**Editorial**

# Detection of Perivascular Adipose Tissue-Associated Macrophages by Zirconium-89 conjugated Imaging Probes – Utility for Diagnosis of Vascular Pathology During Inflammatory Arthritis?

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Mortality risk is nearly doubled in patients with rheumatoid arthritis (RA) compared against the general population [1-3]; a substantial proportion of patient deaths are attributed to cardiovascular disease (CVD) [4]. CVD develops earlier than first anticipated in RA; patient deaths from CVD doubles within the first 5 years of clinical manifestation of RA [5] and the risk of coronary heart disease and myocardial infarction is elevated at least 1 year before arthritis symptom onset [6]. Clearly, superior strategies for preventing RA-associated CVD are needed so that drug and/or risk management approaches are initiated. Key to timely intervention is the development of new diagnostic modalities that have the requisite sensitivity to adequately resolve CVD co-incident with pre-clinical arthritis, a feature that is conspicuously absent in (i) traditional diagnostic techniques (e.g. brachial artery flow-mediated dilation, and carotid intima-media thickness) and (iii) indices of CV risk assessment (e.g. Framingham CV risk score) [7,8]. In view of this fundamental diagnostic deficiency, there is therefore a requirement to develop novel imaging tools to enable the early identification of cells that are associated with increased risk of CVD in patients with inflammatory arthritis.

## Perivascular adipose tissue-associated macrophages (PVAT-mΦ) are a prominent target for imaging

The inflammatory response in RA predicts CVD [9,10] and potentiates the effect of traditional cardiovascular risk factors [11]. With the growing interest in molecular imaging of inflammation in general, novel probes for assessing arthritis-associated CVD need to target cells, enzymes or antigens at pathologically relevant tissue sites in order to appear promising in the clinical field. Inflammation in sub-intimal vascular and perivascular layers is reported to occur more frequently in CVD associated with autoimmune rheumatic diseases (RA, spondyloarthritis, vasculitis and systemic lupus erythematosus) than in patients without autoimmune rheumatic diseases [12]. Chronic low-grade inflammation within the perivascular adipose tissue (PVAT) also causes vascular disorders [13,14]; identifying the perivascular compartment as a new target for the development of a diagnostic tool. The contribution of PVAT to CVD progression in rheumatic disease has been largely overlooked.

PVAT suppresses constriction responses in mouse, rat and human aortae [15-18]. The mechanisms that underlie arthritis-associated cardiovascular pathologies may well be linked to PVAT. A notion supported by studies in obesity and cardiovascular disease that show PVAT's normal inhibitory action on the vascular constriction response is counteracted by oxidative stress and inflammation in the adipose tissue environment [19]. PVAT is a source of adipokines (egadiponectin and leptin) and pro-inflammatory cytokines (eg monocyte chemoattractant protein-1 (CCL2) and tumour necrosis factor-α (TNF-α)), lipids (methyl-palmitate and oleic acid), complement components (C3) and reactive oxygen species [20]. These factors are secreted by macrophage (mΦ)-enriched PVAT [21-23]. Macrophages are also critically involved in the formation and destabilization of atherosclerotic plaques; they orchestrate the progression of CVD e.g. atherosclerosis and coronary artery disease from their inception [24]. Recent evidence identified PVAT-mΦ as critical regulators of adipose tissue homeostasis [25] that also increase inflammation-dependent vascular contractility[26]. PVAT-mΦ are justifiably attractive markers for targeting diagnostics and therapeutics and the development of novel immunological-radiopharma-

ceuticals for molecular imaging of arthritis-associated CVD. This approach has never been tested previously. It has the potential to improve upon current sub-optimal assessments of CVD during autoimmune rheumatic diseases that focus on the systemic compartment [27,28].

