Scientific premise/background and project goal: The SARS-CoV-2 pandemic, with its high mortality, has become an urgent clinical problem. This infection damages several vital organs and may lead to a fatal complication known as a “cytokine storm", resulting in uncontrolled hyperactivation of the innate immune response. A primary emerging concern is that COVID19 may lead to the damage of the stem cell compartment. SARS-CoV-2 may enter human cells after binding mainly to the angiotensin-converting enzyme 2 (ACE2) receptor and Toll-like receptor 4 (TLR4) and utilizes a spike protein (SP) for attachment and entry into the target cells. Our group presented a few months ago a hypothesis, supported currently by published and new additional preliminary data, that SP's interaction with these receptors hyperactivates Nlrp3 inflammasome which may lead to cytokine storm and damage of target cells. To support this, ACE2, similarly to the TLR4 receptor, is expressed not only on innate immunity cells but also on human hematopoietic stem cells (HSCs) and endothelial progenitor cells (EPCs). Moreover, both receptors have been identified on bone marrow-derived small $CD34^{+}/CD133^{+}lin^{-}CD45^{-}$ cells that, as postulated by several groups, may correspond to precursors for both hematopoietic and endothelial lineages. We have noticed that SP's interaction with receptors on HSCs and EPCs hyperactivates Nlrp3 inflammasome what impairs their proliferation and survival. What is crucial for this application this effect was reversed by inhibiting Nlrp3 inflammasome by small molecular inhibitor MCC950. Based on this, we postulate that COVID19 may damage stem cells from hematopoietic/endothelial lineage, which contributes to early and late clinical consequences of this infection.

Description of research: To shed more light on pathological consequences and to propose new potential treatment approaches, we propose three interrelated specific aims. **SA 1**. **Early consequences of SP mediated hyperactivation of Nlrp3 inflammasome in stem/progenitor cells for hematopoietic/endothelial lineage.** We will expose human stem cells at a different level of the specification into hematopoietic and endothelial lineages first to COVID19-derived recombinant SP and following pending obtained data to COVID19 pseudovirus, and test effect of exposure on their in vitro and in vivo hematopoietic and angiopoietic potential. We will also focus on the role of ACE2 and TLR4 in response to SP and elucidate these interactions both at the cellular and molecular level. **SA 2. Late consequences of SP on Nlrp3 inflammasome hyperactivation in hematopoietic/endothelial lineage stem/progenitor cells.** We will employ transgenic mice expressing human ACE2 protein to study the late effects of in vivo exposure first to SP and next to COVID19 pseudovirus on hematopoietic/endothelial stem cell compartment. We will also test a hypothesis that COVID19 may survive in a latent form in HSCs and EPCs, and we will test this hypothesis with cells from patients that survived COVID19 infection. **SA 3. New potential strategies to mitigate the damage of stem/progenitor cells induced by hyperactivation of Nlrp3 inflammasome.** We will test the effect of inhibition of hyperactivated Nlrp3 inflammasome via SP binding receptors and employ as readouts in *in vivo* and *in vitro* models of human hematopoiesis and angiopoiesis.

Potential Outcome: We will gain more insight into the consequences of recombinant SP and COVID19 pseudovirus effects on stem cells at a different level of specification for hematopoietic and endothelial lineages. Our application is highly significant as it examines the effects of SARS-Cov-2 on the adult stem/progenitor cells compartment. This has never been examined before and could explain some of the short- and long-term effects of COVID-19. We will work mainly with mature HSCs and EPCs isolated from adult tissues. We hypothesize that stem cell damage in the early stage of infection occurs mainly in response to SP-ACE2 and SP-TLR4 interaction and results in pathological hyperactivation of Nlrp3 inflammasome leading to pyroptosis and the release of other DAMPs and other mediators eventually causing the “cytokine storm”. Late complication of COVID-19 infection could be related to irreversible damage of immature HSCs and EPCs as well as virus latency in these cells. In this application we will also test potential strategies to mitigate hyperactivation of Nlrp3 inflammasome in response to SP-ACE2 and SP-TLR4 interaction – as potential therapeutic strategy to enhance stem cell survival following exposure to COVID-19.