Project goal and significance - Hematopoiesis is regulated by several well-studied peptide-based "factors", including mainly growth factors, cytokines, chemokines, and certain bioactive phosphospingolipids. However, evidence has accumulated that an additional regulatory role plays **purinergic signaling**, which is a primordial form of extracellular signaling mediated by extracellular nucleotides (EXNs) secreted from the cells. The most important member of the family of purinergic mediators is secreted from the cells adenosine triphosphate (ATP) that, as extracellular adenosine triphosphate (**eATP**), becomes a signaling ligand for the P2X family of ionotropic receptors. This family of receptors consists of seven members (P2X1, 2, 3, 4, 5, 6, and 7), which are activated exclusively by eATP, and normal HSPCs highly express three P2X members of this receptor family - P2X1, P2X4 and P2X7. These P2X receptors are highly expressed by both murine as well as human hematopoietic stem/progenitor cells (HSPCs). eATP also activates receptors from the P2Y family, but these receptors, in contrast to P2X receptors, are activated in addition to eATP by several other extracellular nucleotides (e.g., eADP, eUTP, and eUDP), and their role in regulating HSPCs is not significant. Therefore, we will focus on this innovatory proposal on the family of P2X receptors. Furthermore, what is important for this application eATP is processed in the extracellular space by two cell membrane-expressed ectonucleotidases known as CD39 and CD73 to an extracellular metabolite that is extracellular adenosine (**eAdo**). eAdo activates P1 purinergic receptors. This family consists of four G protein-coupled receptor subtypes (A1, A_{2A}, A_{2B}, and A3), and murine and human HSPCs highly express two members of this family, A_{2A} and A_{2B}. Therefore, we will focus on these two P1 receptors in our proposal.

Description of research - Our preliminary data indicate that eATP and eAdo modulate oppositively several processes in HSPCs. Therefore, their action mimics, according to Chinese philosophy opposite "yin–yang" effect, where eATP is positive "yin" and eAdo negative "yang" mediator of purinergic signaling in hematopoiesis. In our proposal, we will shed more light on the common target of eATP and eAdo signaling in HSPCs, that is, intracellular pattern recognition receptor (PPR) - Nlrp3 inflammasome (**Nlrp3**). At the molecular level, while eATP-P2X receptor signaling leads to release in the cytosol of reactive oxygen species (ROS), eAdo-P1 receptor signaling activates intracellular heme oxygenase-1 (HO-1). Based on this, ROS emerges as the Nlrp3 activator and HO-1 as its negative regulator of this important intracellular PRR. Our recent research demonstrated that Nlrp3 plays a vital role in regulating HSPCs trafficking, primes the hematopoietic microenvironment for proper homing and engraftment of HSPCs, and is involved in proliferation, differentiation, and metabolism of HSPCs. We will focus on the role of this PPR regulated oppositely by eATP and eAdo and execute 3 interrelated specific aims: **1**. *To elucidate the role of eATP-P2X receptor signaling in regulating the biology of HSPCs. We will focus on the biological effects of three highly expressed P2X receptors, P2X1, P2X4, and P2X7, on the trafficking and proliferation of HSPCs.* **2**. *To investigate the role of eAdo-P1 receptors in regulating the biology of HSPCs. We will focus on two highly expressed P1 receptors for Ado on HSPCs A_{2A} and A_{2B} and study the eAdo-mediated mechanism that negatively regulate eATP-mediated biological effects on HSPCs.* **3**. *We will shed novel light on the opposite actions of eATP-P2X and eAdo-P1 signaling in regulating of metabolism and expansion of HSPCs.*

Expected results/Innovation - In this proposal, we would like to put together all pieces of the puzzle to understand better the positive (yin) and negative (yang) effects of P2X and P1 purinergic receptor signaling in regulating the biology of HSPCs. We will perform our experiments using murine and human HSPCs, murine strains deficient in purinergic receptors, and employ small molecular modulators of investigated pathways. In parallel, we will analyze in-depth molecular changes at the mRNA and protein levels by employing OMICS strategies. These studies will lead to developing better stem cell mobilization and homing strategy, as well as more efficient ex vivo expansion protocols of HSPCs. We will elucidate the role of the understudied P2X and P1 purinergic receptors, which emerge as a novel critical regulator in hematopoiesis. The proposed research will allow us to understand this process better and lead to the development of novel therapeutic strategies for treating stem cell disorders.