Olaparib:



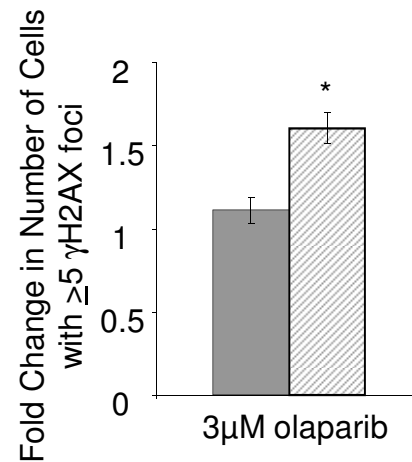
5Gy IR (Positive control):



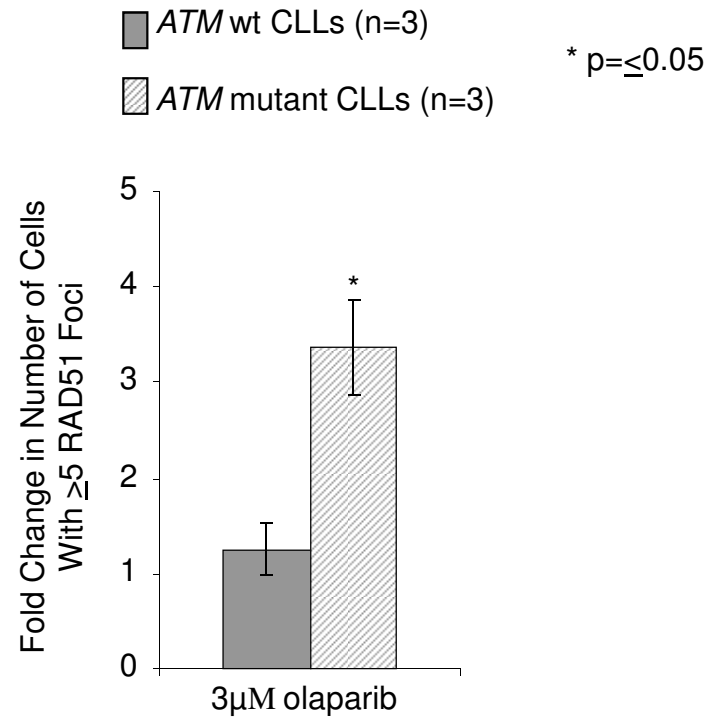
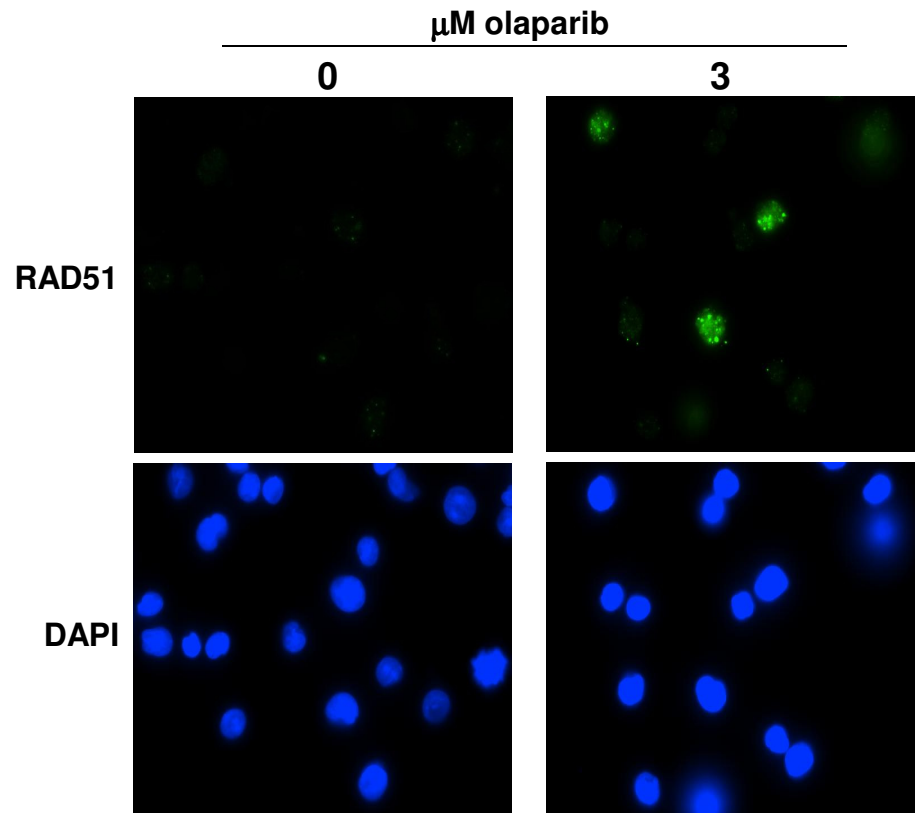
# Accumulation of unrepaired DNA damage ( $\gamma$ H2AX) in *ATM* mutant CLL cells following olaparib treatment



