The SYK/JAK Inhibitor Cerdulatinib (PRT062070) Shows Promising Preclinical Activity in Chronic Lymphocytic Leukaemia by Antagonising B Cell Receptor and Microenvironmental Signalling

Matthew D Blunt, Stefan Koehrer#, Rachel Dobson, Marta Larrayoz, Sarah Wilmore, Alice Hayman, Jack Parnell, Lindsay Smith, Andrew Davies, Peter Johnson, Pamela Conley*, Anjali Pandey*, Jon C Strefford, Francesco Forconi, Freda Stevenson, Graham Packham, Greg Coffey*, Jan Burger# and Andrew J Steele

Cancer Sciences, Southampton University, Southampton, SO16 6YD, UK

*Portola Pharmaceuticals Inc, South San Francisco, CA

Department of Leukemia, The University of Texas MD Anderson Cancer Center, USA

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Introduction

B cell receptor (BCR) mediated signalling is required for chronic lymphocytic leukaemia (CLL) pathogenesis and drugs which target kinases within the BCR signalling complex are revolutionising the treatment of this disease. Recently approved agents for relapsed/ refractory CLL include ibrutinib (targets BTK) and idelalisib (targets PI3Kδ), however these compounds only supress the disease and are not curative. Importantly, a proportion of CLL patients are now developing resistance to these new agents [1], either via mutations in BTK or downstream signalling proteins, or due to as yet unknown mechanisms. A recent publication showed that inhibition of SYK could overcome resistance to ibrutinib [2], identifying SYK inhibition as a promising strategy to treat these patients. An additional resistance mechanism to chemotherapy in CLL is via IL-4 mediated JAK/STAT signalling, which has been shown to protect against cytotoxic agents [3]. Moreover, we have recently shown that IL-4 potentiates BCR mediated signalling and acts as a resistance mechanism to idelalisib and ibrutinib [4]. Cerdulatinib (produced by Portola Pharmaceuticals) is a novel dual SYK/JAK inhibitor which has the potential to overcome the resistance mechanisms described previously and is in phase I clinical trials for several leukaemia\lymphomas including CLL following relapse whilst on ibrutinib.

Aim

To investigate the effect of the dual SYK and JAK inhibitor, Cerdulatinib, on primary human CLL cells.

Methods

Fifty three primary CLL samples were treated with cerdulatinib in the presence or absence of IL-4/CD40L or anti-IgM. Apoptosis was assessed using propidium iodide/ Annexin V staining by flow cytometry and B cell receptor and cytokine receptor induced signalling was assessed by immunoblotting and flow cytometry.

Results

We have demonstrated by immunoblotting that CLL cells treated with soluble or bead immobilised (BI) anti-IgM (Figure 1A-B) or anti-IgD (Figure 1C-D) induced phosphorylation (p) of pAKT^{S473}, S6K^{T389}, pS6 ribosomal subunit$^{S235/236}$, pERK$^{T202/Y204}$ and pAKT^{T308} (BI anti-Ig only). These BCR-induced signals were inhibited by cerdulatinib in a dose dependent manner and most strongly between 0.3-1μM. These results are consistent and comparable to idelalisib and ibrutinib used here as controls to inhibit BCR signalling (Figure 1A-B, 1C-D). Cerdulatinib also strongly inhibited BCR-induced calcium flux at 1μM using anti-IgM (Figure 1E) and anti-IgD (Figure 1F).

CLL cells are thought to secrete chemokines such as CCL3 and CCL4 in order to recruit T cells and monocytes into the lymph node. CLL cells treated with BI anti-IgM (Figure 2A-B) secreted CCL3 and CCL, and at concentrations achievable in patients, cerdulatinib markedly reduced chemokine production, indicating that cerdulatinib may affect T cell/ monocyte recruitment by CLL cells into the lymph node.

