Atherosclerosis Imaging Using Positron Emission Tomography (PET): Insights and Applications

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ABSTRACT

Positron emission tomography (PET) imaging is able to harness biological processes to characterise high-risk features of atherosclerotic plaque prone to rupture. Current radiotracers are able to track inflammation, microcalcification, hypoxia and neoangiogenesis within vulnerable plaque. ^{18}F -fluorodeoxyglucose (^{18}F -FDG) is the most commonly used radiotracer in vascular studies and is employed as a surrogate marker of plaque inflammation. Increasingly, ^{18}F -FDG and other PET tracers are also being used to provide imaging endpoints in cardiovascular interventional trials. The evolution of novel PET radiotracers, imaging protocols and hybrid scanners are likely to enable more efficient and accurate characterisation of high-risk plaque. This review explores the role of PET imaging in atherosclerosis with a focus on PET tracers utilised in clinical research and the applications of PET imaging to cardiovascular drug development.

$\alpha 7$ nAChR	$\alpha 7$ nicotinic acetylcholine receptors
^{18}F -FDG	^{18}F -fluorodeoxyglucose
^{18}F -FMCH	^{18}F -methylcholine
^{18}F -FOL	Aluminum fluoride-18-labeled 1,4,7-triazacyclononane-1,4,7-triacetic acid conjugated folate
^{18}F -HX4	^{18}F -2-(4-((2-nitro-1H-imidazol-1-yl)methyl)-1H-1,2,3-triazol-1-yl) propanolol
^{18}F -NaF	^{18}F -sodium fluoride
^{18}F -CH	^{18}F -Flourocholine
ACS	Acute coronary syndrome
CAD	Coronary artery disease
CANF	C-type atrial natriuretic factor
CD	Cluster of differentiation
CT	Computed tomography
CTCA	Computed tomography coronary angiogram
CVD	Cardiovascular disease
CXCR4	CXC chemokine receptor
DOTATATE	[1,4,7,10-tetraazacyclododecane- <i>N,N',N'',N'''</i> -tetraacetic acid]-D-Phe ¹ ,Tyr ³ -octreotate
FAS	fatty acid synthase
FMISO	^{18}F -Fluoromisonadazole
FR- β	Folate receptor- β
FRS	Framingham risk stratification
GLUT	Glucose transporters
HIF-1 α	Hypoxia-inducible factor 1 α
HIV	Human immunodeficiency virus
hsCRP	High sensitivity C-reactive protein
IVUS	Intravascular ultrasound
LDL-C	Low-density cholesterol
MRI	Magnetic resonance imaging
NOTA	1,4,7- triazacyclononane-1,4,7-triacetic acid
NSTEMI	Non-ST elevation myocardial infarction
OCT	Optical coherence tomography
PBR	Peripheral benzodiazepine receptors
PET	Positron emission tomography
PTSD	Post-traumatic stress disorder
RGD	Arginylglycylaspartic acid
SSTR2	Somatostatin receptor 2
STEMI	ST elevation myocardial infarction
SUV	Standardised uptake value
T2DM	Type 2 diabetes mellitus
TBR	Target to background ratio
TBRmax	Maximum target to background ratio
TCFA	Thin capped fibroatheroma
TIA	Transient ischaemic attacks
TSPO	Translocator protein
VH	Virtual histology

Nomenclature of Targets and Ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in <http://www.guidetopharmacology.org>, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Harding et al., 2017), and are permanently archived in the Concise Guide to PHARMACOLOGY 2017/18 (Alexander et al., 2017).