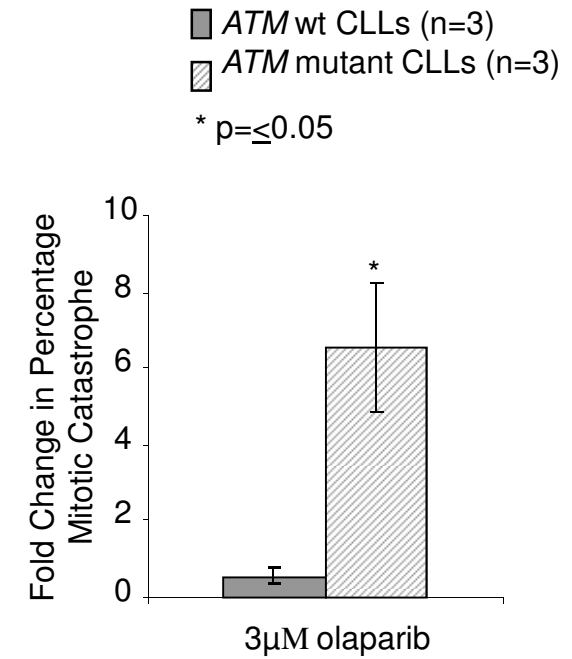
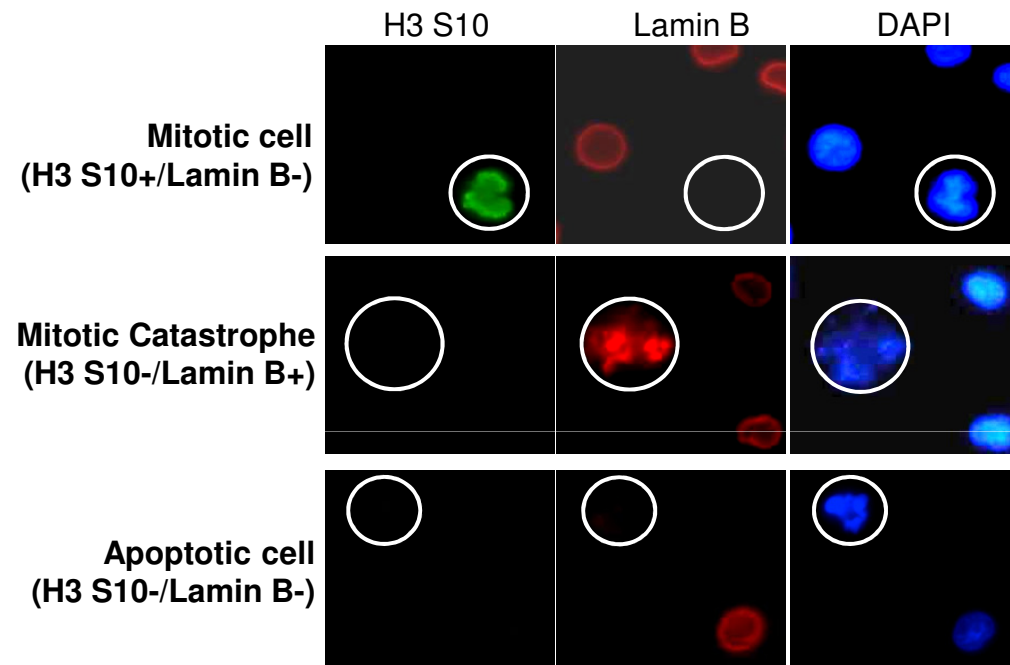
■ *ATM* wt CLLs (n=3)      \*  $p \leq 0.05$   
▨ *ATM* mutant CLLs (n=3)



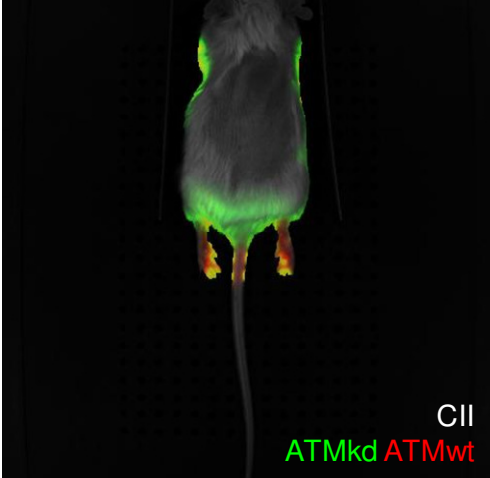
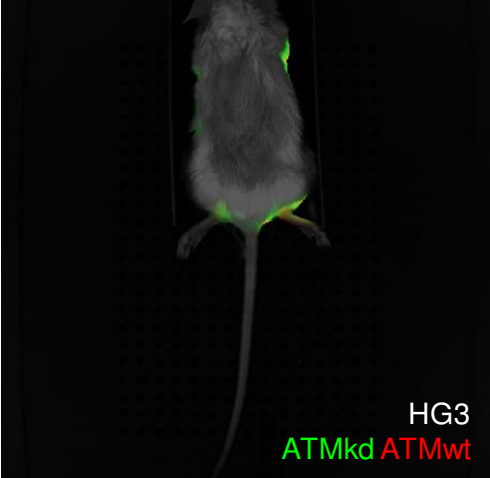
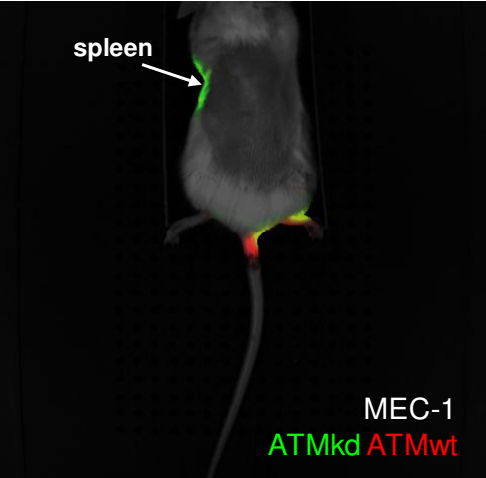
# Persistent repair proteins (RAD51) at the sites of unrepaired DNA DSBs in *ATM* mutant CLL cells following PARPi



