Project goal and significance Hematopoietic stem cell transplantation is a well-established life-saving medical procedure. Its success rate strongly depends on the number of transplanted stem cells and the speed of their engraftment after infusion to the myeloablated recipient. Therefore, clinical outcomes will benefit from accelerating homing and engraftment rate of transplanted hematopoietic stem/progenitor cells (**HSPCs**) into recipient bone marrow (**BM**). This is important when the number of available stem cells is low, as seen after poor harvest from BM, poor mobilization efficiency of the donor, and the low number of HSPCs present in the available umbilical cord blood (UCB) unit for an adult recipient. Evidence indicates that in addition to orchestrating mobilization of HSPCs, as we reported in the past, ComC also plays an important novel role in directing their homing and engraftment after hematopoietic transplantation. It is known that HSPCs express receptors for ComC cleavage fragments. This should not be surprising as both hematopoiesis and the immune system derived from the common stem cell for hemato/lymphopoietic lineages. The scientific premise for this proposal is based on our exciting data indicating that mice that are deficient for the third ($C3^{-/-}$) and fifth ($C5^{-/-}$) protein engraft poorly with donor-derived HSPCs. Moreover, our preliminary data indicate that myeloablative conditioning for hematopoietic transplantation by radio- or chemotherapy induces a state of sterile inflammation in a transplant recipient, that leads to activation of ComC both in circulating peripheral blood (PB) as well as in the BM microenvironment. The known targets for ComC released active fragments are *i)* membrane and mitochondria as a source of reactive oxygen species (**ROS**), and *ii)* ROS activated intracellular pattern recognition receptors (PRR) - **Nlrp3 inflammasome.** In this innovatory proposal, we will shed novel light on the role of ComC and its downstream mediators regulating the speed and efficiency of homing and engraftment of transplanted HSPCs. Also, HSPCs and cells in BM microenvironment express receptors for ComC cleavage fragments that may modulate the responsiveness of HSPCs to BM chemoattractants. On the other hand, these fragments facilitate hematopoietic microenvironment to promote optimal homing and engraftment. Moreover, evidence accumulated for an extrahepatic expression of C3 and C5 in other types of cells e.g., in lymphocytes known as **complosome**. Our recent research demonstrated that complosome is also expressed in HSPCs, and we will address in our application a biological consequence of this surprising expression. **Description of research -** to study the role of the liver- and HSPCs-derived ComC in homing, engraftment, and post-transplant expansion of HSPCs, and to design optimal hematopoietic transplantation protocols, we propose 3 interrelated aims: *1) to learn which of the ComC activation pathways is crucial in response to radio- chemotherapy-mediated myeloablative conditioning for transplantation to promote homing and engraftment of HSPCs? 2) to elucidate the effect of ComC cleavage fragments on infused HSPCs "navigating" to recipient BM*, and *3) to identify ComC activated events in BM microenvironment of myeloablated for transplantation recipient that facilitate homing, engraftment, and subsequent expansion of HSPCs, and to shed more light on the significance of endogenous expression of ComC proteins in HSPCs (complosome) - that as we hypothesize supports engraftment and expansion of transplanted HSPCs in BM.*

Expected results/Innovation - We postulate that the BM navigation of HSPCs is orchestrated by innate immunity response to myeloablative conditioning for transplantation. Our recently published and preliminary results indicate a pivotal involvement of ComC activation that, as we propose triggers the Nlrp3 inflammasome activation in both transplanted donor cells as well as in the recipient BM microenvironment. The proposed research will shed more light on these mechanisms. We will employ available KO animals, molecular modulators of ComC, Nlrp3 inflammasome, and state-of-the-art strategies to evaluate the trafficking and molecular responses in HSPCs. We will also perform highly innovative studies to address the role of intracellular expression of complement elements in HSPCs. Since several small-molecule modulators employed in our studies are available for use in the clinic, our results could find potential future applications in hematopoietic transplantations. We will also employ state-of-the-art strategies to purify HSPCs and cellular elements comprising hematopoietic stem cell niches and evaluate mRNA and protein expression changes by using RNA-Seq and perform mass spectrometry (MS) based proteomic and metabolomic analyses. Our observations would also be highly relevant not only in hematopoietic settings but also when other types of stem cells are employed to treat tissue/organ injuries in regenerative medicine.