In Vivo Nanoparticle-Mediated RNAi in Bone Marrow Enhances Hematopoietic Stem Cell Mobilization and Harvesting

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Statement of Purpose: Hematopoietic stem cell (HSC) transplantations (HSCT) are essential for restoring a patient's immune system destroyed by radiation or chemotherapy. However, HSCT remains a dangerous procedure for patients with life-threatening diseases, with a major complication being harvesting HSCs from blood to restore the recipient's immune system. Here, we developed a nanoparticle (NP) platform to induce RNAi in bone marrow (BM) in vivo to increase HSCs in blood for harvesting and transplantation (Fig 1A,B).

Methods: siRNA targeting stromal-derived factor-1 (SDF-1) and luciferase (Luc) were encapsulated in NPs consisting of lipids and modified polyethylenimine via rapid microfluidic mixing. NPs were characterized using DLS, SEM, and cryo-TEM. BM endothelial cells (BMECs) were treated with NPs at siRNA dosages of 1-60 nM in vitro. C57/BL6 mice were treated with NPs at a siRNA dosage of 1.0 mg/kg to determine in vivo SDF-1 knockdown. HSCs were harvested from blood 1-week after initial NP treatment and characterized via flow cytometry as Lin-Sca-1-c-Kit+ (LSK) cells.

Results: 60 nm polymer-lipid NPs containing siSDF-1 were formulated via microfluidic mixing (Fig 1C). NPs containing fluorescent siRNA were uptaken by BMECs as per confocal micrographs (Fig 1D). NPs containing siSDF-1 induced potent gene knockdown in BMECs, compared to siLuc controls (Fig 1E). In vivo, silencing of SDF-1 induced a nearly 300% increase in the number of HSCs harvested from blood, compared to controls (Fig 1F).

Conclusions: We have developed a NP platform that, potentially for the first time, induces potent gene knockdown in the bone marrow microenvironment in vivo. Clinically, this platform can be used to silence BM factors to increase the number of HSCs in blood available for harvesting and subsequent transplantation.

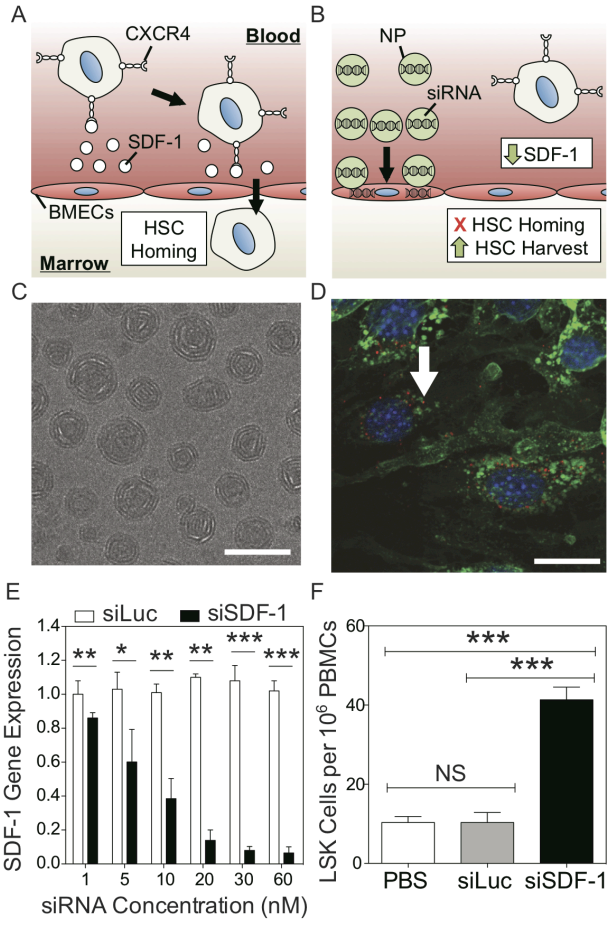

Fig. 1: (A) HSC homing into marrow via SDF-1. (B) SDF-1 knockdown via *in vivo* NP-mediated RNAi reduces homing and increase HSCs within blood. (C) Cryo-TEM micrograph of lipid-polymer NPs encapsulated with siRNA. Scale bar: 100 nm. (D) NP-mediated siRNA (red) uptake in BMECs. Scale bar: 10 μm (E) NP mediated SDF- 1 knockdown in BMECs. (F) Lin^- $Sca\text{-}1^-c\text{-}Kit^+$ (LSK) HSCs isolated from blood per 10^6 peripheral blood mononuclear cells (PBMCs) 1- week post-SDF-1 knockdown. *P<0.05. **P<0.01. ***P<0.001. NS: not significant.