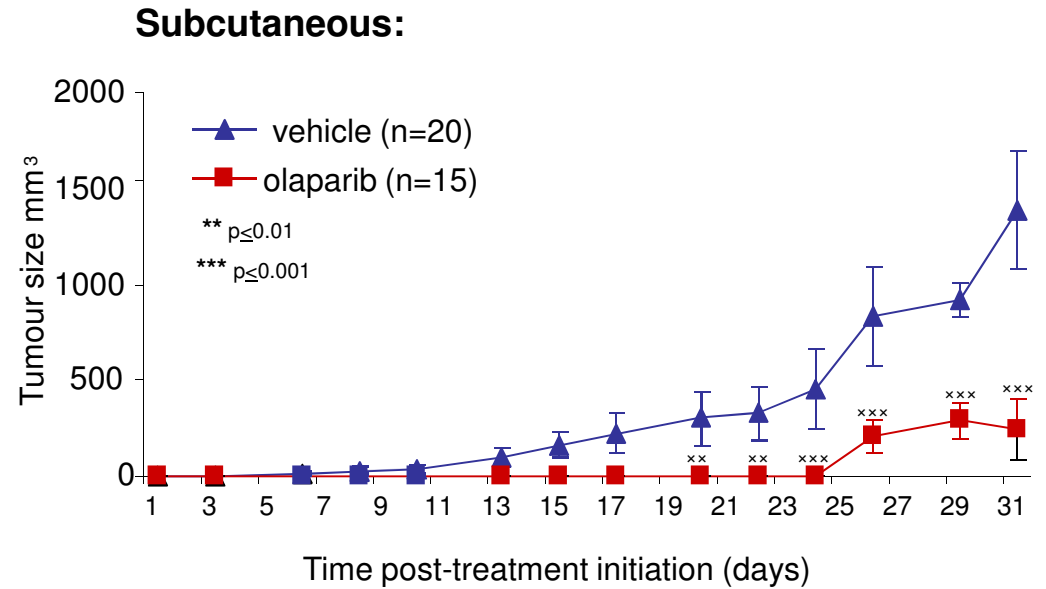
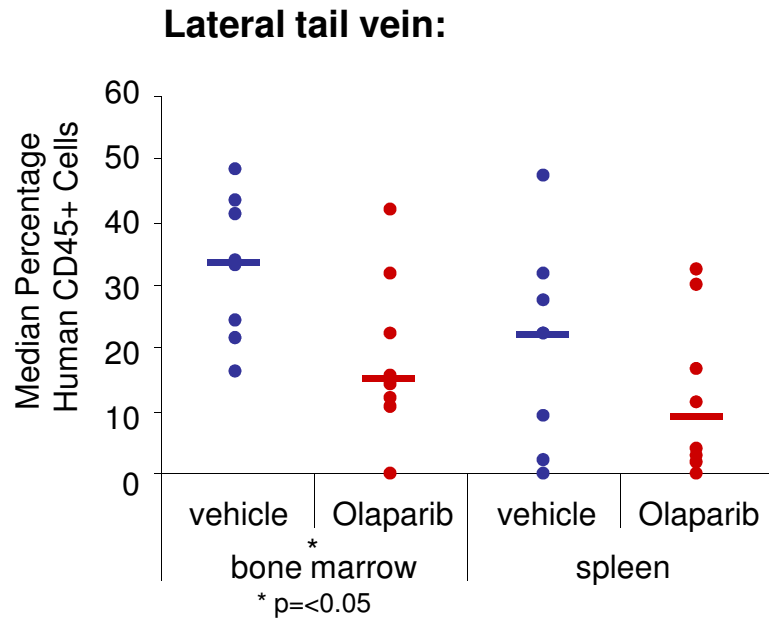
# Olaparib induces mitotic catastrophe in *ATM* mutant CLL cells



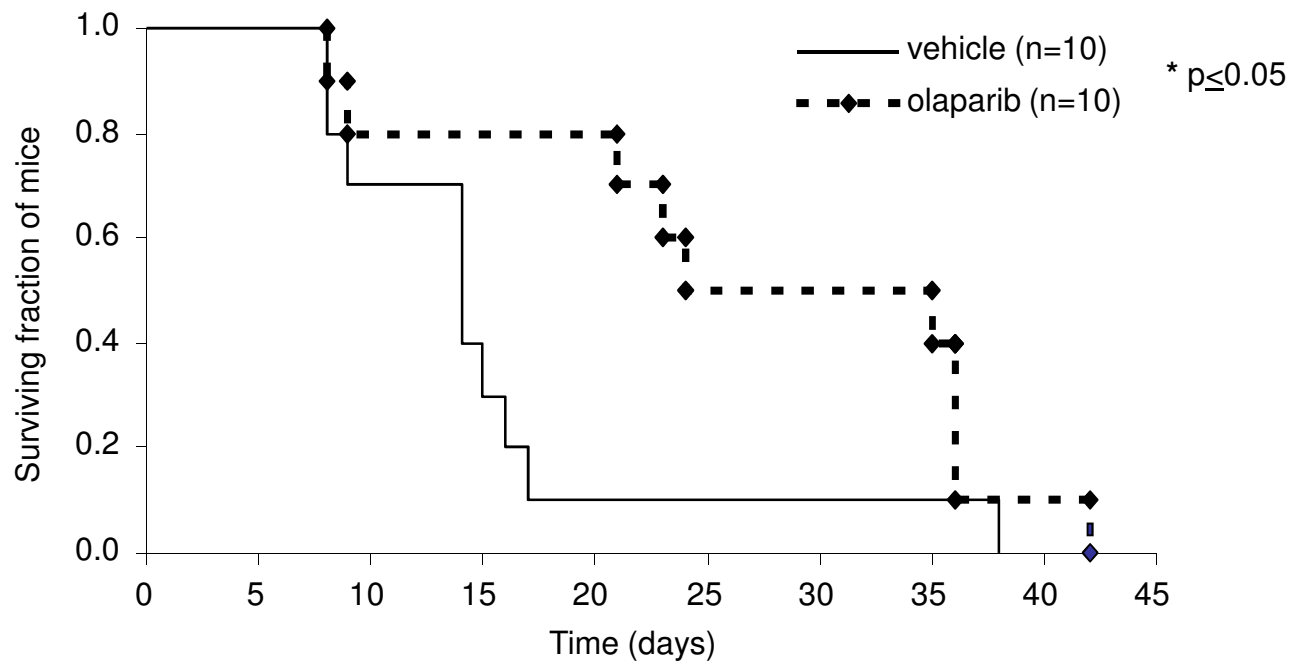
Engraftment of ATM null CLL cell lines in immuno-compromised mice