Introduction

Recent advances in imaging technology have enabled positron emission tomography (PET) to emerge as a powerful tool for studying the pathophysiology of atherosclerosis. Despite great strides made in the fields of diagnostics and therapeutics, atherosclerosis is the commonest underlying cause of acute cardiovascular events, and remains the leading cause of mortality and morbidity worldwide. This in part brings to light the limitations of current diagnostic techniques, which rely heavily on structural changes. Structural changes occur late in the pathophysiology of atherosclerosis and do not always lead to adverse clinical outcomes. More recently, there has been a paradigm shift towards using molecular imaging techniques such as PET to identify features of ‘high-risk vulnerable plaque’ early with the aim of preventing future cardiovascular events. Due to its utilisation in interventional trials, PET imaging also has the potential to bridge the translational divide that exists between laboratory scientific discoveries and their applications to clinical practice. In this era of precision medicine, the development of molecular imaging techniques will hopefully enable more accurate risk prediction and targeted therapy, ultimately leading to improvements in clinical outcomes.

In this review we focus on the application of positron emission tomography (PET) imaging to vulnerable plaque identification, cardiovascular drug development and risk stratification in cardiovascular disease.

The concept of the ‘Vulnerable Plaque’

Ever since the initial post-mortem studies suggested rupture of vulnerable plaque accounted for an overwhelming majority (~ 75%) of myocardial infarctions (Falk et al., 1995, Virmani et al., 2006, Davies, 1996), there has been a growing interest in further characterising precursor lesions that are prone to rupture. Identifying these high-risk precursor lesions are particularly important because a number of clinical studies have shown that the degree of luminal stenosis cannot predict the timing or location of acute coronary events (Hackett et al., 1988, Ambrose et al., 1988).

Furthermore, the majority of coronary events occur at locations previously identified as having angiographically mild disease (Varnava et al., 2002, Smits et al., 1999).

More recently, large-scale studies of patients who underwent CT coronary angiography have shown that 48-54% of patients who went on to experience a coronary event did not have obstructive coronary artery disease at prior imaging (Hoffmann et al., 2017, SCOT-HEART, 2018). This highlights the importance of identifying high-risk features that may confer vulnerability to plaque rupture.

An increasing body of evidence suggests that there are two phenotypically distinct vulnerable plaque types (Libby et al., 2019); the ‘rupture prone plaque’ and the ‘erosion prone plaque’. The histological characteristics of ‘rupture prone plaque’, often termed ‘thin-capped fibroatheroma’ (TCFA), are well defined. They typically have a macrophage rich inflammatory component. Inflammation plays a pivotal role in both the initiation of atherosclerosis and in its propagation (Libby, 2012).

Mediated by risk factors, a pro-inflammatory state initially leads to the expression of

vascular cell adhesion protein-1 and P-selectin (Vita, 2011). This facilitates the recruitment of monocytes and lymphocytes and the secretion of further pro-inflammatory cytokines (Boring et al., 1998). The accumulation of atherogenic lipoproteins and lipid-rich macrophages (foam cells) eventually leads to hypoxia within the vessel intima and the formation of a necrotic core, another feature of unstable plaque. A stabilising collagen-rich fibrous cap covers this core; however, in unstable lesions this fibrous cap undergoes degradation and thinning due to an imbalance between matrix metalloproteinases and collagen synthesis (Aikawa and Libby, 2004).

Local hypoxia and inflammation also stimulate microvessel formation (neovascularisation) within the plaque, making it more prone to intra-plaque haemorrhage and destabilisation (Virmani et al., 2005). Another feature of vulnerable plaque is microcalcification, which is associated with plaque rupture (Vengrenyuk et al., 2006). Microcalcification is thought to arise in part due to vascular smooth muscle cells that secrete extra-cellular vesicles. These matrix vesicles then create a nidus for microcalcification to occur in the extracellular space (Kapustin et al., 2015).

Expansive remodelling is another feature associated with TCFAs, where the patency of the lumen remains preserved despite the plaque occupying a large volume within the vessel wall (Varnava et al., 2002).

It is however, important to be aware that studies have shown less than 5% of TCFAs (identified by intravascular imaging) lead to clinical events (Stone et al., 2011) and most TCFAs transition to a more stable state without ever rupturing (Kubo et al., 2010). These findings highlight the diagnostic challenges faced by clinicians as we

are yet to determine the components of the TCFA that make a plaque most vulnerable and what factors drive transition towards plaque instability. Data from clinical studies suggest that a patient's overall plaque burden and disease activity are important factors in determining long-term prognosis (Stone et al., 2011, Kubo et al., 2008, Kubo et al., 2010). A recent study demonstrated that high risk plaque characteristics and overall coronary artery calcification burden, increased the risk of both non-fatal and fatal cardiac events (Williams et al., 2019).

