Poster #: 1310

XXI iwCLL International Workshop on CLL

Evaluation of two novel Cyclin Dependent Kinase 9 inhibitors for the treatment of MCL1 high Chronic Lymphocytic Leukaemia

Lauren Stott[1], Eun Young Han[1,2], Iona Ashworth[1,2], Emma Kennedy[1], Eleanor Farrant-Jayawant[1], Aimilia Vareli[1], John Jones[1,3], Eleni Ladikou[1,3], Rosalynd Johnston[2], Fabio Simoes[1], Simon Mitchell[1], Shudong Wang[4], Chris Pepper[1], Andrea Pepper[1]

[1] Department of Clinical and Experimental Medicine, Brighton and Sussex Medical School, Falmer, United Kingdom; [2] Brighton and Sussex University Hospitals NHS Trust, Brighton, United Kingdom; [3] East Sussex Healthcare NHS Trust, Eastbourne, United Kingdom; [4] Department of Clinical and Health Sciences, University of South Australia, Adelaide, Australia.

OBJECTIVES

- Identify CLL patient samples with high basal and TME-stimulated MCL1 expression, an anti-apoptotic BCL2 family member associated with poor responses to venetoclax.
- Establish toxicity of high MCL1 CLL cells to two novel selective second-generation CDK9 inhibitors, AU4-53 and AU11-8.
- Determine if AU4-53 and AU11-8 reduce high MCL1 expression in the TME.
- Investigate potential additive or synergistic effects of CDK9 inhibition and venetoclax.

CONCLUSIONS

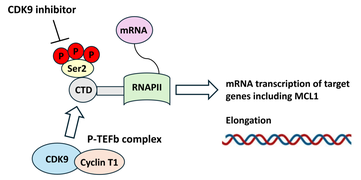
- Basal and TME-stimulated BCL2 family expression is heterogeneous in CLL patients, partly contributing to variability in treatment responses.
- AU11-8 more effectively induces CLL cell apoptosis, reduces high MCL1 expression, and exhibits greater additive effects with venetoclax on the TME-mimicking CD40L co-culture than AU4-53.
- Targeted inhibition of CDK9 may be a promising therapeutic strategy for the treatment of MCL1 high CLL.

NHS University Hospitals Sussex NHS Foundation Trust

brighton and sussex medical school

INTRODUCTION

- CLL is very heterogeneous and responses to treatment are variable, highlighting the need to identify which patients will respond to which drugs.
- Upregulation of anti-apoptotic BCL2 family members in the tumour microenvironment (TME), such as BCL-XL and MCL1, is associated with therapeutic resistance and inferior prognosis(1).
- We have investigated the effect of the TME on BCL2 family expression using *in vitro* models which mimic signals CLL cells receive in the lymph node; activation through TLR9 using ODN 2006, and activation through CD40 using CD40L-expressing 3T3 fibroblasts.
- Direct MCL1 inhibitors have shown promising pre-clinical results in CLL, but these results have failed to translate to the clinic due to the labile nature of MCL1 protein and concerns over cardiotoxicity(2).
- Selective CDK9 inhibitors have been shown to suppress MCL1 indirectly and induce apoptosis of CLL cells, rendering them a promising therapeutic strategy for the treatment of MCL1 high CLL(3,4).

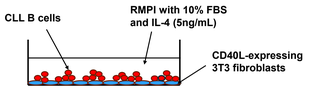


CDK9 regulates MCL1 transcript levels. CDK9, with Cyclin T1, phosphorylates Serine 2 residues on the C-terminal domain (CTD) of RNA Polymerase II (RNAPII), resulting in mRNA transcription of target genes including MCL1. Inhibition of CDK9 reduces MCL1 indirectly through transcriptional repression.

We have tested the toxicity of two novel selective second-generation CDK9 inhibitors in the CD40L 3T3 TME model.