# Olaparib reduces tumour load and size of *ATM* null tumour cells in NOD/SCID xenograft model



# Olaparib increases survival of NOD/SCID mice engrafted with *ATM* null lymphoid tumour cells



## Are other CLL subtypes sensitive to PARPi?

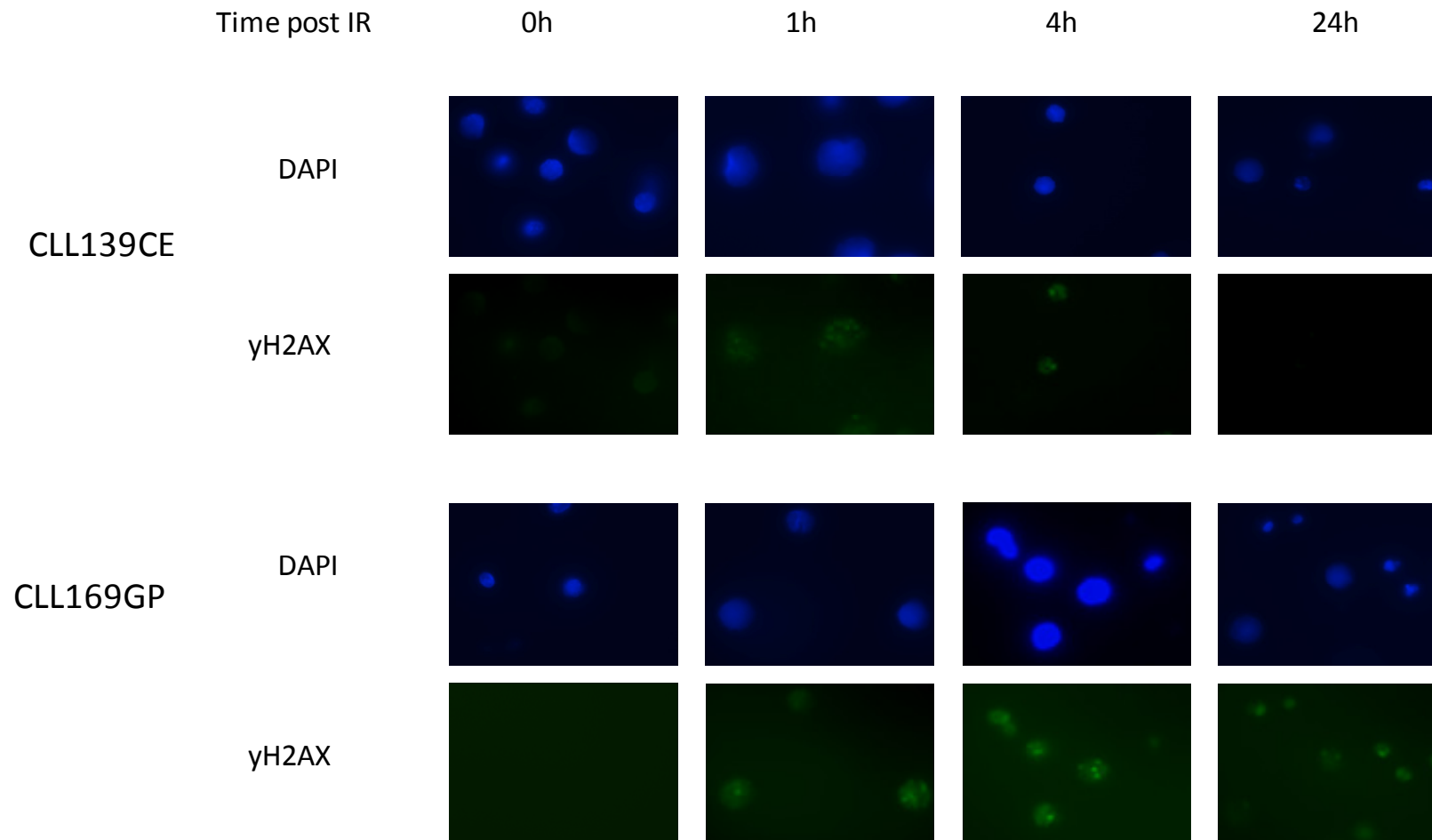
A significant subset of CLL exhibit genomic complexity

(Kujawski L *et al*, Blood 112, 2008; Ouillette P, *et al*, Clin Cancer Research 16, 2010)