Erosion prone plaque is a more recently defined pathological entity which has been identified in approximately a third of patients diagnosed with acute coronary syndromes (Partida et al., 2018). This type of plaque was associated more frequently with non-ST elevation myocardial infarction (Partida et al., 2018) and occurs more commonly in women (Pasterkamp et al., 2017). Erosion prone plaques appear to be phenotypically different to rupture prone plaques because they have an increased fibrous tissue content composed of proteoglycan and glycosaminoglycans (Kolodgie et al., 2004). They also tend to be deficient in inflammatory cells (Virmani et al., 1999), contain an abundance of vascular smooth muscle cells and are associated with platelet rich 'white' thrombus (Libby et al., 2019, Hu et al., 2014).

Due to the availability of radioligands that bind to specific molecular targets on atherosclerotic plaque, PET imaging can be employed to quantify inflammation, hypoxia, neoangiogenesis and microcalcification within vulnerable plaque (Figure 1).

Figure 1: Molecular targets of PET tracers currently used in clinical studies for imaging atherosclerosis

PET imaging

PET is a non-invasive imaging technique and its most widespread use is in investigating patients with oncological conditions. In cardiology, PET imaging is most frequently used for assessing myocardial perfusion and viability. However, more recently PET imaging has been used to characterise atherosclerosis and heart valves (Chin et al., 2015). PET utilises radiolabelled tracers that bind to molecular targets and decay at predicted rates emitting positrons. When a positron collides with an electron, annihilation (γ) photons are created, which are detected by the PET scanner (Rudd et al., 2008a).

Image quality of the PET scan is determined to a large extent by attenuation of the signal by annihilation photon interactions within tissue (Compton scatter).

Attenuation correction is employed to tackle this issue. Apart from this, during 3D reconstruction of the images, corrections are also applied for dead time and random coincidences. Uptake of a given radiotracer can be quantified in a number of ways. Standardised uptake values (SUV) are one way to quantify the PET signal. SUV is a mathematically derived ratio of the radioactivity concentration in a specific tissue and the injected dose of radioactivity per kilogram of the patient's body weight. The target to background ratio (TBR), which is derived from arterial wall SUV, is more commonly used to present PET data in vascular imaging studies.

Although PET allows the detection of picomolar concentrations of radioligands, this advantage is offset by poor spatial resolution compared to other imaging modalities. Particularly with regards to vascular imaging, this can make true quantification of

radiotracer uptake in atherosclerotic lesions (especially in small calibre vessels such as the coronary arteries) challenging due partial volume effects causing signal spill-in and spill-out. This leads to difficulties in demarcating the region of interest (ROI) in the arterial vessel wall (by avoiding the vessel lumen). This challenge is overcome to a certain extent by co-registering PET images with CT or MRI to provide anatomical landmarks enabling better localisation of the signal.

Advances in vascular PET/CT imaging protocols and software have enabled more reliable attenuation correction, faster scanning times and lower radiation doses (Rudd et al., 2008a, van der Valk et al., 2016). Coronary artery imaging using PET can be particularly challenging due to the small calibre of the blood vessels and artefacts created by cardiac and respiratory motion. Techniques such as cardiac gating using electrocardiograms, respiratory gating and list-mode acquisitions have been employed to reduce motion artefacts (Büther et al., 2009). Compensating for respiratory motion has proved to be particularly difficult and methods employed to overcome this such as respiration synchronised image acquisitions and motion tracking with 4DCT (Fayad et al., 2013), remain restricted to research.

PET/MRI has the advantage of significantly reduced radiation exposure compared to PET/CT and provides more detailed plaque characterisation. However, limited scanner provision, accuracy of MR attenuation correction and quality of MR angiography remain significant hurdles to its widespread replacement of PET/CT for imaging atherosclerosis (Robson et al., 2017b, Robson et al., 2017a).

