UNIVERSITÀ DEGLI STUDI DI PADOVA

# PROJECT

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1) Project title

INVESTIGATING THE DIRECT VASCULAR PROATHEROGENIC ACTION OF ANGPTL3

2)Abstract (max 500 words)

Dyslipidaemia is a major risk factor for atherosclerosis and coronary artery disease (CAD), and statins have represented a very effective therapy for preventive the clinical consequences of atherosclerosis. Lately PCSK9 inhibitors have opened a new scenario for the treatment of familial hypercholesterolemia and for preventing CAD. More recently, genetic and pharmacological evidence have reinforced the hypothesis that angiopoietin-like 3 (ANGPTL3) could be a new important target for dyslipidemia and CAD. ANGPTL3 is exclusively produced by the liver and its primary action is to inhibit lipoprotein lipase (LPL), the enzyme responsible for the hydrolysis of circulating triglycerides in the capillaries of adipose and muscle tissue. ANGPTL3 levels correlated positively with femoral artery intima-media thickness (FA-IMT) in healthy human subjects, independently from the classical risk factors. Importantly, the C-terminal fibrinogen-like domain of ANGPTL3 is involved in angiogenesis via binding to integrin αVβ3, a receptor expressed also in macrophages. For this reason, we hypothesize a possible direct and non-lipid related action of ANGPTL3. Thus, the main aim of the present proposal will be to investigate the effect of ANGPTL3 on three additional biological events directly related to cardiovascular diseases, such as:

1) The polarization of macrophage towards an M1 pro-inflammatory phenotype
2) The cholesterol metabolism in macrophages
3) The vascular localization of ANGPTL3 in *in vivo* model of atherosclerosis

The results of the present study could help to better define the biological activity of ANGPTL3 beyond its effect on lipoprotein metabolism and could represent the basis for direct pro-atherosclerotic function of this hepatokine. The results will also make the premises to better understand if the pharmacological inhibition of ANGPTL3 may help to prevent CAD not only by controlling dyslipidemia, but also by affecting its local action in the vascular wall.

Deliverables (D) and Milestone (M)

**D1.1.** Preparing the total RNA, protein and conditioned media of THP-1 derived macrophages incubated with ANGPTL3.
**D1.2.** Preparing the same samples as D1.1 in the presence or absence of the αVβ3 integrin inhibitor RGD peptide.
**M1.1.** Determining the effect of ANGPTL3 on inflammasome activity in THP-1 derived macrophages.
**M1.2.** Determining the involvement of the binding of C-terminal fibrinogen-like domain of ANGPTL3 to integrin αVβ3 on inflammasome activity.

**D2.1.** Preparing samples for measuring the effect of ANGPTL3 on cholesterol efflux and uptake
**D2.2.** Preparing the same samples ad D2.1 in the presence or absence of the αVβ3 integrin inhibitor RGD peptide.
**M2.1.** Determining the effect of ANGPTL3 on cholesterol efflux and uptake from THP-1 derived macrophages.
**M2.2.** Deciphering which cholesterol efflux pathways are affected by ANGPTL3 by the means of different cholesterol acceptors.

**D3.1.** Preparation of fluorescent mouse recombinant ANGPTL3.
**D3.3.** Inducing the atherosclerotic plaque in apoE$^{-/-}$ mice.
**M 3.1.** Detecting ANGPTL3 after i.v. injection in *in vivo* experimental model of atherosclerosis.
**M3.2.** Detecting the localization of macrophage foam cells in mouse atherosclerotic plaques and possible co-localization with ANGPTL3