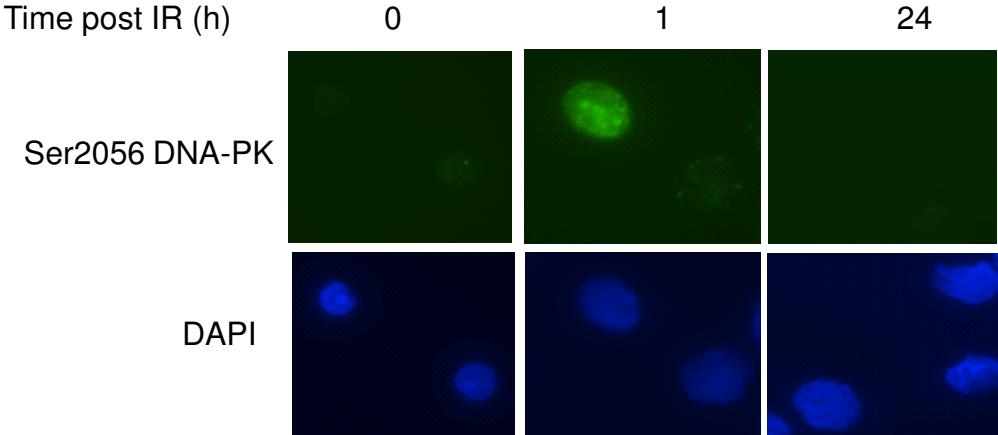
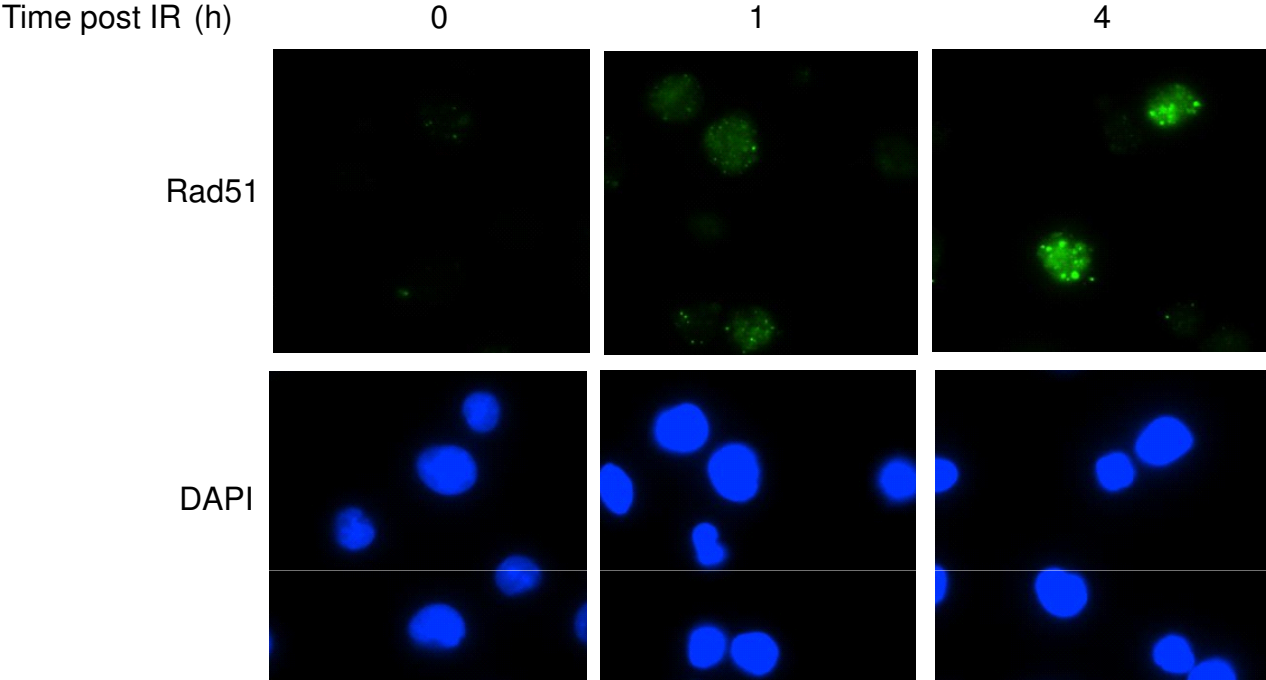
Associated with aggressive disease (progressive, relatively insensitive to treatment)

- cell cycle checkpoint defects?
- DNA repair defects?

# Screening for differences in repair of DNA breaks in CLL (kinetics)

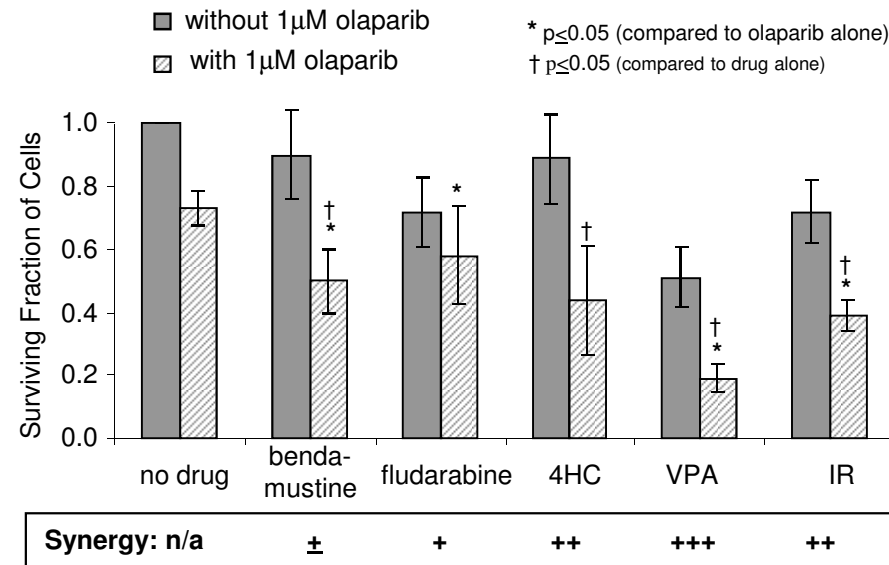


# Functional assessment of DNA repair proteins in CLL

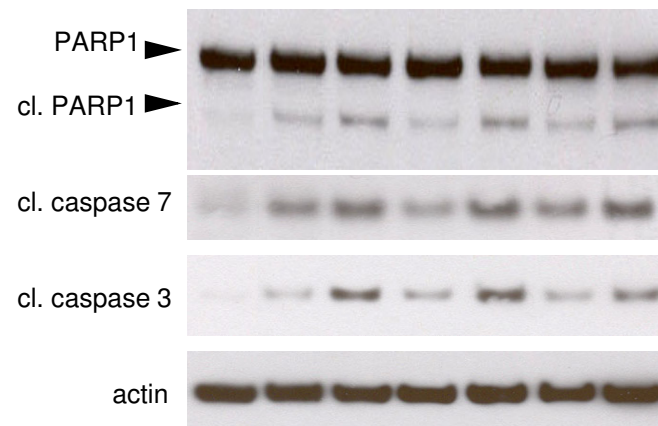


**Do DSB-inducing agents (eg chemotherapy) have a  
combined effect (synergise?)  
with DSB repair inhibiting agents (Olaparib)**