1. Imaging inflammation in atherosclerosis

¹⁸F-FDG PET

¹⁸F-fluorodeoxyglucose (¹⁸F-FDG) is the most commonly used radioligand in PET imaging. Cells that metabolise glucose take up ¹⁸F-FDG via glucose transporters (GLUT 1 and 3). ¹⁸F-FDG is then phosphorylated by the cytosolic enzyme hexokinase and accumulates within the cell as it cannot undergo glycolysis (Mochizuki et al., 2001). Thus ¹⁸F-FDG uptake quantified using PET, corresponds to real-time metabolic activity in tissue. While all metabolically active cells can take up ¹⁸F-FDG, in the case of the ¹⁸F-FDG vascular signal, it appears to correlate mainly to macrophage activity in inflamed plaque. *Ex vivo* histological studies from arterial specimens have shown a strong correlation between ¹⁸F-FDG uptake and macrophage density (Tawakol et al., 2006, Ogawa M, 2012). This finding is thought to be due to increased glucose turnover in macrophages compared to other cells that compose atherosclerotic plaque, leading to upregulation of GLUT transporters on macrophages. The ¹⁸F-FDG signal is also related to local hypoxia which augments glycolysis and leads to up-regulation of GLUT transporters within atherosclerotic plaque via hypoxia-inducible factor 1 α (HIF1 α) gene expression (Pedersen et al., 2013).

¹⁸F-FDG PET and cardiovascular risk prediction

Arterial ¹⁸F-FDG uptake was initially explored in PET/CT studies performed in oncology patients. Although retrospective, these studies demonstrated the possible use of arterial ¹⁸F-FDG PET for cardiovascular risk stratification (Paulmier et al., 2008, Rominger et al., 2009, Figueroa et al., 2013). Studies have demonstrated that higher aortic ¹⁸F-FDG uptake was associated with an increased risk of cardiovascular events

and that the degree of ^{18}F -FDG uptake at baseline could predict the timing of such events (Figuerola et al., 2013, Paulmier et al., 2008). Furthermore, one study demonstrated that considering ^{18}F -FDG uptake in addition to conventional risk factors led to a 27.5% net reclassification in risk prediction compared to using conventional risk factors alone (Table 1).

Table 1: PET imaging and cardiovascular risk prediction

The first prospective clinical study investigating arterial ^{18}F -FDG uptake was done in patients who experienced recent transient ischaemic attacks (Rudd et al., 2002). In this study, the culprit carotid arteries had 27% higher ^{18}F -FDG uptake compared to non-culprit vessels, elucidating that ^{18}F -FDG accumulated in unstable vulnerable plaque that gave rise to symptoms. Subsequent studies have shown that plaques with increased ^{18}F -FDG uptake were associated with ischaemic stroke and early stroke recurrence, independent of carotid artery luminal stenosis (Marnane et al., 2012, Davies et al., 2005). Stroke recurrence may in part be explained by micro-embolic phenomena in unstable plaque (Moustafa et al., 2010); however, more conclusive evidence is required to substantiate this observation. Furthermore, a ^{18}F -FDG PET/MRI study showed that in patients diagnosed with ‘cryptogenic’ stroke, 39% had high-risk plaque features on MRI and these lesions were associated with higher ^{18}F -FDG uptake (Hyafil et al., 2016). These findings are consistent with results from previous studies which have shown that ^{18}F -FDG uptake was higher in plaque that exhibited high-risk features identified on MRI such as a large lipid rich necrotic core and intra-plaque haemorrhage (Silvera et al., 2009, Saito et al., 2013, Chowdhury et al., 2018).