METHODS

- **TME-mediated activation:** Primary CLL cells were stimulated -/+ 1μM ODN 2006 (TLR9 agonist), or co-cultured on CD40L-expressing 3T3 fibroblasts (10:1 ratio) for 24h to mimic the lymphoid microenvironment. Non-transfected fibroblasts (NTLs) served as controls. BCL2 family expression was subsequently quantified by multi-colour flow cytometry.

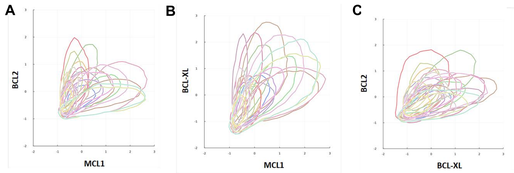


Schematic diagram of the CD40L co-culture used to mimic the lymphoid microenvironment.

- **Apoptosis assays:** Primary CLL cells were treated with concentrations of AU4-53 and AU11-8 ranging between 0.078 and 20μM for 48h on NTLs and CD40L co-culture. Cells were then harvested and stained with Annexin V FITC and 7-AAD for FACS analysis. % apoptosis was normalised to the DMSO control.
- **Synergy assays:** Molar ratios were determined using the LD_{50} values for AU4-53 and AU11-8 and clinically achievable concentrations of venetoclax. Primary CLL cells were treated with each drug individually and in combination at the defined molar ratio for 48h on the CD40L co-culture, before being labelled with Annexin V FITC/7-AAD and analysed by flow cytometry. The drug combination responses were calculated based on the BLISS reference model using SynergyFinder.

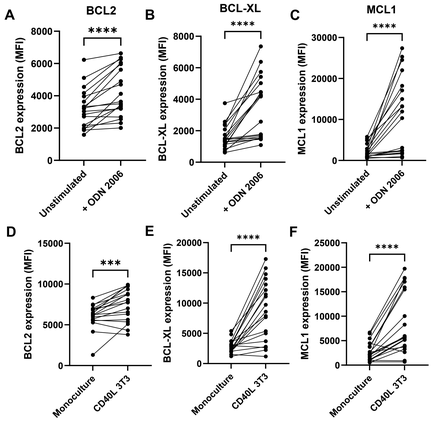
RESULTS

1. Basal expression of anti-apoptotic BCL2 family members is heterogeneous in CLL patients



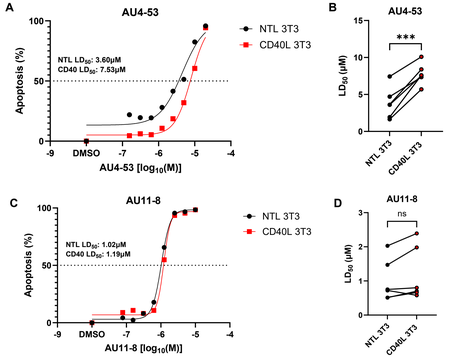
BCL2 family 'fingerprinting' reveals a unique basal anti-apoptotic BCL2 family state within each CLL patient. FACS generated 'fingerprints' based on **A** BCL2 and MCL1 abundance, **B** BCL-XL and MCL1 abundance, and **C** BCL2 and BCL-XL abundance in CLL cells. Cell density is indicated with a contour plot and each patient is shown in a different colour (n=25).

2. MCL1 is significantly upregulated following TME-mediated activation in a subset of CLL patients



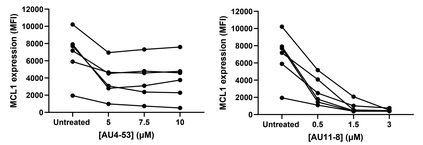
Changes in anti-apoptotic BCL2 family members following **A-C** activation of Toll-like receptor 9 (TLR9) with the TLR9 agonist ODN 2006, and **D-F** CD40 stimulation with the CD40L 3T3 co-culture. ***P≤0.001, ****P≤0.0001. Changes in BCL2 were relatively small and consistent between patient samples, whilst there was more striking heterogeneity in BCL-XL and MCL1 expression following TME-mediated activation, with some patients exhibiting significantly higher BCL-XL and MCL1 than others.