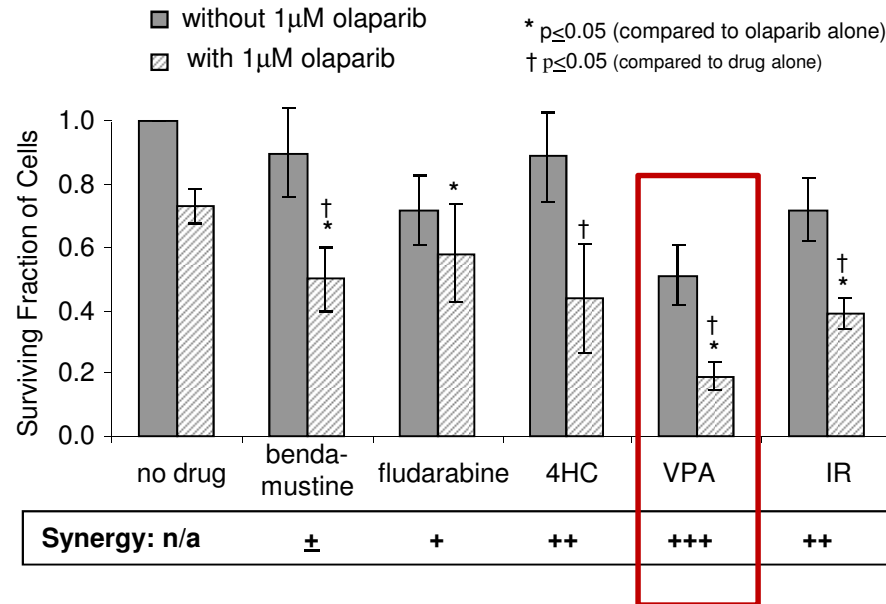
# Olaparib synergises with cytotoxic agents



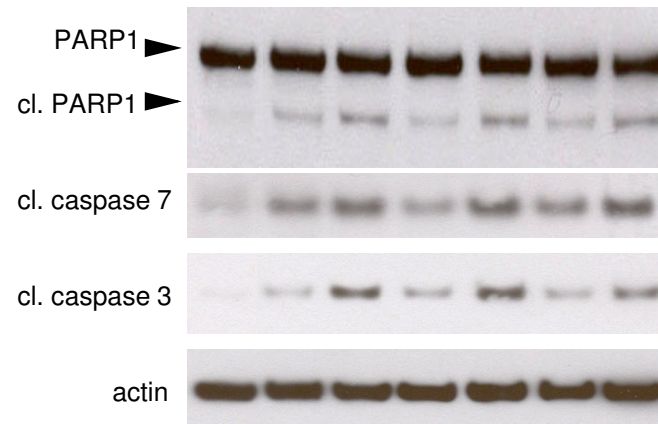
1µM olaparib:	-	-	+	-	+	-	+
50nM 4HC:	-	+	+	-	-	-	-
2.5mM VPA:	-	-	-	+	+	-	-
1Gy IR:	-	-	-	-	-	+	+



# Olaparib synergises with cytotoxic agents



1µM olaparib:	-	-	+	-	+	-	+
50nM 4HC:	-	+	+	-	-	-	-
2.5mM VPA:	-	-	-	+	+	-	-
1Gy IR:	-	-	-	-	-	+	+



## Histone deacetylase inhibitors (HDACi)

Strongest synergism (ATM null CLL) between Olaparib and HDACi Valproic Acid (VPA)

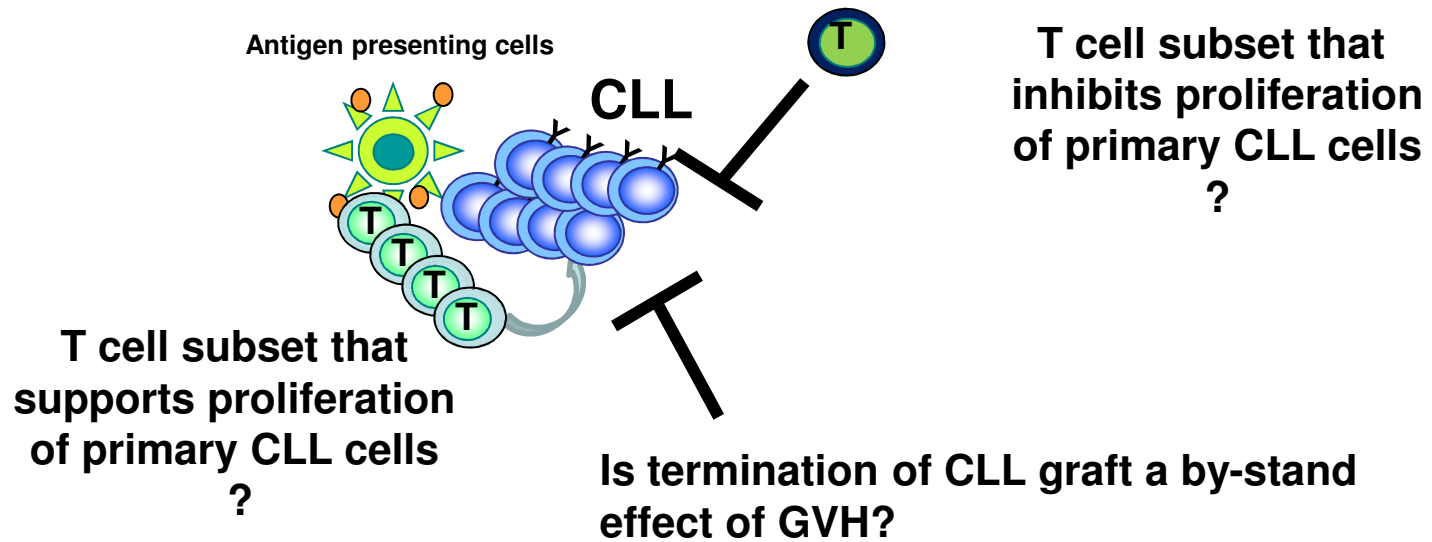
VPA induces DNA damage and inhibit HR repair proteins

- ATM null cells sensitive to HDACi alone (synthetic lethal)
- CLLs with other repair defects?
  
