Cardiovascular Immunotherapy and the Role of Imaging

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Despite significant advances in prevention and treatment, cardiovascular diseases (CVDs) remain the most common cause of death in the United States.[1–3] In part, and compared with treatment developments in oncology, this is because of the lack of current generation CVD precision therapies.[4–6] Most CVDs are caused by atherosclerosis, a disease process that causes thickening of the arterial wall because of inflammation and lipid accumulation, resulting in plaque formation.[7,8] Macrophage dynamics plays a central role in atherosclerosis progression, vessel wall destabilization, and—as has been recently discovered—plaque aggravation because of myocardial infarction.[9–11] Therefore, and despite current standardized treatments, ≈25% of patients who had myocardial infarction or stroke will experience secondary major adverse cardiovascular events, often more harmful than the primary event.[12–14] To break this vicious cycle, innovative and tailored therapeutic strategies are needed.

Various targeted immunotherapies that showed anticancer potential in in vitro screens also displayed in vivo potential in mouse models. Such proof-of-concept studies in validated syngeneic mouse tumor models not only provide information on in vivo efficacy but also disclose the targeted immunotherapies' underlying mechanism of action and safety profile. However, immunotherapies' high costs and the identification of amendable patients compromise clinical translation.[15,16] Continued and focused efforts are needed to understand patient responsiveness and reduce high dropout rates in trials. A parallel effort focused on the development of novel imaging tools that can guide patient selection may offer a potential solution.

Positron emission tomography (PET) can be used to trace immunotherapies. So-called immunoPET uses antibodies, developed for cancer treatment, that selectively recognize a specific epitope on target cells. The antibodies are labeled with radioactive isotopes, such as zirconium-89 or iodine-124, with relatively long decay half-lives of 3.27 and 4.18 days, respectively, to visualize their accumulation in human tissues and study their interaction within the tumor microenvironment. ImmunoPET can be complemented with computed tomography and MRI to determine pathological and morphological changes.[17–19]

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In a preclinical setting, immunoPET has been applied to study a novel combination therapy, involving a STEAP1 antibody conjugate with a chemotherapeutic agent, for the treatment of prostate cancer.[18] In an early stage clinical trial, implementation of immunoPET enabled visualizing bone metastases for the first time. ImmunoPET in a small cohort of 56 patients with advanced breast cancer, who received radiolabeled therapeutic trastuzumab, revealed that in 29% of the patients, antibody internalization was not ample robust.[18,20] Novel combination approaches of immunotherapy and noninvasive imaging enable clinicians and researchers to optimize dosage protocols and obtain deeper insights into tissue distribution and the immune response. In spite of these impressive recent advances in oncology, the principle of empowering a patient's own immune system to fight disease has yet to be applied for reversing atherosclerosis and its clinical manifestations.

Despite the slower development rate of atherosclerosis and CVD treatments, novel immunotherapies are on the rise. The first targeted CVD therapy that has been developed is a PCSK9 (proprotein convertase subtilisin-kexin type 9) antibody for the treatment of hypercholesterolemia.[5] Its approval paved the way for the clinical evaluation of an actual anti-inflammatory CVD immunotherapy directed against interleukin-1β (IL-1β) in the CANTOS trial (Canakinumab Anti-Inflammatory Thrombosis Outcomes Study). The disclosure of the primary end point led to a lot of excitement about the trial's results, suggesting an extensive reduction of secondary cardiovascular events.[21]

In this review, we discuss integration of targeted therapy, immunologic approaches, and noninvasive imaging for the management of CVD. We highlight advancements in contemporary techniques that might improve our understanding of targeted therapies' behavior and their effect on plaque morphology, inflammation, and macrophage dynamics in CVD. Finally, we discuss merging possibilities of various techniques and applications, their limitations, and challenges.

