Targeting WM and MM with BTK Inhibitors

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Specific expression of Bruton's tyrosine kinase (Btk) in osteoclasts (OC), but not osteoblasts (OB), suggests its role in osteoclastogenesis. Although Btk is critical in B cell maturation and myeloid function, it has not been characterized in plasma cell malignancies including multiple myeloma (MM) and Waldenström Macroglobulinemia (WM). We recently investigate effects of PCI-32765, an oral, potent, and selective Btk inhibitor with promising clinical activity in B-cell malignancies, on OC differentiation and function within MM bone marrow (BM) microenvironment, as well as on MM and WM cancer cells. In CD14+ OC precursor cells, PCI-32765 abrogated RANKL/M-CSF-induced Btk activation and downstream PLCγ2, resulting in decreased number of multinucleated OC (>3 nuclei) by tartrate-resistant acid phosphatase (TRAP) staining and inhibition of TRAP5b (ED_{50} = 17 nM), a specific mature OC marker. Importantly, Btk inhibition impaired bone resorption activity, as evidenced by diminished pit formation on dentine slices. Lack of effect of Dexamethasone on osteoclastic activity was overcome by combination of Dexamethasone with PCI-32765. PCI-32765 potently downregulates cytokine and chemokine secretion from OC cultures, i.e., MIP1α, MIP1β, IL-8, TGFβ1, RANTES, APRIL, SDF-1, and activin A (ED_{50} = 0.1-0.48 nM). It significantly decreased IL-6, SDF-1, MIP1α, MIP1β, and M-CSF in 2-week cultures of CD138-negative cells from active MM patients, associated with decreased TRAP staining. In MM cells, immunoblotting analysis showed high Btk expression in the majority of CD138+ myeloma cells from patients, correlating gene expression data. In WM cells, microarray analysis demonstrated increased expression of Btk and its downstream signaling components in WM cells than in CD19+ normal BM cells ($p<0.001$). Btk knockdown by shBtk lentiviruses completely downregulated Btk in MM1R cells, leading to significant decreased adhesion to BMSCs and viability, as well as caspase 3/7 activation. Thus, Btk is required for MM cell growth and survival. Significantly, PCI-32765 blocked SDF-1-induced adhesion and migration of MM and WM cells via blockade of Btk signaling cascade. MM patient cells have detectable pBtk; conversely, PCI-32765 abolished phosphorylation of Btk and its downstream NFκB and STAT3 activation. Quantigene analysis further showed PCI-32765-inhibited MIP1α and IL6R mRNA in MM cells and many NFκB-targeted transcripts in OC-lineages. PCI-32765 mitigated MM cell growth and survival triggered by IL-6 and coculture with BM stromal cells (BMSCs) or OCs. It blocked WM cell proliferation and induced apoptosis. Furthermore, myeloma stem-like cells from MM patients expressed Btk and PCI-32765 (10-100 nM) specifically blocked their abilities to form colonies in methylcellulose (n=5) and viability protected by BMSCs. In contrast, no toxicity was observed in Btk-negative BMSCs and OB. Oral administration of PCI-32765 (12 mg/kg) in mice significantly suppressed MM cell growth ($p< 0.03$) and MM cell-induced osteolysis, resulting in increased bone formation activity ($p<0.01$) on implanted human bone chips in a humanized myeloma (SCID-hu) model. Together, these results strongly support clinical trials of targeting Btk by PCI-32765 in the BM microenvironment to improve patient outcome in MM and WM.