

Abstract Submission

05. Chronic lymphocytic leukemia and related disorders - Biology & Translational Research

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TLR9 SIGNALLING IS A POTENTIAL TUMOUR ESCAPE MECHANISM FOLLOWING BTKI THERAPY FOR THE TREATMENT OF CHRONIC LYMPHOCYTIC LEUKAEMIA.

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Background: CLL cell trafficking is fundamental to CLL progression and an important focus when identifying novel therapeutic targets. Toll-like receptor 9 (TLR9) is an intracellular pattern recognition receptor, and a potential contributor to CLL cell chemotaxis and tumour maintenance. TLR9 recognises **unmethylated CpG motifs** within bacterial/viral/mitochondrial DNA, and TLR9 activation promotes an NFκB and STAT3-driven activation/migratory phenotype in primary CLL cells. We have previously shown cell-free unmethylated DNA to be up to 28-fold higher in CLL patient plasma, relative to healthy controls.

Aims: To investigate TLR9 activation as a potential mechanism of resistance to current B-cell receptor (BCR)-targeted therapeutics.

Methods: Migration assays were performed using primary CLL cells stimulated +/- 1µM ODN 2006 (TLR9 agonist), +/- 1µM ibrutinib (BTK-inhibitor), 2µM ODN INH-18 (TLR9 antagonist) or +/- 2.5-10µM CW15337 (NIK inhibitor). CLL cells migrated through 5µM pore membranes towards a CXCL12-gradient.

Results: TLR9 activation induced a dichotomous migratory response in CLL patient samples. Following stimulation with ODN 2006, 25/42 (60%) patient samples showed an **increase** in CLL cell migration ('Responders') and 17/42 (40%) showed either **no change** or a **decrease** in CLL cell migration ('Non/Reverse Responders'). Interestingly, IGHV-mutated (M-CLL) samples were almost exclusively 'Responders' (i.e., 14/17 [82%]) and IGHV-unmutated (U-CLL) samples were equally likely to be 'Responders' or 'Non/Reverse Responders' (i.e., 11/25 [44%] vs 14/25 [56%] respectively). Whilst there was no difference in the expression levels of TLR9 in M-CLL vs U-CLL cells, U-CLL samples expressed significantly higher basal levels of CD69 (B-cell activation marker), and their TLR9-induced migratory response **negatively** correlated with their basal migration. We therefore hypothesised that U-CLL 'Non/Reverse Responders' may have reached maximal stimulatory capacity through BCR-signalling alone, rendering them unresponsive to further activation. To test this hypothesis, we simulated BCR and TLR9 stimulation in M-CLL and U-CLL cells using mathematical modelling. Our simulations showed M-CLL cells (with low basal BCR-activation) to induce NFκB signalling in response to TLR9 activation and U-CLL cells (with high basal BCR-activation) to be unresponsive. Importantly, when simulating treatment with a Bruton's Tyrosine Kinase inhibitor (BTKi), U-CLL cells **gained sensitivity** to TLR9 activation. These results have since been verified *in vitro* using BTKi-treated patient samples, where we found a subset of U-CLL 'Non/Reverse Responders' to become 'Sensitised' to TLR9 activation in the presence of ibrutinib. In the 'Responder' subgroup, ODN INH-18 (TLR9 antagonist) and ibrutinib inhibited CLL cell migration synergistically. Together, these data implicate TLR9 signalling as a tumour escape mechanism following BTKi therapy. Since both the BCR and TLR9 signalling pathways culminate in NFκB activation, we are currently investigating components of NFκB signalling as potential novel therapeutic targets. Preliminary data using the NIK selective inhibitor 'CW15337' shows CLL cell migration to be inhibited with an IC50 of <1µM.

Summary/Conclusion: CLL patient plasma contains high levels of TLR9 ligand (unmethylated DNA), which may promote CLL cell trafficking and BTKi resistance in select subgroups. We believe that targeting downstream NFκB signalling has

the potential to inhibit both BCR and TLR9 signalling, and to increase treatment efficacy in TLR9 'Responder' and BTKi 'Sensitised' patients.

Keywords: Cell migration, Chronic lymphocytic leukemia, Resistance, Targeted therapy