Poster #: 1301

XXI IWCLL International Workshop on CLL

Selective NIK Inhibition Overcomes Microenvironment-Mediated Resistance and Synergises with Venetoclax in CLL

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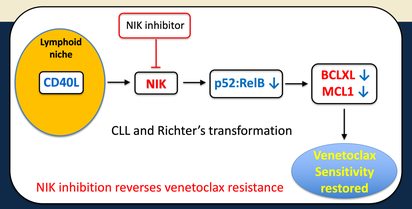
OBJECTIVES

To evaluate the therapeutic potential of targeting NF-κB-inducing kinase (NIK), a central regulator of non-canonical NF-κB signalling. The main aims were:

1. **Determine** the effect of CD40L stimulation (lymph node mimic) on NF-κB activity and BCL2-family expression in CLL.
2. **Assess** the impact of NIK inhibition on CLL cell activation, migration, NF-κB signalling, and anti-apoptotic protein expression, with and without CD40L stimulation.
3. **Test** whether NIK inhibition, in combination with venetoclax, can overcome microenvironment-mediated drug resistance.

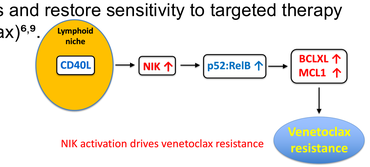
CONCLUSIONS

- **NIK inhibition blocks lymph node-mediated survival signals** in CLL by suppressing CD40L-induced NF-κB activation and anti-apoptotic protein expression.
- **Prevents CLL cell migration and niche re-entry**, reducing the potential for lymph node-resident disease persistence.
- **Restores venetoclax sensitivity** in CLL cells exposed to LN-like conditions and shows strong synergy in CLL and Richter's models.
- **NIK inhibition is a rational clinical strategy to overcome microenvironment-mediated venetoclax resistance**



INTRODUCTION

- **CLL cells in the lymph node (LN) microenvironment** receive CD40L and other signals that activate canonical and non-canonical NF-κB pathways[1–4].
- **NF-κB activation upregulates anti-apoptotic proteins** (BCL-XL, MCL1, BCL2A1) that protect LN-resident CLL cells from apoptosis and drive drug resistance[5–7].
- **NIK is a key driver of non-canonical NF-κB signalling** in CLL LNs and is minimally active in normal tissues[8].
- **Hypothesis:** Selective NIK inhibition will block LN-mediated survival signals and restore sensitivity to targeted therapy (e.g., venetoclax)[6,9].



METHODS

- **Models**: Primary CLL samples, MEC-1, and U-RT1 (Richter's) cells.
- **LN mimic**: Co-culture on CD40L-expressing fibroblasts; NTL fibroblasts as controls.
- **Compounds**: NIK inhibitors (CW15337, Amgen16, B022), venetoclax (ABT-199).
- **Readouts**: NF-κB activity (p100→p52, nuclear NF-κB ELISA), anti-apoptotic protein levels (BCL-XL, MCL1, BCL2), gene transcription (qRT-PCR), cell cycle, CXCL12-driven migration. Drug response ± CD40L; venetoclax + NIK inhibitor synergy (Bliss model) in CLL and U-RT1 cells.

RESULTS

- **NIK inhibitors block non-canonical NF-κB signalling** (Fig. 1A–C)
  - ↓ nuclear p52 and RelB (ELISA)
  - ↓ p100→p52 processing (IB) in CLL ± CD40L
- **NIK inhibition causes G1 arrest and impairs migration** (Fig. 2A–C)
  - ↑ G1 phase accumulation in MEC-1 cells
  - ↓ CXCL12-driven migration
- **CD40L activates canonical & non-canonical NF-κB** (Fig. 3A–D)
  - ↑ nuclear RelA:p50 and p52:RelB
  - ↑ protein/transcription of BCL-XL, MCL1, BCL2A1 (but not BCL2)
- **NIK inhibition downregulates anti-apoptotic BCL2 family transcription and protein expression** (Fig. 4A–B)
  - ↓ BCL-XL, MCL1 proteins; ↓ BCL2A1, BCL2L1 transcription
  - Minimal effect on BCL2
- **NIK inhibitors show potent cytotoxicity in CLL** (Fig. 5)
  - LC_{50} values calculated for 3 NIK inhibitors ± CD40L
- **NIK inhibition reverses CD40L-induced venetoclax resistance** (Fig. 6A–C)
  - Restores venetoclax cytotoxicity in CLL co-cultures
- **NIK inhibitors synergise with venetoclax in Richter's U-RT1 cells** (Fig. 7)

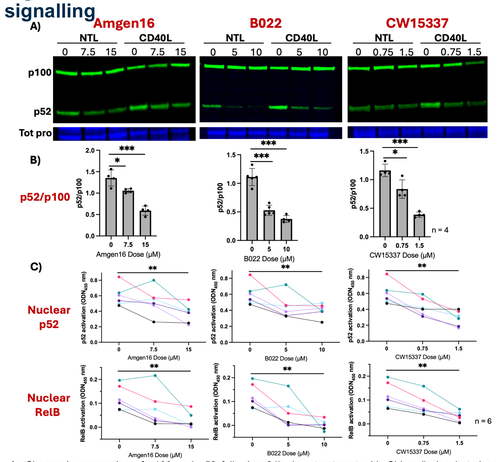
ACKNOWLEDGMENTS

brighton and sussex medical school

University Hospitals Sussex NHS Foundation Trust

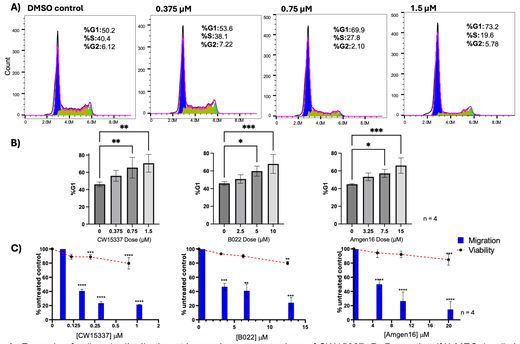
Thank you to all the CLL patients who generously donated their blood for our research

