COMBINATION OF PROPRANOLOL AND A NOVEL CXCR4 ANTAGONIST BURIXAFOR (GPC-100) FOR ENHANCED HEMATOPOIETIC CELL MOBILIZATION

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INTRODUCTION

The chemokine receptor CXCR4, expressed by hematopoietic stem cells (HSC), is a key regulator of their retention in bone marrow. Blockade of CXCL12/CXCR4 signaling induces mobilization of hematopoietic cells including HSCs to peripheral blood (PB).

CXCR4, a G protein-coupled receptor (GPCR) forms heteromers with other GPCRs such as the adrenergic beta-2 receptor (β_2AR, gene: ADRB2), also expressed by HSCs. Previous studies have shown that β_2AR activation enhances CXCR4-mediated retention of lymphocytes in lymph nodes, likely due to the CXCR4- β_2AR heteromer formation[1]. However, role of these heteromers or their cross-talk was not investigated in HSC mobilization for utility in autologous stem cell transplant (ASCT).

In multiple myeloma (MM) patients undergoing ASCT, beta-adrenergic blocker propranolol upregulated CD34$^+$ HSC-like gene signature, which leads to enhanced engraftment[2]. Propranolol was also shown to restore the normal HSC differentiation pathways in MM patient bone marrow samples[3]. Therefore, we investigated the functional cross-talk between CXCR4 and β_2AR, and determined if combination blockade of these receptors by propranolol and the CXCR4 antagonist GPC-100 (Burixafor) enhances HSC mobilization.

Current standards of care for HSC mobilization include G-CSF alone or combined with the CXCR4 antagonist AMD3100. However, insufficient mobilization in up to 35% patients and thus, repeated mobilization attempts pose a major challenge[4]. Treatment related toxicities, especially with G-CSF, have also been reported. This puts patients at the risk of losing survival benefits that ASCT shows over chemotherapy in MM.

GPC-100 has shown rapid and adequate HSC mobilization in MM patients when combined with G-CSF in a phase II study[5]. Here, we demonstrate enhanced in vivo mobilization by GPC-100 when combined with propranolol and propose a new strategy for clinical application in HSC mobilization[7].

RESULTS

Figure 1

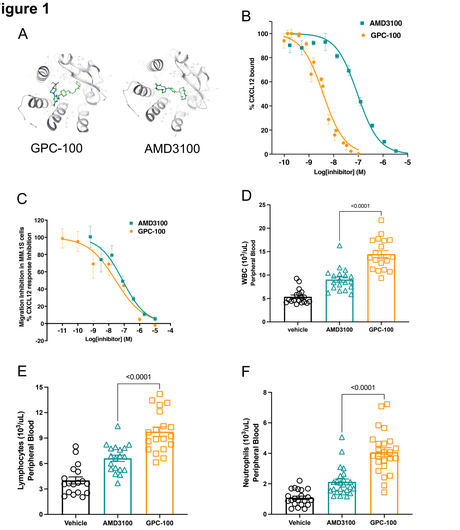

Figure 1. Comparison of GPC-100 and AMD3100. (A) Overlay of proposed binding modes of antagonists obtained from molecular docking with the template of inactive CXCR4 structure and induced-fit algorithm from Schrödinger. (B) Inhibition of CXCL12 binding to CXCR4 in HEK cells in the TagLite® competitive binding assay (CisBio). Ki values: GPC-100 1.6 nM, AMD3100 40 nM. (C) Inhibition of CXCL12-induced migration in MM.1S human myeloma cells. IC_{50} values: GPC-100 28 nM, AMD3100 80 nM. (D) In C57/BL6 mice (6-8 weeks, female), GPC-100 (30 mg/kg IV) or AMD3100 (5 mg/kg SC) induced mobilization of WBC, (E) lymphocytes, and (F) neutrophils following single dose. PB was collected 1 h-post AMD3100 based on literature and 2 h-post GPC-100 based on a time-course study[7]. PB was processed by hematology analyzer (Abaxis) for complete blood count. GPC-100 mobilized significantly greater number of WBC, lymphocytes, and neutrophils than AMD3100.

Figure 2

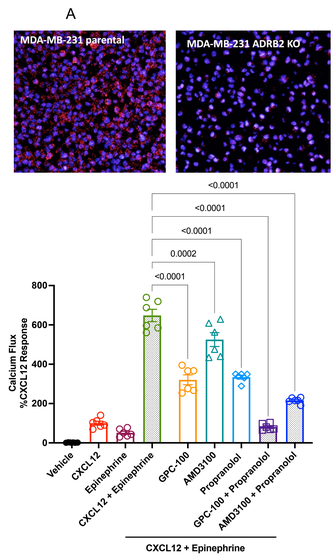

Figure 2. CXCR4 and β_2AR co-localization and crosstalk in MDA-MB-231 cells. (A) Proximity Ligation Assay (PLA) shows endogenously expressed CXCR4 and β_2AR forming heteromers. Specificity is confirmed in ADRB2 knockout cells showing reduced signal. Because PLA detects only protein-protein interactions as a single oligonucleotide (red), only the interacting molecules can produce a robust signal, making it a sensitive and specific assay. (B) CXCR4 and β_2AR agonists, CXCL12 (200 nM) and epinephrine, (10 μM) synergistically increase calcium flux. Complete blockade of this synergy is only achieved with combination of GPC-100 (10 μM) and propranolol (10 μM). % calcium flux is normalized to CXCL12.

