1023 - Targeting non-canonical NF-κB inducing kinase (NIK) in chronic lymphocytic leukaemia

Iona Ashworth[1,2], Thomas A. Burley[1], Emma Kennedy[1], Eleni E. Ladikou [1,2], Lauren Stott[1], Christopher West [3,4], Christopher Fegan[5], Rosalynd Johnston[2], Simon Mitchell[1], Simon P. Mackay[3], Andrea G.S. Pepper[1] and Chris Pepper[1]

[1]Department of Clinical and Experimental Medicine, Brighton and Sussex Medical School, Falmer BN1 9PX, UK. [2]Department of Haematology, Brighton and Sussex University Hospital Trust, Brighton BN2 5BE, UK. [3]Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow G4 0RE, UK. [4]Cancer Research Horizons, The Beatson Institute for Cancer Research, Garscube Estate, Switchback Road, Bearsden, Glasgow G61 1BD, UK. [5]Division of Cancer and Genetics, School of Medicine, Cardiff University, Heath Park, Cardiff CF14 4XN, UK.

Background

Aberrant NF-κB signalling appears to play a key role in the pathogenesis of chronic lymphocytic leukaemia (CLL), including several recurrent genetic mutations in NF-κB-activating genes[1-3]. Furthermore, constitutive NF-κB activity is associated with a more aggressive disease[1,4] and is implicated in the development of resistance to both ibrutinib and venetoclax[5]. NF-κB signalling can be divided into two distinct pathways; both of which regulate a number of essential cellular processes (Fig. 1). Although inhibiting NF-κB signalling is a potentially attractive therapeutic approach, direct pharmacological targeting of the canonical NF-κB pathway (e.g., using IKKβ inhibitors) has so far failed due to their associated toxicities.

Here, we investigated the potential for selectively targeting non-canonical NF-κB, by inhibiting its central kinase, NIK (NF-κB inducing kinase). Given that NIK expression levels are very low under normal physiological conditions, and constitutive activation is common in pathological contexts[6], NIK may represent a tumour-selective therapeutic target. We hypothesised that NIK inhibition may be a promising strategy in the treatment of CLL by preferentially targeting CLL cells in the lymphoid tissue environment where they are particularly reliant on non-canonical NF-κB signalling.

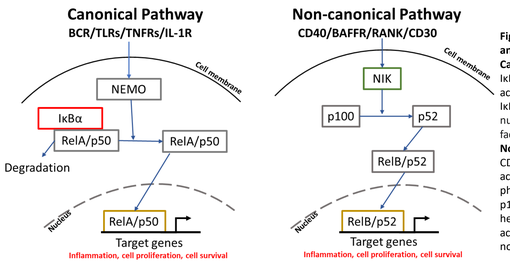


Figure 1. Overview of canonical and non-canonical NF-κB signalling. Canonical: under resting conditions IκBα binds RelA/p50. Upon activation, NEMO phosphorylates IκBα, allowing RelA/p50 to enter the nucleus and act as transcription factors for canonical signalling. **Non-canonical:** NIK is stabilised by CD40/BAFFR/RANK/CD30 activation. NIK causes phosphorylation and processing of p100 to p52. p52/RelB heterodimers enter the nucleus and act as the transcription factor for non-canonical NF-κB signalling.

Methods

We evaluated three NIK inhibitors, CW15337, Amgen16 and B022, in the MEC-1 cell line and in primary CLL samples. We compared our findings with those obtained using ACHP Hydrochloride, a dual IKKβ and IKKα inhibitor.

To establish toxicities, MEC-1 cells and primary CLL cells were incubated at 37°C with serial dilutions of the drugs for 48h then cell viability was assessed using annexin V and 7-AAD labelling.

To investigate the impact of NIK inhibition in culture conditions resembling the lymphoid niche, MEC-1 cells and primary B cells were co-cultured on CD40L-expressing 3T3 fibroblasts, designed to mimic interactions with activated T cells in the lymph node environment[5].

Cell cycle analysis was performed on MEC-1 cells treated with serial concentrations of the drugs for 24h. Cells were fixed with ethanol then stained with propidium iodide.

Transwell inserts were used to study the impact of the drugs on MEC-1 cell migration (Fig.2). 100ng/mL CXCL12 was added to the basal chamber to create a chemokine gradient.

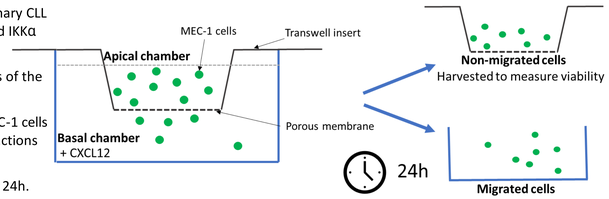


Figure 2. MEC-1 cell migration assay MEC-1 cells were incubated at different drug concentrations for 24h. Cells in the basal chamber (migrated cells) were counted. Cell viability was determined by labelling cells harvested from the apical chamber with annexin V and 7-AAD.