- *TP53* deficient cells also exhibit sensitivity to combined HDACi/PARPi

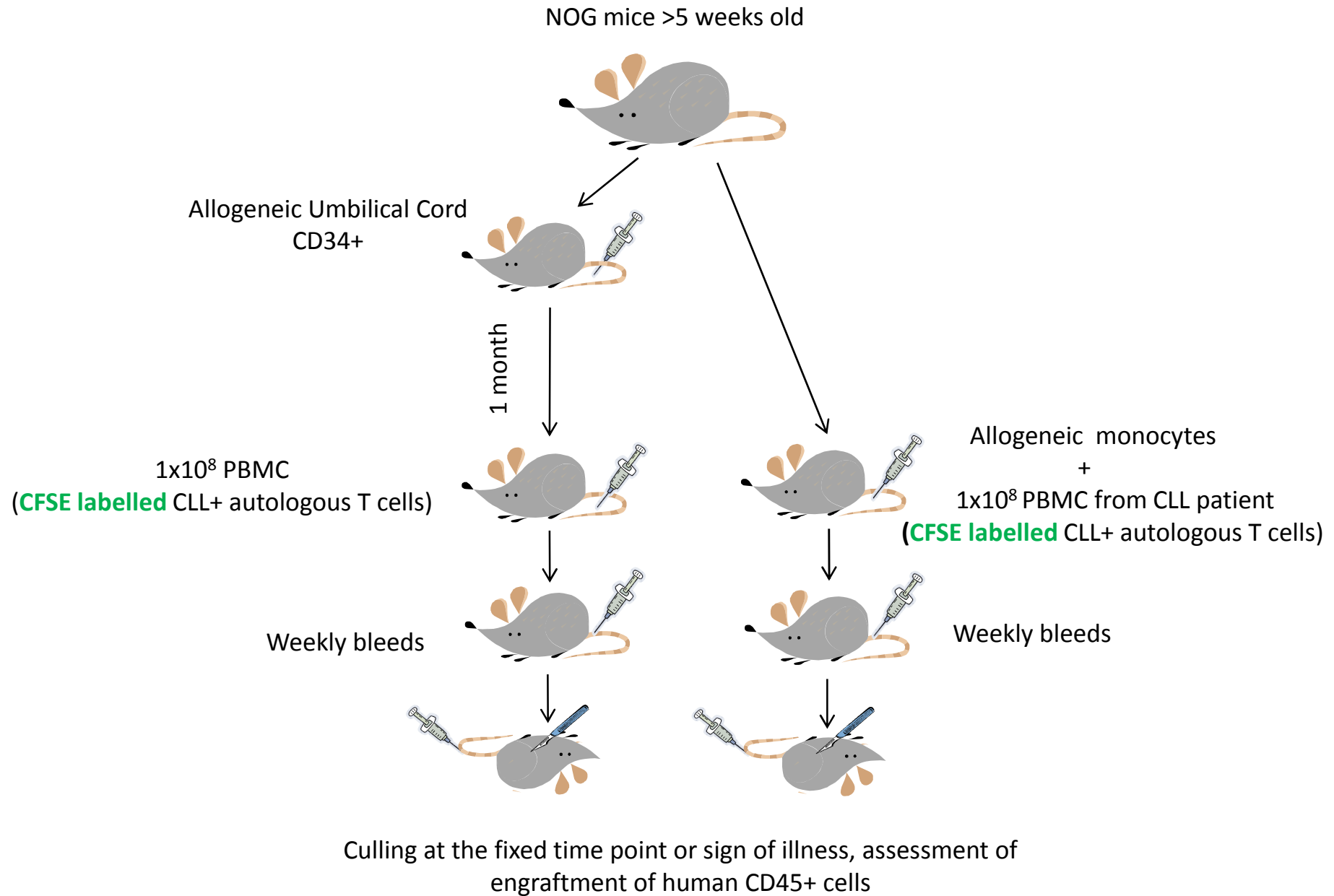
We are currently testing Olaparib in combination with a panel of HDACi (PXD101, Droxinostat, Givinostat, Dacinostat, MC1568, VPA and SAHA)

