The CXCR4 antagonist BL-8040 efficiently induces apoptosis and inhibits the survival of AML cells

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Background

The chemokine CXCL12 and its receptor CXCR4 are key players in mediating the interactions between the bone marrow (BM) microenvironment and Acute Myeloid Leukemia (AML) cells. CXCL12, which is constitutively secreted from the BM stroma and AML cells, is critical for the survival and retention of AML cells within the BM. CXCR4 expression is associated with poor prognosis in AML patients with or without a mutated FLT3 gene. Of note, mutation of FLT3, which is found in approximately 30% of AML patients, is associated with increased CXCR4 expression and activated CXCR4 signaling. Antagonists to CXCR4 inhibit migration of AML cells, induce mobilization of AML cells into the circulation and enhance anti-leukemic effects of chemotherapeutic drugs. The hypothesis that CXCL12/CXCR4 interactions contribute to the resistance of AML cells to signal transduction inhibitors, and chemotherapy-induced apoptosis, is currently being tested in a series of clinical trials in humans.

In-vitro Aims and Methods

The effect of the CXCR4 antagonist BL-8040 and AMD3100 (Mozobil) as single agents or in combination with ARA-C (Cytarabine) or the FLT3-inhibitor AC220 on the survival and proliferation of AML cells in-vitro was examined. Cells: the human AML cell lines HL60 (FLT3-WT) and MV4-11 (FLT3-ITD) and human primary AML cells from patients with or without FLT3-ITD mutations were used. AML cells were incubated in-vitro for 48 hr in the presence of BL-8040 (2μM), AMD3100 (20 μM), ARA-C (50 μM) or AC220 (5 μM) or their combination. The % of apoptotic cells and the level of viable cells were evaluated by FACS using PI.

Results

• In-vitro, treatment of human AML cells either FLT3-WT or FLT3-ITD, with BL-8040 unlike treatment with AMD3100 directly inhibited cell growth and increased cell death.
• A combination of BL-8040 with ARA-C further increased the apoptotic effect of these agents achieving a 96% reduction in cell viability and inducing cell death of AML cells more robustly in FLT3-ITD cells.
• A combination of BL-8040 with AC220 resulted in an additive inhibitory effect on cell growth in the FLT3-ITD cells but not in the FLT3-WT cells.

In-vivo Aims and Methods

In order to study the in-vivo effect of BL-8040, a mouse model of human AML cells was established in NSG mice.

NSG mice were transplanted with the human AML-FLT3-ITD MV4-11 cell line. 24 hr before cell transplantation (day 0), mice were irradiated with 200Gy, and AML cells were intravenously (i.v) injected (10x10⁶ cells/mouse) into the mouse (day 1). Engraftment of AML cells was allowed for 25 days after transplantation. BL-8040 was sc injected daily for 7 days at a dose of 400 mg/mouse (days 25-31). 24 hr after the last injection of BL-8040, mice were sacrificed and the blood, BM and spleen were collected for analysis. The number of human AML cells was assessed by FACS using anti-human CD45 antibody, and the number of apoptotic cells was assessed by Annexin/PI staining.

• BL-8040 reduced the number of alive human AML cells and induced their apoptosis in the blood of transplanted mice.
• BL-8040 reduced the total number and percentage of alive AML cells and induced their apoptosis within the BM.
• BL-8040 prevented the accumulation of AML cells in the spleen and induced their apoptosis.

Conclusions

• The CXCR4 antagonist BL-8040 rapidly and efficiently induces cell death of AML cells both in-vitro and in-vivo.
• The results suggest potential therapeutic advantage of BL-8040 both FLT3-mutated and wild type AML patients by targeting both AML and stromal BM chorage in the BM and survival.
• These results provide the rational basis for BL-8040 therapy in combination with ARA-C and the FLT3 inhibitor AC220.