Results: NF-κB expression (Western blot)

MEC-1 cells in CD40L co-culture showed activation of non-canonical signalling (increase in p52). The negative regulator of canonical signalling, IκBα, was reduced in co-cultured MEC-1 cells, in keeping with an increase in canonical activity on CD40L (Fig.3A).
All three NIK inhibitors showed a reduction in p52.
In contrast, ACHP, a non-selective NF-κB inhibitor, caused a dose-dependent reduction in p100 but not p52. This suggests inhibition of mainly canonical activity, given that canonical signalling results in increased p100 production.

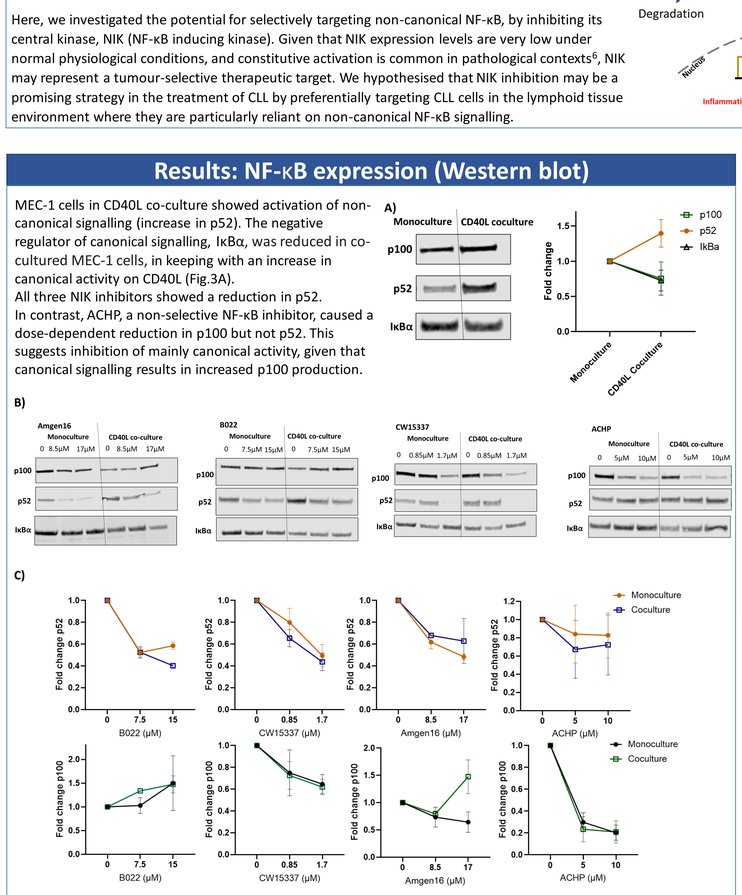
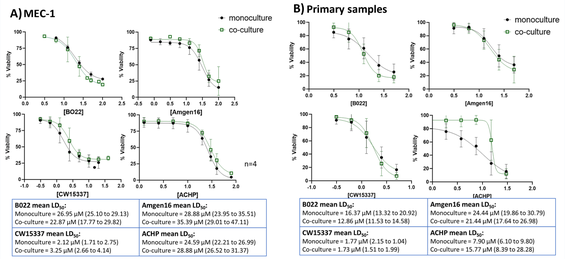


Figure 3. Summary of NF-κB subunit levels in MEC-1 cells treated with different concentrations of drug. MEC-1 cells incubated on and off CD40L. A) Change in expression of p100, p52 and IBα in MEC-1 cells incubated on and off CD40L. Fold changes are normalised to no-drug control. Expression levels normalised to total protein. **B)** Western blot of p100, p52 and IκBα at different drug concentrations with MEC-1 cells incubated on and off CD40L coculture. **C)** Change in p52 (top) and p100 (bottom) expression at different drug concentrations normalised to control. Expression levels normalised to total protein.

Results: drug cytotoxicity

All drugs induced a concentration-dependent cytotoxicity in MEC-1 cells and in primary CLL samples (n = 10).
NIK inhibitors were able to overcome the pro-survival effects of CLL cells grown in CD40L co-culture. In contrast, ACHP, which is a non-selective NF-κB inhibitor, showed resistance in primary CLL samples in CD40L co-culture.