3. CDK9 inhibition induces apoptosis of MCL1 high CLL cells on the CD40L co-culture



A Representative dose response curve to AU4-53 on NTLs and CD40L co-culture. **B** LD_{50} values (dose required to kill 50% of cells) of 6 CLL patients to AU4-53. LD_{50} values were significantly higher on the CD40L co-culture (P=0.004). **C** Representative dose response curve to AU11-8 on NTLs and CD40L co-culture. **D** LD_{50} values of 6 CLL patients to AU11-8. There was no significant difference in LD_{50} values on the NTLs and CD40L co-culture (P=0.1), suggesting that this inhibitor may be able to overcome the cytoprotective microenvironment of the TME.

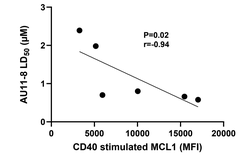
4. AU11-8 more effectively reduces MCL1 expression on the CD40L co-culture



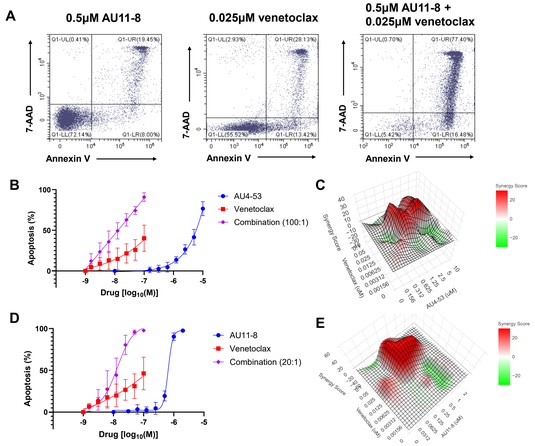
A MCL1 expression on the CD40L co-culture following 24h treatment with AU4-53 for 6 CLL patient samples. There is a larger reduction in some patients than others, which occurs at the lowest dose only (5μM) before plateauing at the higher concentrations. **B** MCL1 expression on the CD40L co-culture following 24h treatment with AU11-8 for 6 CLL patient samples. AU11-8 causes a more significant dose-dependent reduction in MCL1 on the CD40L co-culture in all 6 samples, suggesting that this inhibitor may be more effective at reducing MCL1 expression in CLL cells within the TME.

5. High MCL1 expression correlates with increased sensitivity to AU11-8

There was a significant negative correlation between MCL1 expression and AU11-8 LD_{50} values on the CD40L co-culture (P=0.02, r=-0.94).
This could implicate MCL1 as a potential biomarker of sensitivity to this inhibitor.



6. CLL cell apoptosis is significantly enhanced on the CD40L co-culture with CDK9 inhibitor + venetoclax



A FACS plots showing % apoptosis following treatment with 0.5μM AU11-8, 0.025μM venetoclax, and AU11-8 and venetoclax combined (20:1 ratio) on the CD40L co-culture. **B** % apoptosis with AU4-53 and venetoclax (100:1 ratio) on the CD40L co-culture for 5 CLL patients. **C** Synergy plot indicates additive effects of AU4-53 and venetoclax; BLISS score=4.68, P=0.009. **D** % apoptosis with AU11-8 and venetoclax (20:1 ratio) on the CD40L co-culture for 5 CLL patients. **E** Synergy plot indicates additive effects of AU11-8 and venetoclax; BLISS score=5.17, P=0.009.

REFERENCES

1. DOI: 10.1182/bloodadvances.20160041764
2. DOI: 10.1080/10428194.2019.1622098
3. DOI: 10.1038/s41375-022-01758-z
4. DOI: 10.18632/oncotarget.1568

ACKNOWLEDGMENTS

Thank you to Paul Stanforth for funding this PhD project, and all the CLL patients who kindly donated blood for this research. Also thank you to the UK CLL Forum for awarding me an iwCLL Travel Award.