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# 3735 Combination of Imatinib with CXCR4 Antagonist BKT140 Overcomes the Protective Effect of Stroma and Targets CML in Vitro and in Vivo

**Program:** Oral and Poster Abstracts
**Session:** 631. Chronic Myeloid Leukemia - Biology and Pathophysiology, excluding Therapy: Poster III

**Monday, December 10, 2012, 6:00 PM-8:00 PM**
Hall B1-B2, Level 1, Building B (Georgia World Congress Center)

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**Background**: Chronic myeloid leukemia (CML) is myeloproliferative disease of hematopoietic stem cells that is driven by the constitutively active oncogenic tyrosine kinase BCR-ABL. Inhibition of BCR-ABL activity by tyrosine kinase inhibitors (TKIs) such as imatinib mesylate, revolutionized the treatment of CML and remains a major therapeutic strategy for CML patients. However, existence of quiescent leukemic stem cells (LSCs) that are resistant to TKIs and may be responsible for CML resistance and recurrence continues to pose challenges for CML cure. Thus, novel therapies that eradicate LSCs are in need. The bone marrow (BM) microenvironment is believed to have a role in protecting LSCs and CML cells from TKIs-induced apoptosis. Chemokine receptor CXCR4 and its ligand CXCL12 have a key role in the trafficking and retention of normal and leukemic SCs in the BM niche. Although, CXCR4 signaling is impaired in CML cells, it can be elevated by the imatinib treatment and may induce stroma-promoted chemo-resistance. Therefore, CXCR4 inhibition may antagonize the survival and spread of CML and LSC cells and may restore their sensitivity to TKIs in the BM microenvironment context.

**Methods**: In order to study the role of CXCR4 in CML progression, we over-expressed CXCR4 in K562 cells using a lentiviral vector and generated a cell line with high and stable CXCR4 expression.

**Results**: CXCL12 stimulation did not significantly affect the migration of K562 cells in vitro. In contrast, elevated CXCR4 increased the in vitro survival and proliferation of K562 cells in response to CXCL12 treatment, suggesting an important role of CXCR4/CXCL12 axis in CML growth. In accordance, we found that in vitro treatment with CXCR4 antagonist BKT140 (8 μM) directly inhibited the cell growth by 40-60% and induced cell death of three CML cell lines tested (K562, KCL22 and LAMA84). Combination of BKT140 (8 μM) with IC50 concentrations of imatinib (0.2-0.3 μM) significantly increased the anti-CML apoptotic effect in achieving 90-95% reduction in cell viability in vitro ($p<0.01$). Furthermore, to investigate the interaction between CML cells and BM microenvironment, we evaluated the effect of BM stromal cells on CML growth and response to imatinib treatment in vitro in co-culture experiments. We found that murine BM stromal cells 14F1.1 and MS5, as well as primary human BM stromal cells (BMSCs) significantly increased the proliferation of CML cell lines K562 and LAMA84 ($p<0.01$) and protected them from imatinib-induced apoptosis. We next wanted to assess whether BCL6 is involved in the stroma-mediated TKI resistance as it was recently reported that CML cells up-regulate proto-oncogene BCL6 in response to TKI treatment. Indeed, the BM stromal cells remarkably elevated BCL6 mRNA expression in the CML cells in response to imatinib treatment, suggesting the possible role of BCL6 in stroma-mediated TKI resistance. Addition of BKT140 to the co-culture system reversed the protective effect of the stroma, re-sensitizing CML cells to imatinib and effectively promoting CML cell death. Moreover, combination of imatinib with BKT140 decreased BCL6 mRNA levels in CML cells co-cultured with BMSCS. To further explore the role of CXCR4 inhibition in vivo, we established a xenograft bioluminescent model of CML. Luciferase-transduced K562 cells were intra-peritoneally injected into NOD/SCID mice and tumor burden was quantified using bioluminescence imaging. Both imatinib (0.6 mg/injection) and BKT140 (100 μg/injection), administered intra-peritoneally, effectively reduced disease burden. However, combined treatment demonstrated increased potency in vivo.

**Conclusions**: Taken together, our data indicate the importance of CXCR4/CXCL12 axis in CML growth and CML-BM stroma interaction. CXCR4 inhibition with BKT140 antagonist efficiently synergized with imatinib both in vitro as well as in vivo, over-coming the protective effect of the BM stroma. These results provide the rational basis for CXCR4-targeted therapy in combination with TKI to override drug resistance and suppress residual disease.

**Disclosures:** No relevant conflicts of interest to declare.

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