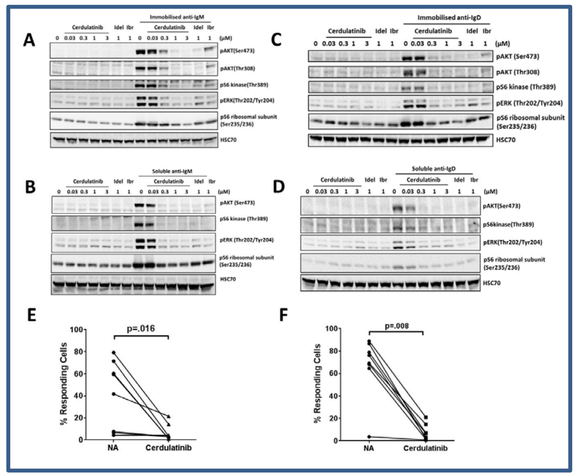

Figure 1. Cerdulatinib inhibits BCR mediated signalling

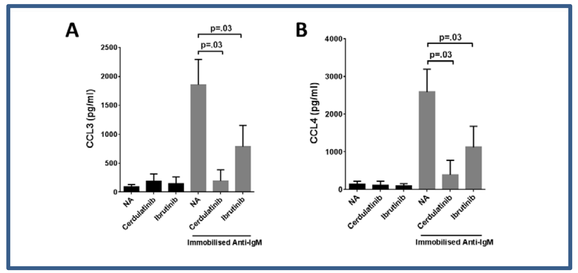

Figure 2. Cerdulatinib supresses chemokine secretion

IL-4 signals via the JAK/STAT-6 pathway in CLL cells and has been shown to be important in mediating protection from chemotherapy. Treatment of CLL cells with cerdulatinib abrogated IL-4 induced STAT6 phosphorylation (Figure 3A and B). In contrast, the Syk inhibitors fostamatinib and PRT062607 were unable to inhibit IL-4 induced STAT6 phosphorylation (Figure 3C). In addition, cerdulatinib inhibited IL-4 increased surface IgM expression (Figure 3D) and inhibited IL-4 decreased CXCR4 expression (Figure 3E) after 24 hours in the presence of QVD.

CLL samples from 24 patients were treated with the dual Syk/JAK inhibitor cerdulatinib for 24, 48 and 72 hours and viability assessed by flow cytometry. Cerdulatinib induced apoptosis in a concentration and time dependent manner (Figure 4A). Treatment of CLL cells with cerdulatinib was found to induce cleavage/activation of the pro-apoptotic caspase 3 protein and also increased levels of the 85kDa PARP sub-fragment; a marker of apoptosis (Figure 4B). Cerdulatinib induced apoptosis was inhibited by co-treatment with the caspase inhibitor ZVAD (Figure 4B-C), indicating that cerdulatinib induced apoptosis of CLL cells occurs via a caspase dependent mechanism.

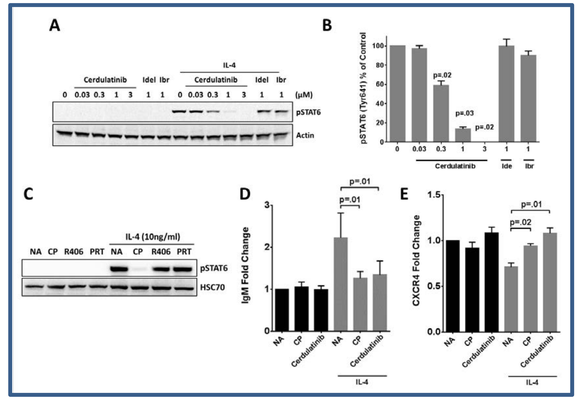

Figure 3. Cerdulatinib inhibits IL-4 induced signalling and abrogates IL-4 increased IgM expression

We subsequently assessed the ability of cerdulatinib to induce apoptosis between samples with different prognostic markers at 48h. IGHV unmutated CLL samples or samples expressing higher levels CD49d+ or ZAP70+, were more sensitive to drug-induced killing (Figure 4D-F). Interestingly, cerdulatinib also induced greater levels of cell death in samples expressing higher levels of sIgM (MFI >50) (Figure 4G).