Targeted Therapies

Atherosclerosis is initiated by the arterial wall accumulation of atherogenic lipoproteins, namely low-density lipoproteins (LDL).[22,23] This is accompanied by monocyte recruitment and macrophage accumulation in the vessel wall.[24–26] Macrophages' uncontrolled phagocytosis of LDL leads to the generation of foam cells,[27] inducing early stage plaque deposition in childhood.[13,28] In advanced disease, the inflamed atherosclerotic plaque can rupture and induce an acute coronary syndrome or myocardial infarction.[7,29–31]

Current therapies are focused on lipid lowering and ineffectively reverse atherosclerosis. Targeting the immune system's capacity to resolve inflammation represents a compelling alternative strategy for treating atherosclerosis. At present, 3 main atherosclerosis immunotherapy approaches are under investigation, focusing on lipoprotein metabolism,

inflammation, and tissue repair. We will discuss all these approaches in the content of preclinical and clinical studies.

Lipid Lowering

To this day, the best clinically established approach to treat atherosclerosis has been lowering LDL cholesterol[32] by gold standard statin therapy.[33] However, 2 recent statin trials, JUPITER (Justification for the Use of Statins in Primary Prevention: An Intervention Trial Evaluating Rosuvastatin trial) and ASTEROID (A Study To Evaluate the Effect of Rosuvastatin on Intravascular Ultrasound-Derived Coronary Atheroma Burden), have clearly shown that protection from CVD is limited. In the JUPITER trial, it was shown that despite standard treatments, 25% to 40% of patients who had a prior heart attack remain at increased risk of secondary major adverse cardiovascular events, that is, myocardial infarctions or stroke, within the first 5 years.[30,34] Consistent with this finding, the ASTEROID trial showed that after 2 years of treatment with rosuvastatin, a mere 0.6% reduction in plaque volume was achieved.[35] It is clear that atherosclerosis pathophysiology extends beyond lipids because 2 of 3 of patients experiencing a heart attack have normal LDL cholesterol levels. Hence, more effective approaches are being developed.

The first targeted therapy for the treatment of hypercholesterolaemia is an antibody against PCSK9.[5] PCSK9 is a circulating protein that regulates hepatic and serum LDL cholesterol levels.[36,37] Evolocumab, a human monoclonal antibody that blocks the interaction between PCSK9 and the LDL receptor, was evaluated in the FOURIER (Further Cardiovascular Outcomes Research With PCSK9 Inhibition in Subjects With Elevated Risk) randomized clinical trial. In combination with statin therapy, up to 59% of patients show LDL reductions from a median of 92 to 30 mg/dL. Moreover, during the 26-month study, an associated 15% decrease in cardiovascular risk was observed. ORION-1 (Trial to Evaluate the Effect of ALN-PCSSC Treatment on Low-Density Lipoprotein Cholesterol), a phase II trial designed to assess a small interfering RNA therapy directed against PCSK9, found reductions in LDL levels, ranging from 27.9% to 52.6%, depending on the regimen. Both studies suggest that targeting PCSK9 may help maintain consistent and effective reductions in LDL cholesterol levels.[38–40]

Resolving Inflammation

It has been shown that in ≈4 of 10 patients that survived heart attack, the risk of secondary events is directly related to increased inflammation.[34] Chronic, nonresolving inflammatory atherosclerosis is a critical factor and a main culprit for atherosclerotic plaques progression, rupture, and thrombosis.[40]

IL-1β is a proinflammatory cytokine which is released by macrophages and plays a major role in vascular inflammation and modulation of atherosclerotic calcification.[41,42] In June 2017, it was announced that the primary end point of the CANTOS phase III study was met. Subcutaneous injections of canakinumab (ACZ885), a human monoclonal antibody that selectively neutralizes IL-1β, significantly lowered systemic inflammatory biomarker levels in postmyocardial infarction patients, reducing cardiovascular risk in patients with a prior heart attack and inflammatory atherosclerosis.[21,43] The detailed trial's results' will be fully disclosed at an upcoming medical congress in the fall of 2017.

