BL-8040: BEST-IN-CLASS CXCR4 ANTAGONIST FOR TREATMENT OF ONCOLOGICAL MALIGNANCIES

Overview and Mechanism of Action

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BL-8040: Novel CXCR4 Antagonist For Hematological Cancers

• **Indications:**
  – AML & other hematological cancers
  – Stem cell mobilization for autologous and allogeneic transplantations

• **Mode of Action:** CXCR4 antagonism

• **Status:** Phase II

• **Product Highlights:**
  – Short synthetic peptide with high affinity to the CXCR4 receptor
  – Induces apoptosis in cancer cells overexpressing CXCR4
  – Sensitizes cancer cells to chemo- and bio-based anti-cancer therapy
  – Mobilizes stem cells and cancer cells from bone marrow to the peripheral blood
  – Safety profile demonstrated in phase I/II study in multiple myeloma patients

• **Orphan drug designation was granted by the FDA for:**
  – AML treatment
CXCR4 Involvement in Tumor Initiation, Progression and Metastasis

- CXCR4 is overexpressed in more than 70% of human tumors
- CXCR4 overexpression often correlates with disease severity
- CXCR4 is the bone marrow retention factor for hematopoietic stem cells (HSC)
- CXCR4 mediates the adhesion of cancer cells to stromal cells which is critical for their survival
- CXCR4 directly stimulates tumor outgrowth, invasion and formation of blood vessels
- CXCL12 / SDF1 is constitutively produced by bone marrow stromal cells and stromal fibroblasts within the tumor microenvironment
**BL-8040 Mechanism Of Action**

- BL-8040 binds CXCR4 with high affinity (1-2 nM)
- BL-8040 has long receptor occupancy (>24 hours) resulting in extended inhibition of CXCR4
- BL-8040 is an inverse agonist of CXCR4
- BL-8040 induces apoptosis of tumor cells that are dependent on CXCR4 for survival
- BL-8040 mobilizes tumor cells from their protective microenvironment, resulting in increased sensitivity to anti-cancer agents
BL-8040 Induces Apoptosis in Tumor Cells

- BL-8040 induces apoptosis in tumor cells which are dependent on CXCR4 for their survival.

- Binding of CXCL12 to CXCR4 promotes cell survival via several pathways:
  - Activation of the protein kinases PI3K, AKT, p38 and ERK1/2 – all implicated in tumor cell survival.
  - Increase of NFκB mediated transcription of cell survival-related genes.
  - Post-translational inactivation of the cell death machinery through inactivation of the pro-apoptotic protein BAD.

- In AML cells induction of apoptosis has been described following blockage of CXCR4 via altering levels of different BCL-2 family members.
BL-8040 is Effective in a Variety of Hematological Cancer Models

- **Leukemia** – BL-8040 inhibits tumor growth in mice leukemia models including ALL, AML & CML.

- **Lymphoma** – BL-8040 synergizes with Rituximab and Bendamustine to stimulate lymphoma cell death and inhibit tumor growth in-vivo.

- **Mobilization** – BL-8040 mobilizes stem cells from bone marrow

- **Thrombocytopenia** and recovery of the bone marrow after transplantation.

- **Multiple myeloma (MM)** – BL-8040 inhibits tumor development; treatment of well established tumors inhibits their growth and stimulates tumor necrosis in vivo; BL-8040 synergizes with Bortezomib to stimulate MM cell death in-vitro
BL-8040 vs. Mozobil

Common characteristics

• BL-8040 and Mozobil inhibit CXCR4 by blocking its binding to its cognate ligand
• BL-8040 and Mozobil mobilize hematopoietic stem cells from the bone marrow to the bloodstream, for autologous stem cells transplantation.

Differentiating characteristics

• BL-8040 binds CXCR4 with higher affinity: 1-2 nM vs. 84 nM
• BL-8040 binds extracellular domains in the CXCR4 receptor while Mozobil binds transmembrane regions
• Receptor occupancy (longer than 24 hr.) is much longer than Mozobil, suggesting extended inhibition of CXCR4
• BL-8040 directly induces apoptosis in tumor cells
The number of progenitor cells in the blood were determined by a colony-forming cell (CFC) assay, evaluating the number of mobilized CFCs following treatment.
BL-8040 has Direct Anti-Cancer Activity

BL-8040, in contrast to Mozobil, showed potent cytotoxicity against human leukemia and myeloma cells in vitro in a dose-dependent manner.

**Cell lines**
- NB4-Acute Promyelocytic Leukemia
- HL60-Acute Promyelocytic Leukemia

**Primary cells**
- MM-Multiple Myeloma
- AML-Acute Myelogenous Leukemia
BL-8040 Increases Cytarabine Cytotoxicity

- Effect of BL-8040 +/- Ara-C on human primary AML cells in comparison to Mozobil, in vitro
- Similar results were observed in:
  - FLT3-ITD AML cell line (MV4-11)
  - FLT3-WT AML cell line (HL60) and human primary FLT3-WT AML cells

* p<0.05 vs control
** p<0.05 vs Ara-C alone
Summary: BL-8040-based therapeutic for hematopoietic cancers

- BL-8040’s therapeutic and anti-cancer properties were demonstrated in a broad range of *in vitro* and *in vivo* studies.

- Results obtained with BL-8040 support multiple treatment modalities targeting cancers of hematopoietic origin:
  - BL-8040 mono-therapy promotes CXCR4-dependent selective tumor cell death
  - Blocking outgrowth of residual tumor stem cells
  - Blocking survival and angiogenic signals
  - Disruption of the adhesive tumor-stromal interaction that confers survival and drug-resistance signals
  - Blocking spreading following cancer disease treatment
  - Combination therapy potentiates cancer cell death and decreases metastasis
Indications for BL-8040

• BL-8040 currently has two lead indications:

  • **AML:**
    • Proof of concept for improved objective response (CR+CRi 46%) was established by Mozobil in phase I/II clinical study in combination with chemotherapy (MEC) in patients with r/r AML.
    • BL-8040 has shown significant efficacy in preclinical AML models.
    • BL-8040 currently investigated in relapsed/refractory AML patients as part of a phase II study.

  • **Stem cell mobilization in preparation for autologous bone-marrow transplant:**
    • BL-8040 was successfully tested in phase I/II study for stem cell mobilization in MM, showing significantly improved results compared to Mozobil historical data. Based on these results the FDA has granted an IND to the compound.
    • BL-8040 will be investigated as a single agent for SC mobilization.

• **BL-8040 has also shown significant efficacy in preclinical models of oncological indications associated with over-expression of CXCR4, including:**
  • Hematological tumors: AML, ALL, NHL, CML, MM
  • Solid tumors: NSCLC, melanoma, Neuroblastoma, Breast and Prostate cancers