Scientific premise/background and project goal: Delayed engraftment of hematopoietic stem/progenitor cells (HSPCs), or even failure to engraft, is still a significant problem, mainly if the number of HSPCs is limited (e.g., poor bone marrow [BM] harvest, poor mobilization procedure, low-quality umbilical cord blood (UCB) unit). However, a better understanding of these mechanisms is crucial to successful patient outcomes following hematopoietic transplantations. Therefore, safe strategies to enhance homing of transplanted HSPCs by manipulating *ex vivo* their seeding efficiency are needed to solve this important clinical problem. Nevertheless, the mechanisms that direct homing and engraftment of HSPCs after transplantation to BM are still poorly understood despite significant progress in the field. We will shed more light on these processes and propose new innovative strategies by ***i)*** improving the responsiveness of infused HSPCs so that they can better migrate toward gradients of the main BM chemoattractant, stromal-derived factor 1 (SDF-1), and the two supporting BM chemoattractants, sphingosine-1-phosphate (S1P) and extracellular adenosine triphosphate (eATP), and ***ii)*** based on the fact that myeloablative conditioning for transplantation fuels a state of "sterile inflammation" in BM that directs the homing of transplanted HSPCs, we will manipulate this state in BM microenvironment to promote better homing and engraftment.

Description of research: To address the first task, our pioneering observations indicate that receptors for HSPCs chemoattractants, including CXCR4 for SDF-1, $S1P_1R$ for S1P, and P2X7 for eATP have to be included in membrane lipid rafts (**MLRs**) for optimal migration and BM homing. These MLRs are assembled in response to NADPH oxidase 2 (**Nox2**) activation that generates reactive oxygen species (**ROS**) that directly trigger activation of **Nlrp3 inflammasome.** Thus, migration of HSPCs and their homing properties are regulated in a Nox2-ROS-Nlrp3 inflammasome axis-dependent manner. **In Aim1,** we will focus on this axis as a potential target for enhancing the migration of these cells. We will activate in controlled manner elements of this axis and prevent its active state from possible negative inhibitory signals. **To address a second task,** we will enhance homing/engraftment of HSPCs after transplantation by modification of the hematopoietic microenvironment of the transplant recipient to be more permissive for seeding efficiency for transplanted cells. To address this, our data indicate that myeloablative conditioning for transplantation induces in the BM microenvironment a state of "sterile inflammation," and several mediators released in response to this treatment affect the homing of HSPCs. Here again, as we postulate is involved the Nox2-ROS-Nlrp3 inflammasome axis. We will activate in controlled manner elements of this axis and prevent this axis from potential inhibitory signals. Molecular and cellular mechanisms involved in these phenomena will be studied in **Specific Aim 3.** We will employ available KO animals, molecular modulators of Nox2, Nlrp3 inflammasome and purinergic signaling, and state-of-the-art strategies to evaluate the trafficking of HSPCs. Moreover, several small-molecule modulators employed in our studies are available for use in the clinic and new compounds are in the production pipeline, and these could find application in hematopoietic transplantations. We will also employ state of art strategies to purify HSPCs and cells comprising hematopoietic stem cells niches from Nox2-KO and Nlrp3-KO mice and to evaluate changes in mRNA and protein expression by employing NGS sequencing on the NextSeq2000 platform and proteomic analysis using High-resolution mass spectrometer FT/MALDI-ICR-MS (Solarix 2xR 7T, Bruker) coupled to nano-UHPLC ESI-Q-TOF MS/MS mass spectrometer.

Potential Outcome: The seeding efficiency (homing) to BM preceding engraftment of HSPCs is crucial for hematopoietic recovery after transplantation, and strategies are needed to improve this parameter. **Improving the seeding efficiency after transplantation is an important goal that, if optimized, could significantly improve clinical outcomes.** Trafficking of HSPCs is orchestrated by innate immunity response to myeloablative conditioning for transplantation. Our recently published results indicate an involvement of **Nox2–ROS-Nlrp3 inflammasome axis.** We will activate **in controlled** manner elements of this axis. This is an important point that has to be taken into consideration in all the strategies employed to modulate sterile inflammation in BM, that will be presented in this application. We postulate that proposed strategies to modulate MLR and biological effects of Nox2-ROS-Nlrp3 inflammasome axis would find a practical application into the clinic to accelerate homing and engraftment of transplanted cells.