# Development of a murine xenograft (pre-clinical) model for primary CLL

## A current model of primary CLL engraftment

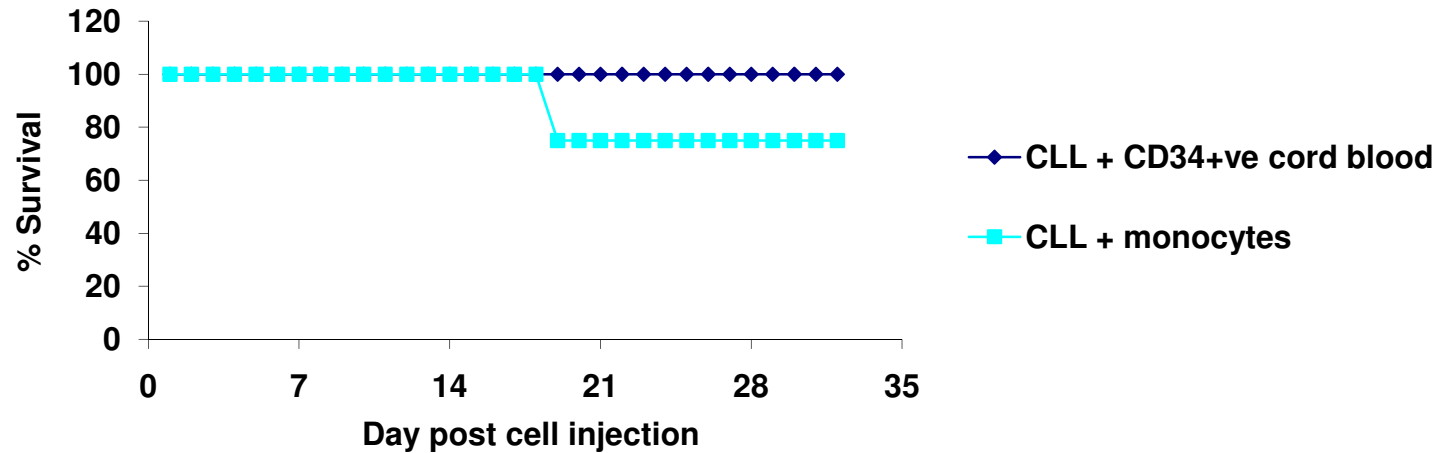


# Primary ATM null CLL Xenograft

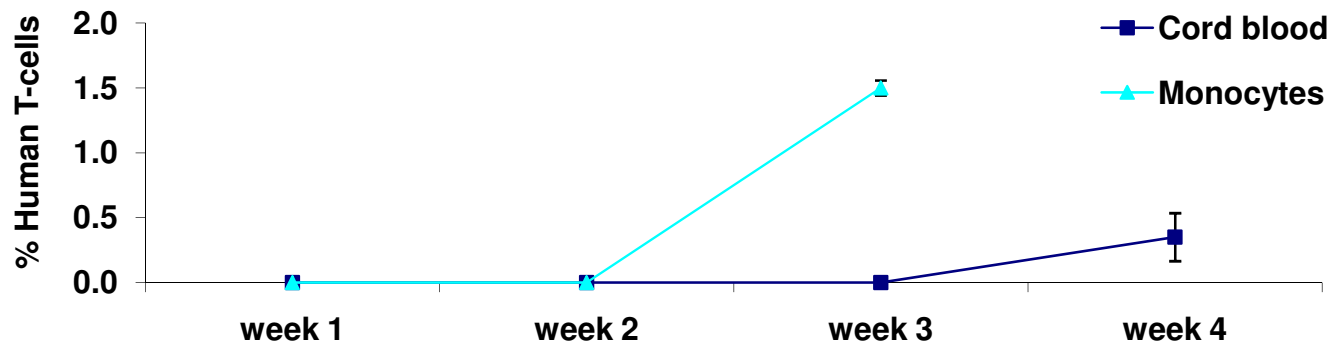


# Umbilical cord CD34+ /CLL ('humanised') xenograft lasts longer

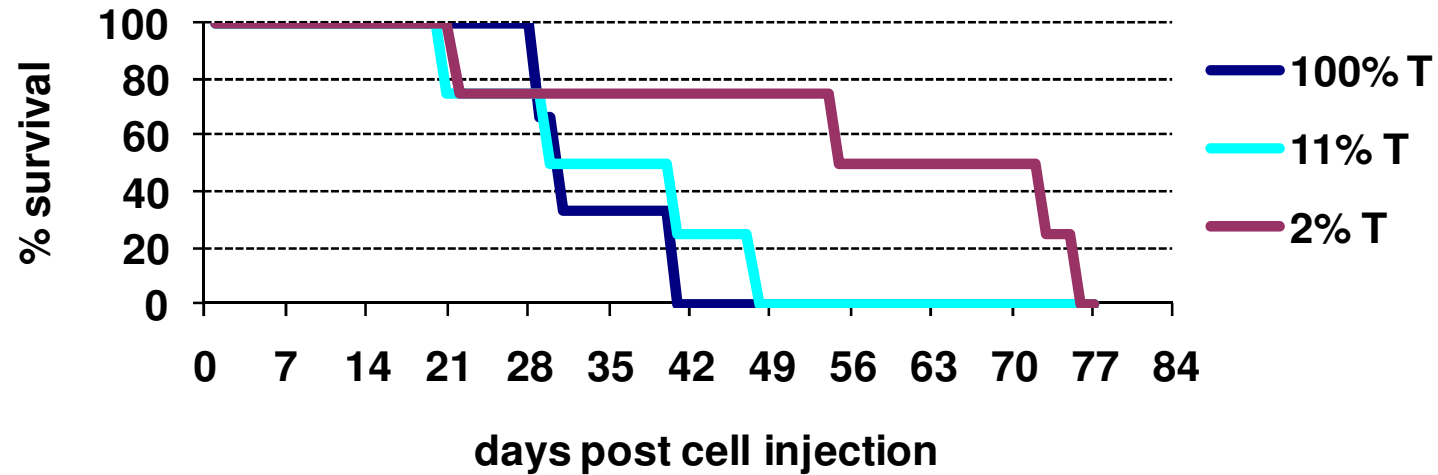
## Survival of 'engrafted' mice



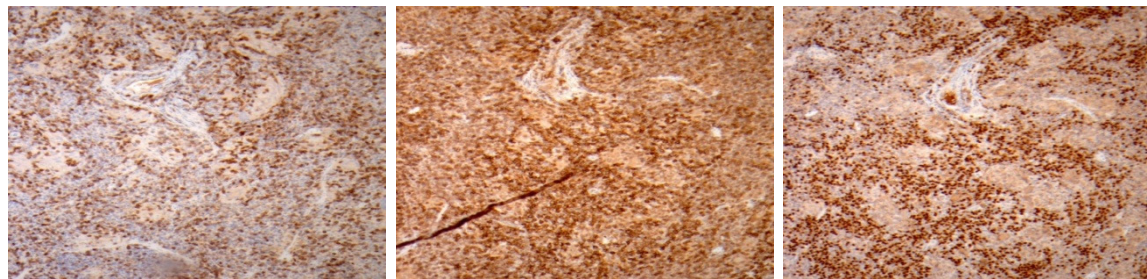
## Total T-cells in peripheral blood with humanisation



## Partial depletion of autologous T cells allows CLL engraftment up to 11 weeks



(Mouse B2) 2% T cells injected; spleen at the time of harvest

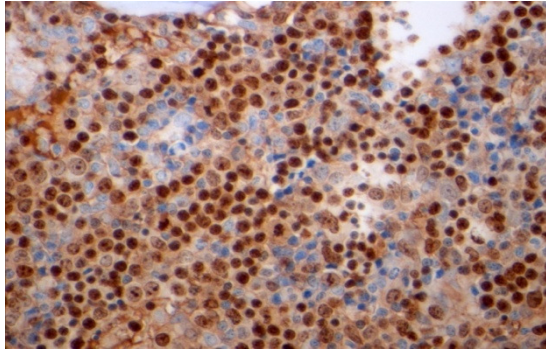


CD3

CD5

PAX5

## CLL cells are highly proliferative at the end of harvest



Ki-67 staining of the spleen of a mouse with CLL+2%T cells engraftment

## Summary

- Olaparib targets cycling, *ATM* mutant CLL and MCL cells
- Most applicable to highly proliferating, aggressive tumour cells
- Non cycling and normal cells (including B and T cells) are not sensitive
- Viable therapeutic option for fragile patients (where fludarabine-based approaches are not an option)
- Prophylactic application
  
- Other CLLs with genomic instability (DNA repair defective) may be candidates for PARPi
  
- HDACi/PARPi may increase tumour targeted killing of aggressive tumours (including *ATM*wt CLL)

## Acknowledgements

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