Figure 1. NIK inhibitors block non-canonical NF-κB signalling



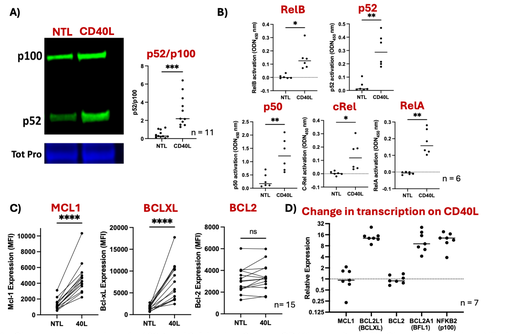
A: Change in expression of p100 and p52 following 24h drug treatment with CL cells incubated on non-transfected (NTL) or CD40L-expressing fibroblasts. Numbers at top of each lane are drug concentrations in μM. **B:** Summary of change in p52/p100 following drug treatments in CD40L-stimulated cells. **C:** Changes in nuclear p52 and RelB following treatment with the three NIK inhibitors in 6 CD40L-stimulated CLL samples. *** p < 0.001 ** p < 0.01 * p < 0.05.

Figure 2. NIK inhibition causes G1 arrest and impairs migration



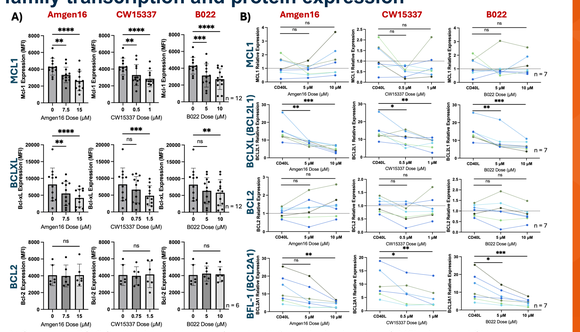
A: Example of cell cycle distribution at increasing concentrations of CW15337. **B:** Proportion (%) MEC-1 cells in G1 at increasing drug concentrations. **C:** Effect of NIK inhibitors on MEC-1 cell migration towards CXCL12. **** p < 0.0001 *** p < 0.001 ** p < 0.01 * p < 0.05.

Figure 3. CD40L activates canonical & non-canonical NF-κB



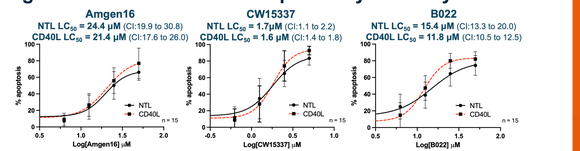
A: Change in expression of p100 to p52 following 24h on CD40L co-culture. **B:** Changes in nuclear NF-κB subunits following CD40L stimulation in 6 CLL patients. **C:** CD40L-induced changes in BCL2 family proteins (n = 15). **D:** Changes in transcription on CD40L (n = 7). **** p < 0.0001 ** p < 0.01 * p < 0.05 ns not significant.

Figure 4. NIK inhibition downregulates anti-apoptotic BCL2 family transcription and protein expression



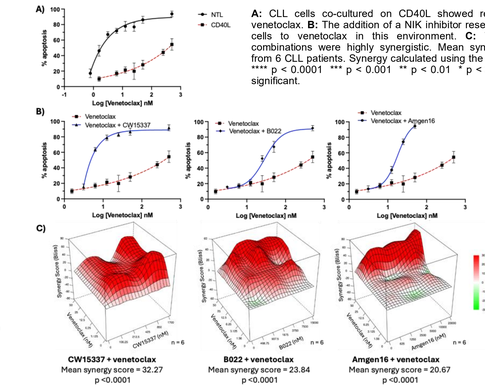
A: Changes in BCL2 family total protein levels following NIK inhibitor treatments in CD40L-stimulated CLL.
B: Transcriptional changes following NIK inhibitor treatments in 7 CLL patients. **** p < 0.0001 *** p < 0.001 ** p < 0.01 * p < 0.05 ns not significant.

Figure 5. NIK inhibitors show potent cytotoxicity in CLL



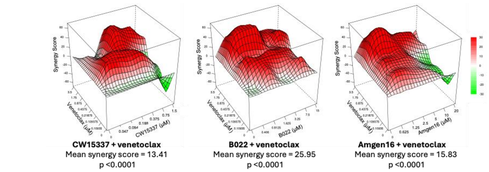
Dose responses to the 3 NIK inhibitors in 15 CLL patients following incubation on CD40L-transfected and non-transfected (NTL) fibroblasts. LC_{50} presented as mean values, 95% confidence interval in brackets.

Figure 6. NIK inhibition reverses CD40L-induced venetoclax resistance



A: CLL cells co-cultured on CD40L showed resistance to venetoclax. **B:** The addition of a NIK inhibitor resensitised CLL cells to venetoclax in this environment. **C:** These drug combinations were highly synergistic. Mean synergy scores from 6 CLL patients. Synergy calculated using the Bliss model. **** p < 0.0001 *** p < 0.001 ** p < 0.01 * p < 0.05 ns not significant.

Figure 7. NIK inhibitors synergise with venetoclax in Richter's U-RT1 cells



Drug combinations were highly synergistic in the U-RT1 Richter's cell line (n = 3). Synergy scores calculated using the Bliss model.

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