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

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Background

Aberrant NF-κB signalling appears to play a key role in the pathogenesis of CLL, including several recurrent genetic mutations in NF-κB-activating genes[1-3]. 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]. Inhibiting NF-κB signalling is therefore a potential therapeutic approach. However, given the key role of canonical NF-κB signalling on multiple cellular processes, direct pharmacological targeting of NF-κB (e.g. through IKKβ inhibitors) has so far failed due to associated toxicities.

We investigated the potential for targeting NF-κB inducing kinase (NIK), the central kinase regulating non-canonical NF-κB signalling, in CLL. We evaluated three NIK inhibitors, CW15337, Amgen16 and BO22, in the MEC-1 cell line and in primary CLL cells. Given that NIK expression levels are very low under physiological conditions, and constitutive activation is usually only present in pathological contexts[6], NIK may represent a tumour-selective therapeutic target. We hypothesised that NIK inhibition may represent a promising strategy in the treatment of CLL by targeting CLL cells in the lymphoid tissue environment where they are particularly reliant on non-canonical NF-κB signalling.

Results

All three NIK inhibitors caused a dose-dependent G1 arrest in the cell cycle in the MEC-1 cell line. Mean proportion of MEC-1 cells in G1 with no drug = 48%, whilst at the highest drug concentration = 69%, 68% and 66% for CW15337, Amgen16 and BO22, respectively. All three agents were cytotoxic in both in the MEC-1 cell line (LC_{50} = 1.63μM, 30μM and 19.3μM for CW15337, Amgen16 and BO22, respectively) and in primary samples (LC_{50} = 2.05μM, 15.23μM and 14.4μM for CW15337, Amgen16 and BO22, respectively). CW15337 was the most potent drug in both MEC-1 and primary samples. Nuclear expression of the non-

canonical NF-κB subunit, p52, was correlated with sensitivity to CW15337 (p = 0.01; r^2 = 0.39).

When co-cultured with CD40L-expressing fibroblasts, as a model of the lymphoid niche, NIK inhibitors were able to overcome this cytoprotective environment and cause cell death. This was seen with both MEC-1 and primary samples. Furthermore, all three agents significantly inhibited MEC-1 cell migration against a chemokine gradient ($p < 0.001$) in a dose-dependent manner. Co-culture on CD40L-expressing cells induced both canonical and non-canonical subunit expression in nuclear extracts, which promoted *in vitro* resistance against fludarabine and ABT-199 (venetoclax) but not CW15337. Furthermore, the combination of CW15337 with fludarabine or ABT-199 showed cytotoxic synergy. Mechanistically, CW15337 caused the selective inhibition of non-canonical NF-κB subunits and the transcriptional repression of BCL2L1, BCL2A1 and MCL1 anti-apoptotic gene transcription. Taken together, these data suggest that the NIK inhibitor, CW15337, exerts its effects via suppression of the non-canonical NF-κB signalling pathway, and reverses venetoclax resistance in the context of CD40L stimulation through repression of BCL2 family proteins.

In conclusion, 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. The inhibition of MEC-1 migration suggests that NIK inhibitors could block re-entry of CLL cells to the lymph node microenvironment. Furthermore, they may be effective in targeting residual disease in the lymph node by suppressing the non-canonical NF-κB signalling pathway, which resensitises CLL cells to venetoclax.

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