Figure 3

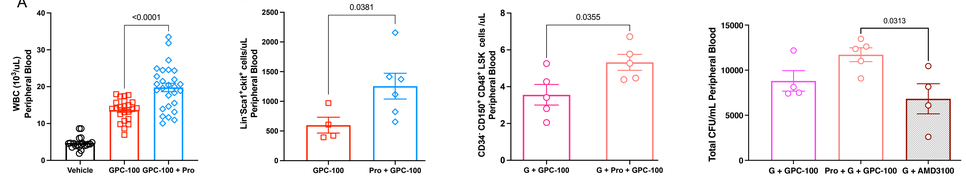

Figure 3. Propranolol improves GPC-100 induced mobilization in vivo. (A) Seven-day pretreatment with propranolol (20 mg/kg IP, days 1-7) enhances GPC-100 (co-administered on day 7) induced mobilization of WBC and (B) HSCs or Lin$^-$Sca-1$^+$ cKit$^+$ (LSK) cells in PB. (C) Addition of propranolol (days 1-7) to G-CSF (0.1 mg/kg SC BID, days 2-6) and GPC-100 (day 7) increases mobilization of primitive HSCs or CD34$^-$CD150$^+$CD48$^+$ LSK cells. (D) Hematopoietic progenitors represented by colony forming units (CFUs) in peripheral blood showed a greater PB count in mice treated with the combination of propranolol, G-CSF and GPC-100 compared to the standard of care, G-CSF plus AMD3100. AMD3100 was administered on day 7 for all combination treatments.

Figure 4

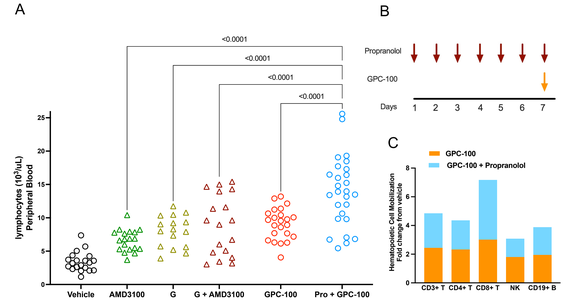

Figure 4. Lymphocyte mobilization by propranolol and GPC-100 combination treatment. (A) Lymphocyte count was determined on the hematology analyzer. Propranolol plus GPC-100 mobilized significantly greater number of lymphocytes compared to GPC-100, AMD3100 and the standards of care, G-CSF +/- AMD3100. (B) Propranolol was administered for 7 days, followed by GPC-100 co-administration on day 7. (C) Lymphocyte subset analysis was performed with flow cytometry. Propranolol improved GPC-100 induced mobilization of T cells, including CD4$^+$ and CD8$^+$ T-cell subsets, NK cells (Nkp46$^+$) and CD19$^+$ B cells.

Figure 5

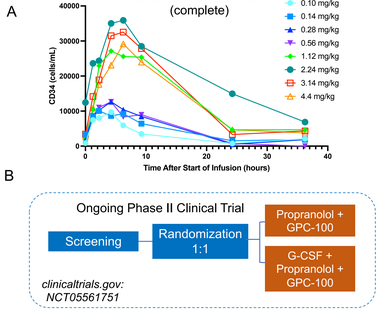

Figure 5: Clinical studies with GPC-100. (A) In a phase I randomized, double-blind, placebo-controlled study, GPC-100 (0.1 to 4.4 mg/kg IV) was administered to 64 healthy subjects (8 cohorts) in a single-ascending-dose design. At maximal doses, GPC-100 induced a 3-14-fold increase in PB CD34+ HSC count within 4-6 h. (B) Two-arm Phase II clinical trial investigating G-CSF +/- Propranolol + GPC-100 in ASCT-eligible MM patients is ongoing.

CONCLUSION

GPC-100 shows superior binding to CXCR4, more potently inhibits CXCL12-induced migration in MM cells and is a better mobilizer in vivo than AMD3100.

Endogenously expressed CXCR4 and β_2AR can form heteromers in native environment that have functional consequences such as calcium flux synergy, highlighting the importance of these receptors as druggable targets.

Propranolol and GPC-100 combination can provide sufficient HSCs harvest with the possibility to eliminate G-CSF. This reduces risk of side effects such as severe bone pain and splenic rupture[8]. MM patients report a poor health-related quality of life, and some have undergone treatments that negatively affect G-CSF mobilization. An alternate approach of using an oral beta blocker like propranolol removes the risks and burden of daily subcutaneous G-CSF injections, as well as toxicity from repeated mobilization attempts.

Triple combination of propranolol, G-CSF and GPC-100 significantly increased mobilization of primitive, functional HSCs compared to G-CSF plus AMD3100, and can potentially treat patients where other mobilization regimens have failed.

Propranolol significantly improved GPC-100-induced mobilization of lymphocytes with a robust impact on CD8$^+$T cells. This may have potential utility in adoptive cell therapies, where success relies on the ability of T cells to persist and exert function in an immunosuppressive tumor microenvironment. In our ongoing phase II trial, immune cell subset analysis will be performed to confirm these findings in patients.

Propranolol is a safe, accessible, and inexpensive option to supplement the mobilization therapies for greater stem cell yields in fewer apheresis sessions and reduce the financial burden on patients and healthcare systems. In conclusion, these studies support combination of GPC-100 and propranolol for HSC mobilization for ASCT in MM.

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Study is registered on clinicaltrials.gov as NCT05561751.

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