## Advanced imaging using PET/CT for early diagnosis and monitoring of therapy in the rheumatic diseases

Accurate and early diagnosis of disease is challenging and often beyond the scope of a single imaging modality. High detection capability and detailed anatomical context (in high spatial resolution) are required at sites of pathology. Positron emission tomography (PET) is a non-invasive imaging methodology that has extremely high detection capability; it locates molecular signatures that are expressed at low levels (nM-pM/g tissue). The added benefit of a computed tomography (CT) scan, performed at the same time using the same machine, provides accurate anatomical anchorage points for image co-registration. PET/CT is rapidly becoming an excellent ancillary tool to assess disease activity and prognosis in RA, systemic lupus erythematosus, ankylosing spondylitis patients [29-32]. PET-probes used to study these musculoskeletal diseases were made using short-lived radioisotopes ($^{18}$F ($t_{1/2}$=110 minutes) and $^{11}$C ($t_{1/2}$=28 minutes)). The rapid decay of the radioisotopes limits their clinical utility. For example, $^{11}$C cannot be transported over long distances and access to a cyclotron and radiochemistry facilities are necessary for the on-demand preparation of the radiotracer. $^{18}$F exhibits favourable physical decay characteristics. However, because of its rather short half-life it cannot be used for in vivo imaging of biomolecules (e.g. monoclonal antibodies and nanoparticles) with slow pharmacokinetics and peptides for which longer imaging studies may prove informative. $^{89}$Zr ($t_{1/2}$=3.27 days) is safer to handle, cheaper to produce, more stable in vivo than most isotopes. Several cancer-based clinical investigations using $^{89}$Zr-labeled antibody constructs show promising results [33], this imaging approach also has the potential for clinical translation in musculoskeletal medicine.

## Advantages of $^{89}$Zr over other isotopes for Immuno-PET

Antibody based positron emission tomography (Immuno-PET) imaging is of increasing importance to visualize and characterize tumour lesions, identify patients who may benefit from a particular therapy and monitor the outcome of therapy. In recent years the field has focused on $^{89}$Zr, a radiometal with near ideal physical and chemical properties for Immuno-PET. The clear energy disparity between the decay of $^{89}$Zr (via positron emission (511 keV)) to $^{89m}$Y versus the decay of $^{89m}$Y to $^{89g}$Y (gamma ray emission (909 keV)) enables clear detection of the 511 keV photon signal by PET. This makes reconstruction of $^{89}$Zr-based PET images relatively straightforward, unlike other isotopes such as $^{124}$I or $^{86}$Y that are also used for Immuno-PET. Additionally, the average energy of positrons emitted by $^{89}$Zr is low, compared to $^{124}$I or $^{86}$Y, resulting in images of better resolution. The high specificity and affinity of radiolabeled antibodies makes them attractive candidates as imaging agents. The slow pharmacokinetics of intact antibodies ($t_{1/2}$= 3 to 4 days) matches the half-life of $^{89}$Zr. The long-lived positron-emitting

characteristic of $^{89}$Zr is perfect for tracking the localization of monoclonal antibodies with excellent image quality over an extended timecourse. $^{89}$Zr is a residualizing isotope. This means that a $^{89}$Zr-antibody-antigen complex remains inside the target cell once is internalized, a property that permits the sustained accumulation of a $^{89}$Zr-Immuno-PET probe in a target cell. The continuous cellular uptake of an $^{89}$Zr-Immuno-PET probe is accompanied by the clearance of non-localized activity from the body. This intensifies the signal/noise ratio and improves image contrast for mAbs that accumulate slowly at their target site. Given the clear advantages of $^{89}$Zr-Immuno-PET it is surprising that research in this area has extensively focused clinical use in cancer trials. Data for musculoskeletal research is limited to one recent pre-clinical investigation; it used a $^{89}$Zr-fibroblast activation protein antibody conjugate in PET/CT imaging and biodistribution studies to correlate the uptake of radioactivity in the joints with arthritis severity in experimental collagen-induced arthritis[34].

The main tenets of this article are that

· $^{89}$Zr-Immuno-PET probes provide highly sensitive, quantitative imaging at a molecular level that reveal the important pathophysiological processes underlying inflammation alterations in the profile of PVAT-derived mediators and/or the mΦ population underpins the exacerbation of vascular dysfunction during inflammatory arthritis[35-39].

The multi-milligram amounts of antibody, normally required for the most widely cited $^{89}$Zr-radiolabelling procedure, is likely to be cost-prohibitive during the research and development stage for Immuno-PET probes [40]. The recent article by Knight et al reported a reliable $^{89}$Zr-radiolabeling procedure that provided high radiochemical yields at the microgram scale [41]. This novel approach will perhaps facilitate the comprehensive and detailed investigation, particularly for the most contemporary antibodies. More research is also needed in the area of PVAT-mΦ biology in order to discover new and specific molecular targets for predicting, halting or preventing vascular pathology and dysfunction during inflammatory arthritis.

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