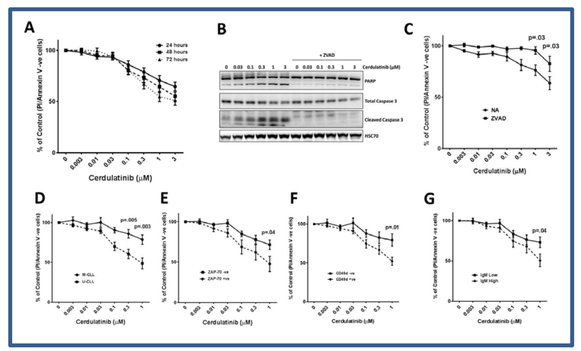

Figure 4. Cerdulatinib induces apoptosis in CLL cells in a time and concentration dependent manner

We used BI anti-IgM or IL-4 and CD40L to mimic signals from the lymph node environment in vitro. Cerdulatinib was able to overcome BCR and IL-4/CD40L promoted survival of CLL cells (Figure 5A-B).

BI anti-IgM and IL-4/CD40L treatment induced expression of anti-apoptotic proteins MCL-1 and BCLX$_L$, shown by immunoblotting in a representative sample (Figure 5C-D) in line with previously published data. Simultaneous inhibition of Syk and JAK by cerdulatinib decreased MCL-1 and BCL-X$_L$ protein expression but had no discernible effect on Bcl-2 protein expression.

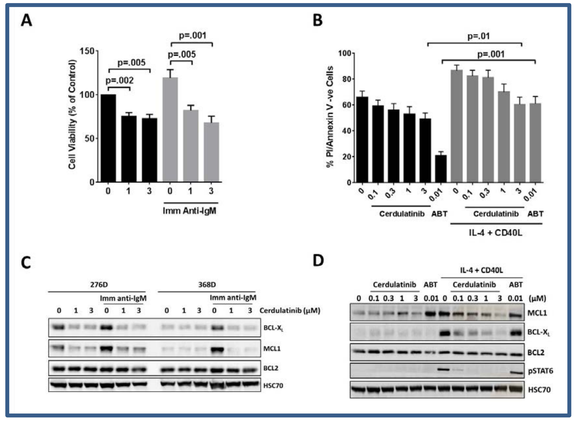

Figure 5. Cerdulatinib induces apoptosis irrespective of microenvironmental support

Since we showed that cerdulatinib could inhibit MCL-1 and BCL-X$_L$ expression induced by IL-4/CD40L and anti-IgM ligation, but not BCL-2, we investigated whether cerdulatinib would synergise with venetoclax in vitro to augment CLL cell killing. CLL cells were stimulated with IL-4/CD40L for 6 hours then treated with cerdulatinib or ABT-199, alone or in combination. In the presence of CD40L/IL-4, the combination of cerdulatinib with ABT-199 further enhanced apoptosis, indicating synergistic effects (Figure 6A). Synergistic interactions between cerdulatinib and ABT-199 were evaluated as indicated (Figure 6B). Points below the diagonal line represent synergistic interactions, above the line are additive.

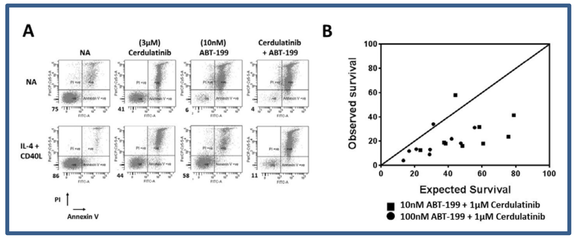

Figure 6. Cerdulatinib is synergistic with ABT-199 in the presence of IL-4/CD40L

Conclusions

Cerdulatinib is a novel dual SYK/JAK inhibitor which is able to overcome BCR- and microenvironmental- mediated signalling and is currently in clinical trials for CLL.

References

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