^{18}F -FDG PET imaging may also have a role to play in individuals with subclinical atherosclerosis. A recent study conducted to define thresholds of ^{18}F -FDG uptake for arterial inflammation showed that the majority (>52%) of individuals with atherogenic risk factors had higher ^{18}F -FDG uptake (van der Valk et al., 2016, Fernández-Friera et al., 2019). It also demonstrated that healthy individuals (those who had calcium scores of zero) had significantly lower arterial ^{18}F -FDG uptake than those with a diagnosis of cardiovascular disease. Studies in individuals without a history of cardiovascular disease demonstrated that in both, intermediate and low Framingham risk score groups, vascular inflammation measured using ^{18}F -FDG PET/CT was higher in individuals with high sensitivity C-reactive protein (hsCRP) levels (Yang et al., 2013, Yoo et al., 2016). Interestingly, the correlation between hs-CRP and vascular ^{18}F -FDG uptake in patients with cardiovascular disease has been inconsistent, with at least 5 studies failing to show a statistically significant correlation (Duivenvoorden et al., 2013, Rudd et al., 2009, Rogers et al., 2010).

Vascular inflammation as measured by arterial ^{18}F -FDG PET/CT has also been used to risk stratify other at-risk groups such as human immunodeficiency virus (HIV) positive individuals (Subramanian et al., 2012, Yarasheski et al., 2012), those with non-alcoholic fatty liver disease (Lee et al., 2017) and severe dermatitis (NCT02926807). In these studies the individuals with HIV and fatty liver disease had higher arterial uptake of ^{18}F -FDG compared to matched controls. More recently a ^{18}F -FDG PET/CT study investigated the association between stress and cardiovascular risk. The study demonstrated that activity in the amygdala (a region of the brain associated with stress) correlated with increased arterial inflammation as measured by

^{18}F -FDG PET/CT (Tawakol et al., 2017). Another trial is currently underway that explores this theme further. It aims to recruit individuals with post-traumatic stress disorder (PTSD) to explore the relationship between the activity in the fear system of the brain (amygdala and anterior cingulate cortex) and arterial inflammation as measured by ^{18}F -FDG PET/MRI (NCT03279393).

Apart from playing a role in cardiovascular risk prediction, due to validation of the relationship between arterial ^{18}F -FDG uptake and vascular inflammation (Bucci et al., 2014) and the excellent inter-scan reproducibility of ^{18}F -FDG PET/CT imaging in large arteries (Rudd et al., 2009, Rudd et al., 2007b, Rudd et al., 2008b), the arterial ^{18}F -FDG signal is increasingly being used as a surrogate endpoint in cardiovascular interventional studies (Tarkin et al., 2018).

^{18}F -FDG PET in cardiovascular intervention trials.

A number of experimental studies involving patients with CVD or cardiovascular risk factors have examined the effect of various statin therapies on arterial inflammation using ^{18}F -FDG PET imaging (Ishii et al., 2010, Wu et al., 2012, Rudd et al., 2007a, Subramanian et al., 2013, Tahara et al., 2006). Notably, a rapid dose-dependent reduction in arterial ^{18}F -FDG uptake was observed in a double-blind trial that compared treatment of high-dose to low-dose atorvastatin (Tawakol et al., 2013). ^{18}F -FDG has since been used to explore similar effects of other lipid lowering therapies such as evolocumab, a PCSK9 inhibitor. This study failed to demonstrate a reduction in vascular inflammation (Stiekema et al., 2018). Table 2 summarises cardiovascular interventional trials done using PET imaging.

Table 2: PET imaging in cardiovascular interventional trials

In the Dal-PLAQUE trial (Fayad et al., 2011), the effects of dalcetrapib, a modulator of cholesteryl ester transfer protein activity was investigated in patients with CVD or cardiovascular risk factors. This study failed to show significant changes in arterial inflammation with treatment, a result which was later mirrored in a phase III clinical trial that was terminated early due to futility (Schwartz et al., 2012).

More recently ^{18}F -FDG PET/CT has been used to study the efficacy of novel anti-inflammatory agents in cardiovascular disease. Studies have looked at the p38 mitogen-activated protein kinase inhibitors, losmapimod (Elkhawad et al., 2012) and BMS-582949 (Emami et al., 2015). These studies done in patients with established cardiovascular disease, did not show significant reductions in arterial ^{18}F -FDG uptake with treatment. Similar results were demonstrated in a ^{18}F -FDG PET/CT study investigating another potential anti-inflammatory agent, rilapladip, a lipoprotein associated phospholipase A₂ inhibitor (Tawakol et al., 2014).

