

## STEM CELL MOBILIZATION AND TISSUE REPAIR AND REGENERATION

### BACKGROUND OF THE INVENTION

Adult tissue stem cells including hematopoietic stem cells (HSC) are unique and rare cells responsible for regeneration of different tissues: blood, muscles, hair follicles, skin keratinocytes, pancreatic and neural cells (Orlic et al. 2001, Krause et al. 2001). Stem cell transplantation has been tested in clinical trials for tissue regeneration with a various but low degree of success. This is due to the fact that even after enrichment with the most up-to-date approaches, the resulting HSC populations are not homogeneous. A large proportion of cells may still have no HSC potential, molecular heterogeneity within different HSC subsets and other uncertainties make cell transplantation less feasible for tissue regeneration than HSC mobilization.

Primitive stem cells exhibit differential motility responses to the chemokine, stromal derived factor-1 (SDF-1) and lysophospholipid mediator sphingosine-1-phosphate (S1P) recently found to play a critical role in stem cell mobilization. Mobilization of stem cells from bone marrow into peripheral blood prior to harvesting is currently being used in clinical settings of allogeneic stem cell transplantation instead of bone marrow. The most common mobilizing agent for clinical uses is granulocyte colony stimulating factor (G-CSF). Other molecules have mobilizing effect on bone marrow cells (AMD3100, IL8, GM-CSF and others) their effect is shown to be indirect and not stem cell specific.

G-CSF, for example, acts on mature bone marrow cells; cells release proteases cleaving the adhesion factors responsible for the retention of cells in bone marrow. AMD3100, the CXCR4 inhibitor, approved recently for stem cell mobilization induces a more specific mobilization of cells into the circulation than G-CSF via disruption of the CXCR4-SDF1 interaction of bone marrow cells with their microenvironment; not only stem cells, but their immature progenitors and even malignant cells in Multiple Myeloma and Acute Promyelocytic leukemia express CXCR4 and therefore migrate into peripheral blood. (Kareem, et al. 2009).

Thus, all current drugs affect multiple cell populations, releasing into circulation high numbers of cells and causing changes in the bone marrow microenvironment. Therefore, these approaches cannot be used for multiple rounds of stem cell mobilization for tissue regeneration. Furthermore, both G-CSF and AMD3100, while mobilizing cells that can promote tissue repair, can also impair homing of mobilized stem and progenitor cells to sites of tissue damage. AMD3100 by blocking CXCR4 (Dai et al., 2010), the receptor for SDF-1, a primary chemotactic factor released by injured tissues, and G-CSF by cleaving CXCR4 (Honold, et al., 2006).

Under homeostatic conditions many physiological mechanisms including stem cell mobilization are found to be controlled by circadian oscillations; maximal mobilization of HSC into blood stream was found in mice at 5 hr after the onset of light with a reversed circadian HSC mobilization time (early night) demonstrated for human (Lucas, et al. 2008).

A stem-cell-specific mechanism increasing physiological level of cell mobilization is required for tissue regeneration and a drug that can be applied for multiple rounds of mobilization i.e. repeatedly causing stem cell egress from bone marrow without side effects and without impairing CXCR4-mediated homing of mobilized cells to damaged tissues is needed.

## SUMMARY OF THE INVENTION

This invention provides an oligopeptide having the sequence Phe-Xaa-His-Phe-Asp-Leu-Ser-His-Gly-Ser-Ala-Gln-Val-Ser-Asp-Lys-Pro (SEQ ID NO: 1), wherein Xaa is Pro or Ala. This invention provides a method of mobilizing stem cells from bone marrow of a subject, comprising administering to the subject an amount of (SEQ ID NO: 1) effective to mobilize the stem cells. This method is useful for promoting bodily tissue regeneration in a patient in need of tissue regeneration treatment. Alternatively, the mobilized stem cells can be collected for transplant. It behaves as bidirectional regulator of hematopoietic cell growth, the effects is being dependent on both differentiation status and the presence of serum and some cytokines like SCF, IL3 and FGF among others. In contrast to other growth suppressor molecules such as macrophage inflammatory protein-1 $\alpha$  (MIP-1 $\alpha$ ) or TGF- $\beta$ 1 the growth-inhibitory effects of the peptide is not apparent throughout the whole stem cell hierarchy, but only on the very primitive long term repopulating cells (LTRCs) engrafting cobblestone forming cells (CAFC), SCID repopulating cells (SRC) and colony forming cells (CFU-GEMM). The invention also features a method of modulating apoptosis in a cell able to form colony containing all types of hematopoietic cells.

### BRIEF DESCRIPTION OF FIGURES

FIG. 1. Number of early HSC-CFU-GEMM and their progenitors CFU-GM 1 hr after subcutaneous injection of Compound A.

FIG. 2. Mobilization of CFU-GM and CFU-GEMM into peripheral blood 1 hr after single subcutaneous injection of Compound A.

FIG. 3. Mobilization of CFU-GEMM and CFU-GM after multiple subcutaneous injections of Compound A.

FIG. 4. Mobilization of CFU-GEMM and CFU-GM into peripheral blood after multiple subcutaneous injections of Compound A (dose response).

FIG. 5. S1P gradient regulates HSC mobilization and its disruption abrogates Compound A effect on CFU-GEMM egress into peripheral blood.

FIG. 6. Presence of human recombinant bFGF in Matrigel plug induces angiogenesis.

FIG. 7. Angiogenesis in Matrigel plugs after subcutaneous injections of peptides.

FIG. 8. Compound A effect on angiogenesis in db/db mice with diabetes in Matrigel Plugs.

FIG. 9. Mobilization of CFU-GEMM and CFU-GM into peripheral blood after multiple subcutaneous injections of Compound A in db/db mice with high glucose level

FIG. 10. TNF production by THP-1 cells in response to 20 ng/ml LPS

FIG. 11. Compound A (10 nM) exerted a similar inhibitory effect on IL-8 production

FIG. 12. Colony-forming units (CFU-GEMM) per 200 EML-CI cells plated.

FIG. 13. Caspase-3 activity measured in relative units AFC in EML cell lysates after 3 hr of HS and SCF deprivation.

FIG. 14. CFU-GEMM number per 200 EML cells plated after 3 hr of serum and SCF deprivation; effect of Compound A and S1P3 receptor antagonist Cay 10444.

FIG. 15. Compound A effect decreasing Caspase-3 activity during serum starvation is abrogated by Cay 10444-S1P3 receptor antagonist.

FIG. 16. HPLC histogram of Compound C and Compound A spiked in the medium.