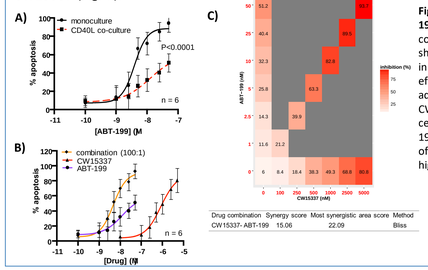
B022 mean LD_{50}: Monoculture = 26.95 μM (25.10 to 29.13) Co-culture = 22.87 μM (17.77 to 29.82)	Amgen16 mean LD_{50}: Monoculture = 28.88 μM (23.95 to 35.51) Co-culture = 35.39 μM (29.01 to 47.11)
CW15337 mean LD_{50}: Monoculture = 2.12 μM (1.71 to 2.75) Co-culture = 3.25 μM (2.66 to 4.14)	ACHP mean LD_{50}: Monoculture = 24.59 μM (22.21 to 26.99) Co-culture = 28.88 μM (26.52 to 31.37)

B022 mean LD_{50}: Monoculture = 16.37 μM (13.32 to 20.92) Co-culture = 12.86 μM (11.53 to 14.58)	Amgen16 mean LD_{50}: Monoculture = 24.44 μM (19.86 to 30.79) Co-culture = 21.44 μM (17.64 to 26.98)
CW15337 mean LD_{50}: Monoculture = 1.77 μM (2.15 to 1.04) Co-culture = 1.73 μM (1.51 to 1.99)	ACHP mean LD_{50}: Monoculture = 7.90 μM (6.10 to 9.80) Co-culture = 15.77 μM (8.39 to 28.28)

Figure 4. Comparative dose response curves to different concentrations drugs in monoculture and CD40L co-culture. A) Dose responses in MEC-1 cell line. **B)** Dose responses in primary CLL samples. LD_{50} values (the concentration of drug required to kill 50% of the cells) are presented as mean values, with 95% CI in brackets.

Results: synergy with ABT-199

Co-culture of CLL B cells on CD40L-expressing fibroblasts caused marked resistance to the venetoclax (ABT-199). This can be reversed by the co-administration of the NIK inhibitor, CW15337 (Fig. 7).



Drug combination	Synergy score	Most synergistic area score	Method
CW15337- ABT-199	15.06	22.09	Bliss

Figure 7. Synergy with ABT-199 (venetoclax). A) CLL cells co-cultured on CD40L showed a significant increase in resistance to the cytotoxic effects of ABT-199. **B)** The addition of the NIK inhibitor, CW15337, resensitised CLL cells to the effects of ABT-199 and **C)** the combination of these two drugs was highly synergistic.

Results: cell cycle analysis

All the drugs caused a concentration-dependent G1 arrest in the MEC-1 cell cycle. Concentrations of each drug resulting in G1 arrest were in keeping with cytotoxicity data

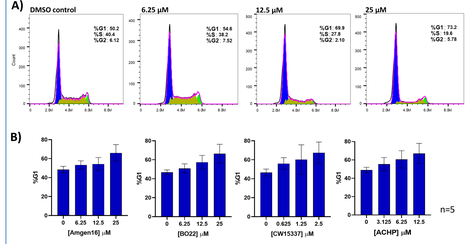
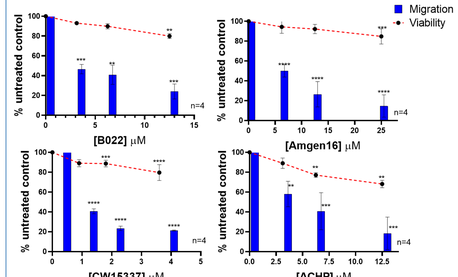


Figure 5. Summary of cell cycle distribution of MEC-1 cells following treatment with a range of concentrations the drugs.
A) Example of cell cycle distribution at increasing concentrations of Amgen16.
B) Graph showing proportion (%) MEC-1 cells in G1 (Y-axis) and different drug concentrations (X-axis).

Results: cell migration



All drugs significantly inhibited cell migration of MEC-1 cells in a concentration-dependent manner (Fig.6). Concentrations of drug required to inhibit MEC-1 cell migration also caused a statistically significant reduction in cell viability, but this could not explain the marked inhibition in MEC-1 cell migration.

Figure 6. Evaluation of effects of NIK inhibition on MEC-1 cell migration. **** p < 0.0001 *** p < 0.001 ** P < 0.01

Conclusions

- **NIK inhibitors can overcome the cytoprotective environment conferred by co-culture, suggesting that they could be effective at targeting disease in lymphoid tissues, where CLL cells are more reliant on NF-κB signalling.**
- **Primary CLL cells in CD40L co-culture showed resistance to the pan NF-κB inhibitor, ACHP.**
- **The inhibition of MEC-1 migration suggests that NIK inhibitors could block re-entry of CLL cells to the lymph node microenvironment.**
- **NIK inhibitors may be effective in targeting residual disease in the lymph node by suppressing the non-canonical NF-κB signalling pathway.**
- **Co-administration of a NIK inhibitor resensitises CLL cells to venetoclax.**

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References

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Contact information

Dr Iona Ashworth
Email: i.ashworth1@uni.bsms.ac.uk