^{18}F -FDG PET/CT has also been used to study the off-target effects of pioglitazone in atherosclerosis. One study showed that treatment with pioglitazone resulted in a decrease in ^{18}F -FDG uptake in the left main coronary trunk (Nitta et al., 2013).

Interestingly, in a randomised controlled study done in patients with stable coronary artery disease, those who had ^{18}F -FDG uptake above the median at baseline showed a significant reduction in inflammation when treated with pioglitazone in addition to a

statin (Choo et al., 2018). This highlights the potential for using ^{18}F -FDG to tailor therapy to populations who may most benefit from the treatment.

The results of two interventional trials assessing the effect of (a) etanercept on vascular inflammation and atherosclerosis in psoriasis patients (NCT01522742) and (b) colchicine on vascular inflammation in patients with stable CAD (NCT02162303); are currently awaited. A ^{18}F -FDG study investigating the lowest threshold for benefit from LDL-C lowering in patients with stable cardiovascular disease is also currently underway (NCT03355027).

^{18}F -FDG PET/CT has also been employed to study potential circulating blood biomarkers of inflammation. In two recent studies, arterial ^{18}F -FDG uptake was associated with circulating resistin levels (Choi et al., 2011) and adipocyte fatty acid-binding protein levels (Yoo et al., 2011), independent of other cardiovascular risk factors. This highlights the scope for using PET imaging to improve future CVD diagnostic techniques by aiding the development of potential circulating blood biomarkers.

Challenges in using ^{18}F FDG PET for plaque imaging

The main limitation of using ^{18}F -FDG in imaging atherosclerosis is that as the tracer is taken up by all glucose metabolising cells, its signal is not inflammatory-cell specific. This is particularly important when imaging coronary arteries, as myocardial cells take up ^{18}F -FDG avidly. This makes it difficult to assess and quantify ^{18}F -FDG uptake in the coronary arteries accurately (Tarkin et al., 2017, Saam et al., 2010,

Cheng et al., 2012). Similarly, ^{18}F -FDG uptake by the thyroid and structures in the neck in close proximity to the carotid arteries can pose a challenge when imaging these vessels. Thus, there is clearly a need to develop alternative PET tracers with greater specificity for arterial inflammation.

Choline imaging

^{18}F -Fluorocholine (^{18}F -FCH) and ^{18}F -methylcholine (^{18}F -FMCH) are used clinically to aid the diagnosis and staging of prostate cancer. Choline, an integral component of cell membranes, is taken up by activated macrophages and incorporated into their cell membranes following phosphorylation. As with ^{18}F -FDG imaging, initial data on arterial uptake of ^{18}F -FMCH comes from retrospective analyses of scans performed in patients undergoing imaging for oncological indications (Bucerius et al., 2008). A recent prospective study undertaken in stroke patients demonstrated that arterial ^{18}F -FMCH uptake was higher in the culprit carotid arteries and correlated with the degree of macrophage infiltration in endarterectomy samples (Vöö et al., 2016).

Currently two feasibility studies employing ^{18}F -FCH PET/MRI are underway. The first of these studies will utilise ^{18}F -FCH PET/MR along with optical coherence tomography (OCT), to validate this tracer in patients with active coronary artery disease (NCT03252990). The second study aims to recruit patients with stable and unstable cardiovascular disease and compare arterial ^{18}F -FCH uptake between the two disease states. In this study patients with symptomatic carotid artery disease who undergo carotid endarterectomy surgery will have ^{18}F -FCH uptake compared to CD68 staining (a marker of plaque inflammation) on histological samples (NCT02640313).

