Cardiopulse article:

CHECKPOINT ATHERO: a new LeDucq Foundation Network of Excellence project

Or

CHECKPOINT ATHERO: development immune checkpoint-based therapeutics to combat atherosclerosis

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Immune checkpoints: a potent class of immunomodulators

Immunotherapy using immune checkpoint modulation has revolutionized the way cancer patients are treated. Antibody therapies against inhibitory immune checkpoints such as CTLA-4 and PD-1, have shown revolutionary response rates in patients with a diverse array of cancers. Immune checkpoint therapy is now indicated for >50 (sub)types and stages of cancer, and the eligibility of cancer patients to receive this therapy has risen to >40% and continues to increase. The clinical success underlines the widespread therapeutic potential of this class of immune modulators[1].

Immune checkpoint proteins, including *co-stimulatory* and *co-inhibitory* molecules, are master regulators of the immune response that allow cognate interactions not only between T cells and antigen presenting cells (APCs), but also between immune cells and tissue resident cells. The sequence, the location and the extent of these interactions condition the very establishment of an immune response and its resolution or chronicity[2]. Co-stimulatory immune checkpoints, including the CD28-CD80/CD86, CD40L-CD40, Ox40-Ox40L, CD27-CD70 and GITR-GITRL dyad mostly exert pro-inflammatory actions, whereas co-inhibitory immune checkpoints, including the CTLA4-CD80/CD86, PD1-PDL1/2, CD200/CD200R dyads, as well as VISTA mostly exert immune regulatory actions, although there are quite some exceptions.

Co-stimulatory and co-inhibitory immune checkpoints in atherosclerosis

Not only in cancer, but also in atherosclerotic CVD, immune checkpoints play a pivotal role, as revealed by many papers who have studied the role of this class of molecules in human and experimental models of atherosclerosis. For example, the CD40L-CD40 dyad is a powerful driver of atherosclerosis and its inhibition reduces atherosclerosis and induces plaque stability[3,4]. A small molecule inhibitor that targets CD40-TRAF6 signaling reduces atherosclerosis without causing immunosuppressive side-effects[5]. This SMI's specific delivery to macrophages using HDL nanobiologics resulted in atherosclerotic plaques with a stable plaque phenotype and was proven safe in non-human primates[6]. Of interest are the cell-type specific roles of this immune checkpoint dyad in atherosclerosis. Deficiency of CD40L in T-cells reduces atherosclerosis, whereas deficiency in platelet CD40L does not affect atherogenesis, but ameliorates athero-thrombosis[4]. Deficiency of CD40 on dendritic cells reduces atherosclerosis, mostly by affecting Th1 driven inflammation[4], whereas deficiency of myeloid CD40 ameliorates atherosclerosis by dampening macrophage activation and necrotic core formation[7]. Furthermore, circulating sCD40 and sCD40L levels are associated with CVD[8].

The inhibitory immune checkpoint dyad CD200-CD200R, once identified as a GWAS-hit correlated to CVD, was found to restrain monocyte-macrophage infiltration in atherosclerosis, and CD200R expression on monocytes is negatively correlated with coronary artery disease severity in patients[9].

Expression of the co-stimulatory immune checkpoint 'Glucocorticoid Induced TNF-Related Protein' (GITR) in atherosclerotic plaques is associated with cerebrovascular disease and sGITR levels in blood predict cardiovascular events. GITR-deficient mice display a reduction of atherosclerosis by hampering monocyte infiltration[10]. Also, inhibition of the co-stimulatory immune checkpoint Ox40L, originally identified as a gene present in the CVD risk locus reduces atherosclerosis and facilitates plaque regression by affecting both T and B cell responses[11].

Immune checkpoints: immunotherapeutic potential to combat atherosclerosis?

Results of the CANTOS, COLCOT and LoDoCo2 trials demonstrate that immune regulation on top of optimal lipid management reduces CV events in humans, emphasizing the immune system's central role in CVD[12-14], and underlining the importance of targeting inflammation in atherosclerosis.

While a wealth of data provides evidence for a key role for immune checkpoints in atherosclerosis, *the cardiovascular field lags woefully behind cancer in studying and advancing immune checkpoint-based therapies*. In the oncology field, antagonizing co-inhibitory immune checkpoints is used to break tolerance and elicit a vigorous immune response to accomplish CD8+ T-cell mediated killing of tumor cells. In contrast, employing immune checkpoint-based targets as immunotherapy for CVD requires inhibition of co-

stimulatory immune checkpoints or activation of co-inhibitory immune checkpoints to reduce inflammation and induce tolerance, thereby inhibiting atherosclerosis development and progression.