In recent years, various preclinical studies focused on the development of anti-inflammatory therapeutic strategies to reduce macrophage accumulation in plaques.[44,45] Cholesterol-lowering drugs called statins, also known as 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, exhibit tangible anti-inflammatory actions at high doses.[46] However, oral statins are rapidly metabolized and secreted through the liver. Consequently, low amounts of drug are systemically available to exert anti-inflammatory effects at the vessel wall. Our group has developed a targeted approach to inhibit plaque macrophage proliferation in apolipoprotein E–deficient mice with advanced atherosclerosis.[47,48] To that aim, simvastatin was incorporated in high-density lipoprotein nanoparticles[49] to achieve targeted delivery to plaque macrophages.[47,50] A rapid reduction in macrophage burden was achieved as a result of a 1-week intravenous regimen. When supplemented with an ensuing 8-week oral statin treatment, the rapid inhibition of plaque inflammation could be maintained, resulting in favorable vessel wall remodeling.[47,51]

Although the plaque macrophage reduction in the above-mentioned study was achieved by targeting proliferation, blunting monocyte recruitment is an attractive alternative therapeutic strategy.[52] Dutta et al[53] have shown an effectiveness in impairing monocyte migration from the spleen to the vessel wall in atherosclerotic apolipoprotein E–deficient mice. The same group of principle investigators observed similar findings using polymeric (7C1) small interfering RNA–loaded nanoparticles with specific avidity toward endothelial cells. Concurrent delivery of 5 distinct small interfering RNAs inhibited adhesion molecule function and induced an ≈40% reduction in neutrophil, monocyte, and macrophage infiltration into atherosclerotic plaque lesions.[54]

Tissue Repair and Remodeling

Maintaining tissue homeostasis and repairing injuries are fundamental survival mechanisms. In case of mechanical injury or infections, inflammation occurs in response to damage-associated and pathogen-associated molecular patterns. The activated immune system is primed to eliminate dead and dying cells from the site of injury.[55–59]

Key players in the regulation of the tissue repair and regeneration are macrophages,[25,60,61] which—on detection of anti-inflammatory cytokines that are released from the injured cells—shift into repair mode. IL-4 and IL-13 are the 2 main activators of host defense responses and prompt an anti-inflammatory and tissue repair macrophage phenotype.[58,62,63] These 2 cytokines are mainly secreted by T-helper 2 lymphocytes at the site of infection or injury.[62,64] On the other side, tissue repair macrophages are characterized by expressing high levels of IL-10, IL-1ra, and TGF-β (transforming growth factor-beta). The latter drives tissue repair through targeting myofibroblast, by stimulating angiogenesis, enhancing production of extracellular matrix components, and thus recruitment of IL-13–producing leukocytes to the site of tissue injury.[55]

In a recently study by Bosurgi et al, it has been reported that IL-4 and IL-13 together with apoptotic cells are critical players in wound repair after helminth infections in the

lungs or in the gut.[65–67] RNA sequencing analyses identified increased expression of 61 wound healing genes involved in cell proliferation, chemotaxis, and cell adhesion.[66] Notably, epidemiological studies found that helminth infections reduce atherosclerosis risk,[68,69] suggesting the anti-inflammatory cytokines' unique role in inflammation resolution and tissue repair through, for example, the synthesis of collagen.[70]

Integration of Imaging

In the oncology field, noninvasive imaging is increasingly applied to study and assess immunotherapies, in the early phases of preclinical development, in clinical trials, as well as to select patients and predict therapeutic outcome. As described in the introduction, immunoPET with therapeutic antibodies is a promising tool for diagnostic and therapeutic treatments.

