ImmunoTools *special* Award 2015

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Capturing Cardiovascular Disease in vitro

Cardiovascular diseases are a major cause of mortality and morbidity. Atherosclerosis is a chronic inflammatory disease of the large arteries triggered by damage to the vessel wall followed by cholesterol and immune cell accumulation in the innermost layer of the artery. Rupture of a so-called vulnerable atherosclerotic plaque results in a cardiovascular event such as heart infarct, embolism or stroke. Statins – the main family of cholesterol-lowering drugs –reduce cardiovascular event risk by only 30%. The search for newer and better therapies is however hampered by empty drug development pipelines and suboptimal bench-to-bedside translation. A huge percentage of therapeutic candidates fail in the preclinical phase. Moreover, one third of all medication successfully tested in animal models turns out to have unforeseen effects in men.

Our research tackles these problems by modeling a cardiovascular disease sensor *in vitro*. Ultimately, we aim at designing a "**plaque on a chip**" device that can be used for drug and functional food screenings, pathway analyses, but also for diagnostic or prognostic purposes (serving as surrogate endpoint of cardiovascular events). We hypothesize that patient plasma, a pool of thousands of known and unknown factors, represents that unique individual's health state, cardiovascular risk profile, etc. Adding patient plasma to our *in vitro* model of cardiovascular disease results in a direct functional readout: instead of laboratory results giving different values for a small set of molecules we are looking at the relevant cellular behavior as a sensor.

Using human macrophages, endothelial cells and vascular smooth muscle cells - the three main cell types in atherosclerosis, we have recapitulated key processes in atherosclerotic disease (ROS production, apoptosis, lipid uptake, phagocytosis and efferocytosis, cell migration, polarization, …) in a 96-well format. We developed a **High Content Analysis Assay platform to evaluate these critical functional assays** in a standardized, automated and high-throughput manner. For this purpose, we use a

confocal fluorescence image capturing system able to scan multiwell plates and perform digitalized image analysis. We are currently finalizing the setup of our assays. As an example of our validation experiments, we were able to show that macrophages incubated with serum from heart infarct patients produce significantly more ROS than macrophages incubated with control patient serum.

As a next step, we will screen plasma samples from a cohort of 30 Acute Myocard Infarct and matched control patients. Based on the outcomes of these assays we will design an algorithm that determines a "cardiovascular risk score". This risk score is a direct reflection of the functional reaction of all relevant cell types in atherosclerosis to the individual patient plasma.

ImmunoTools human flow cytometry antibodies will first of all be used to assess purity, quality and consistency of our macrophage cultures. More importantly however, the **ImmunoTools Reagents will enable us to add an extra dimension** to our High Content Analysis Assay platform: with the ELISA kits for human TNFa, IL-10 and IL12p40 we will determine cytokine secretion by macrophages which have been exposed to the patient plasma samples of our cohort. These experiments are very interesting from a "basic science" point of view. Secretion of signature cytokines will be immediately linked to a comprehensive analysis of macrophage functions, and to cardiovascular health status. These results are likely to generate new insights into macrophage biology, specifically in the context of cardiovascular health. Most importantly, the cytokine secretion data will allow for a refinement of our cardiovascular risk score algorithm, increasing its value for future applications.

ImmunoTools *special* AWARD for **Lieve Temmerman** includes 15 reagents

FITC - conjugated anti-human CD3, CD19, CD66b,

PE - conjugated anti-human CD56,

PerCP - conjugated anti-human CD14,

APC - conjugated anti-human CD16,

human ELISA-set for 96 wells, human IL-10, human IL-12p40 diff, human TNF-alpha (each 3 reagents) DETAILS more AWARDS