Project Summary

Platelets play pivotal roles in hemostasis and platelet-related disorders. Activated platelets facilitate recruitment of monocytes and neutrophils to arterial wall and promote atherogenesis. Platelets also initiate the formation of thrombi on ruptured or eroded atherosclerotic plaques, causing clinical complications of atherosclerosis. A striking example occurs in myeloproliferative neoplasms (MPNs) such as essential thrombocytosis (ET) and primary myelofibrosis (MF), in which increased platelet production is associated with prominent arterial thrombosis or athero-thrombosis. Aberrant platelet production has also been linked more broadly to cardiovascular risk in the general population. Substantial residual risks of cardiovascular disease in the general population and in patients with MPNs still remain, even with the access of current preventive and therapeutic interventions.

HDL may be protective against coronary heart disease. A major hypothesis for the protective role of HDL is that it promotes cholesterol efflux from lesional atheroma cells. Recent studies indicate a key role of ATP-binding cassette transporters, ABCA1 and ABCG1 in promoting cholesterol efflux from macrophage foam cells to HDL. ABCG4 also promotes cholesterol efflux to HDL but ABCG4 is not expressed in macrophages. We found *Abcg4* to be selectively expressed in bone marrow megakaryocyte progenitor cells (MkPs). *Abcg4*$^{-/-}$ MkPs showed defective cholesterol efflux to HDL and increased plasma membrane cholesterol. *Abcg4*$^{-/-}$ BM transplantation into hypercholesterolemic *Ldlr*$^{-/-}$ mice resulted in thrombocytosis, accelerated atherosclerosis and arterial thrombosis. Increased platelets reflected an expanded pool of MkPs and megakaryocytes, resulting from increased expression of the thrombopoietin (TPO) receptor (MPL) on the cell surface of MkPs. This reflected blunting of the negative feedback regulation of MPL by the E3 ubiquitin ligase, c-CBL. Further studies suggested that membrane-anchored LYN Kinase is inhibited by association with cholesterol-rich membrane microdomains in *Abcg4*$^{-/-}$ cells, resulting in impaired activation of c-CBL. We propose that HDL promotes cholesterol efflux from MkPs via ABCG4, activates LYN and c-CBL, promotes ubiquitination and degradation of MPL and limits MPL-mediated proliferation signaling in response to TPO. HDL inhibits platelet production and suppresses thrombocytosis.

Since ABCG4 deficiency decreases LYN activity and results in thrombocytosis and accelerated atherogenesis, activation of LYN kinase by the drug tolimidone will be assessed for anti-atherogenic and anti-thrombotic activity in *Abcg4*$^{-/-}$ BMT →*Ldlr*$^{-/-}$ mice. Increased platelet phosphatidylserine (PS) exposure, as seen in Abcg4-/- mice, may lead to increased thrombin generation and contribute to accelerated atherosclerosis and arterial thrombosis. This will be tested, using recombinant annexin V to shield platelet surface PS. The impact of human CBL/ABCG4 SNPs associated with platelet counts on megakaryopoiesis will be examined using human cord blood samples.

While thrombosis is a major risk in ET and MF patients, this has not been modeled in animals. We will assess the impact of activating mutations in MPL (MPL-W515L) on platelet production, atherogenesis and arterial thrombosis in *Ldlr*$^{-/-}$ mice fed a high fat high cholesterol diet. We will assess the relative role of platelets, neutrophils and monocytes in the accelerated atherosclerosis and or arterial thrombosis in this model. We also will assess the possibility that increased platelet microparticles, platelet PS exposure and thrombin formation, increased platelet/leukocyte interaction and activation and increased monocyte recruitment into atherosclerotic lesions are mechanistically linked to accelerated atherosclerosis and arterial thrombosis in the setting of genetically increased platelet production. We will assess rHDL infusion and pharmacological activation of LYN kinase by tolimidone as novel treatments to limit thrombocytosis, reduce atherosclerosis and possibly increase survival in MPNs.

We expect that the proposed studies will provide novel insights and therapeutic strategies for atherosclerosis and athero-thrombosis associated with aberrantly increased platelet production.