In the context of atherosclerosis, radiolabeling may help in understanding the targeted therapy's behavioral parameters, such as pharmacokinetics and plaque (macrophage) specificity.[71] At the same time, imaging can also be used to study immunotherapeutic agents' effects on vessel morphology and plaque stability through readouts reporting on vessel wall thickness and permeability, inflammation, and macrophage proliferation.[72,73]

Compared with other available anatomic imaging methods, such as ultrasound,[74] magnetic resonance, or computed tomography, PET imaging[75–77] has the advantageous ability to visualize minuscule concentrations of tracers that target specific molecular process. PET vascular inflammation imaging with fluorodeoxyglucose has become important in atherosclerosis research[78] because of fluorodeoxyglucose's accumulation in cells with glycolytic rate.[79] Several studies showed that fluorodeoxyglucose uptake, when measured in the arterial wall in vivo, reflects the level of macrophage accumulation within the atheroma.[80] Consequently, fluorodeoxyglucose-PET imaging is increasingly used to evaluate therapeutic approaches targeting atherosclerosis.[81,82]

Although fluorodeoxyglucose-PET imaging adequately addresses certain pathophysiological and treatment-related questions, the specificity for inflammation with this agent is not clearly defined because of the variable affinity for glucose of all cells in the body. In addition, high fluorodeoxyglucose activity in the blood pool and tissues near the vessel wall complicates quantification. Thus, there are opportunities for other imaging agents for atherosclerosis, such as vascular microcalcification imaged with ^{18}F-sodium fluoride[83,84] or somatostatin receptors imaging, present in high concentration on inflammatory leukocytes, imaged with ^{68}Ga-DOTATATE.[76] Further investigation of the performance characteristics of these new and other agents[75] are ongoing.

Combined and simultaneous PET/magnetic resonance is an exciting novel imaging modality that can assess disease activity alongside assessments of cardiac anatomy, function, and tissue composition during a single scan.[77,85,86] The lower associated radiation doses may be of particular importance for the clinical imaging of younger patients. In this research arena, beyond the ability to easily combine and coregister already established magnetic resonance and PET imaging techniques in a single scan, many researchers are seeking novel applications that may advance the state-of-the-art even further. Although technological and operational obstacles

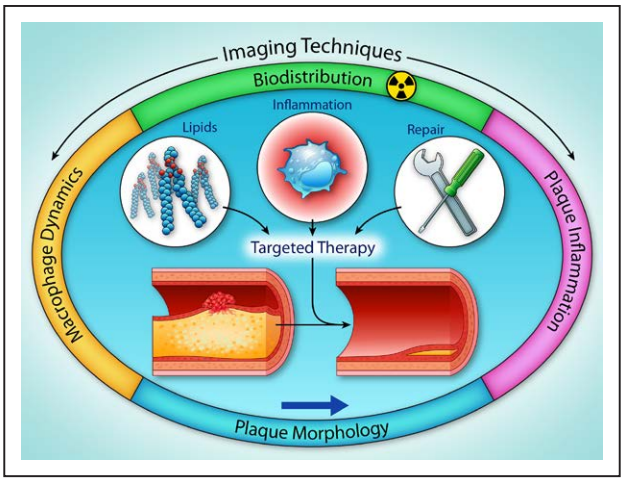

Figure. Cardiovascular immunotherapy can be integrated with noninvasive imaging to probe biodistribution and targeting, as well as to monitor therapeutic efficacy, systemically and at the level of atherosclerotic lesions.

persist, these are rapidly being overcome, positioning PET/magnetic resonance as a useful new imaging modality for the investigation of CVD. Further clinical trials are now required to explore and disclose this technique's full potential.

Final Remarks

In this review, we have highlighted the most recent advances in immunotherapy for the management of CVD. A large amount of basic and translational atherosclerosis research has yielded important new insights in the disease's molecular pathogenesis. Similar to oncological applications and with the first immunotherapy on the clinical horizon, atherosclerosis immunoimaging will likely also become a reality, as outline in the Figure. The high costs associated with this approach may make it a treatment modality that may become available to high-risk patients who do not benefit from the current standard of care.

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Disclosures

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