CHECKPOINT ATHERO Network of Excellence

The Leducq International Network CHECKPOINT ATHERO aims to unlock the full translational potential of immune checkpoint-based immunotherapeutic targets and drugs in atherosclerosis. In the next 5 years, this consortium aims to obtain an integrated understanding of cell-type specific immune checkpoint responses in human and mouse atherosclerosis, which can be readily applied to develop novel biomarkers and targeted therapies of 5 candidate immune checkpoints. More specifically, the CHECKPOINT ATHERO network will define the unique signature of the immune checkpoint landscape in humans/patients with CVD, delineate underlying mechanisms of cell type specific, immune checkpoint mediated dysregulation in atherosclerosis and develop targeted immune checkpoint-based therapeutics for atherosclerosis, using nanotechnology and antibody-based approaches (Figure 1). Understanding the role of immune checkpoint proteins in CVD is crucial, not only for advancing therapies for CVD aimed to lower inflammation, but also for identifying potential CVD risks caused by immune checkpoint-based therapies in cancer that provoke immunity.

To accomplish these goals, we have formed an international consortium consisting of experts with complementary expertise in this area. We brought together expert clinical investigators in the fields of inflammation and atherosclerosis with leading investigators in cardiology, vascular biology, immunology, basic, clinical, and commercial antibody development and nano-immunotherapy. The team is composed of Drs. Esther Lutgens (Mayo Clinic, Rochester, MN, USA), Willem Mulder (Radboud University, Nijmegen, the Netherlands), Claudia Monaco (University of Oxford, Oxford, UK), Isabel Gonçalves (Lund University, Malmö, Sweden), Coleen McNamera (University of Virginia, Charlottesville, VA, USA), Johan Kuiper (Leiden University, Leiden, the Netherlands), and Randolph Noelle (Dartmouth Geisel school of Medicine, Hanover, NH, USA).

The CHECKPOINT ATHERO team is confident to be able to identify underlying regulatory mechanisms of our candidate immune checkpoints in atherosclerosis and to assess novel interventional approaches, that will ultimately lead to safe, targeted immunotherapeutics to combat cardiovascular disease.

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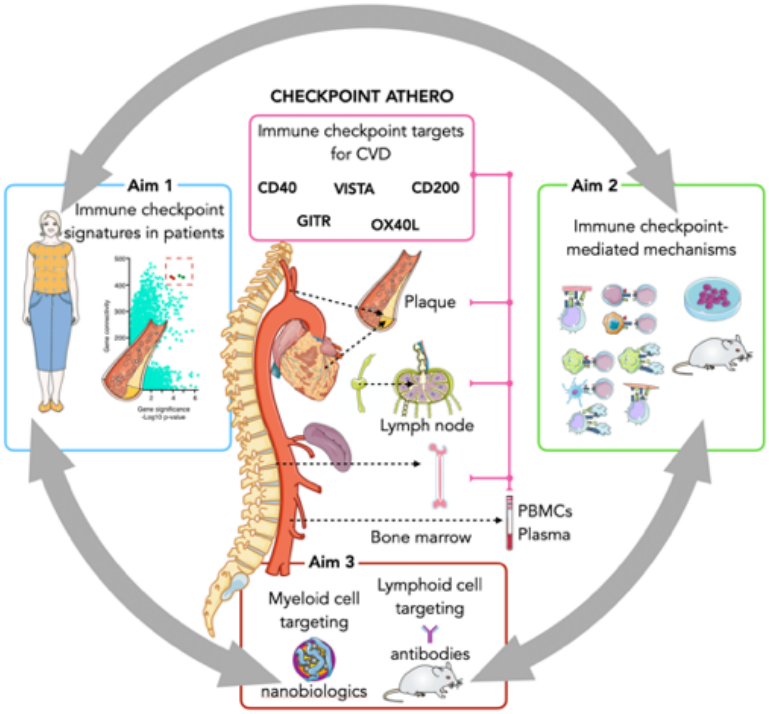

Figure 1:
The CHECKPOINT ATHERO consortium will define the unique signature of the immune checkpoint landscape in humans/patients with CVD by employing single cell technologies on atherosclerotic plaque tissue and PBMCs available in our cohorts (**aim 1**), delineate underlying mechanisms of cell type specific, immune checkpoint mediated dysregulation in atherosclerosis using human cell-based in vitro systems and mouse models (**aim 2**) and develop targeted immune checkpoint-based therapeutics for atherosclerosis, using nanotechnology and antibody-based approaches (**aim 3**).