Somatostatin receptor imaging

Somatostatin receptors are G-protein coupled transmembrane receptors, which mediate a broad range of functions in the body when bound by somatostatin and other endogenous circulating inhibitory peptides. ^{68}Ga -[1,4,7,10-tetraazacyclododecane-*N,N',N'',N'''*-tetraacetic acid]-D-Phe¹, Tyr³-octreotate (DOTATATE) is a PET tracer with highly specific binding affinity for [somatostatin receptor 2](#) (SSTR2), currently in clinical use for imaging neuroendocrine tumours. Somatostatin receptor 2 is expressed in activated macrophages, and therefore, ^{68}Ga -DOTATATE represents an attractive molecular probe by which to detect plaque inflammation (Armani et al., 2007).

Preclinical data from a murine model of atherosclerosis (Li et al., 2013) and retrospective analysis of imaging datasets from oncology patients (Malmberg et al., 2015, Rominger et al., 2010) demonstrated the feasibility of vascular ^{68}Ga -[DOTATATE](#) imaging. These retrospective clinical studies showed that vascular ^{68}Ga -DOTATATE signals were moderately correlated with the presence of coronary artery calcification and other cardiovascular risk factors, but did not specifically co-localise to areas of high ^{18}F -FDG uptake (Li et al., 2012). Importantly, there was low background uptake of the radiotracer by myocardial cells, permitting tracer uptake analysis within coronary arteries.

In 2015, a dedicated vascular PET/MRI study done in patients scheduled for carotid endarterectomy surgery showed that arterial ^{64}Cu -DOTATATE uptake was higher in

the culprit carotid artery compared to the non-culprit vessel (Pedersen et al., 2015). Findings from this study suggested that the ^{64}Cu -DOTATATE signals in culprit carotid arteries were associated with upregulation of CD163 gene expression, which is a marker of alternatively activated macrophages. Thus ^{68}Ga -DOTATATE potentially provides a more specific tracer to study inflammation in atherosclerotic plaque.

A more recent study provided further clinical, histological and gene-expression level data to support the use of ^{68}Ga -DOTATATE for imaging macrophage-related inflammation in atherosclerosis (Tarkin et al., 2017). In this study, patients with stable and unstable cardiovascular disease underwent prospective ^{18}F -FDG and ^{68}Ga -DOTATATE PET/CT imaging. ^{68}Ga -DOTATATE accurately identified culprit from non-culprit coronary and carotid lesions, as well as stable coronary lesions with high-risk plaque morphology. In contrast, ^{18}F -FDG imaging in the coronary vasculature was significantly hampered by excessive myocardial signal spillover. The study also confirmed specific ^{68}Ga -DOTATATE binding to SST2 receptors in CD68-positive macrophage-rich carotid plaque regions, and demonstrated that carotid SSTR2 mRNA correlated with in vivo ^{68}Ga -DOTATATE PET signals.

Translocator Protein Imaging

The ^{11}C -labelled PET tracer PK11195, is a selective radioligand of the translocator protein (18KDa). This [translocator protein](#) (TSPO) was shown to be upregulated in activated macrophages in pre-clinical models (Scarf and Kassiou, 2011). This finding was further corroborated by studies performed using explanted human carotid

endarterectomy specimens, which demonstrated that the translocator protein was found on CD68 positive macrophages (Fujimura et al., 2008, Lomare et al., 2011). Following investigations in vasculitis patients (Pugliese et al., 2010, Lomare et al., 2011), a study done on patients with carotid atherosclerosis showed that patients with symptomatic carotid artery plaque (stroke/TIA) in the preceding three months had increased arterial ^{11}C PK11195 uptake compared to asymptomatic individuals (Gaemperli et al., 2012).

Newer, non-carbon-11 TSPO tracers are currently being evaluated. ^{18}F -GE-180 (*S,N,N*-diethyl-9-[2- ^{18}F -fluoroethyl]-5-methoxy 2,3,4,9-tetrahydro-1H-carbamazole-4-carboxamide) is one such tracer that has been used in animals models which appears to have lower non-specific binding and a higher signal-to-noise ratio (Dickens et al., 2014, Boutin et al., 2015). PBR28 ([^{11}C]N-acetyl-N-(2-methoxybenzyl)-2-phenoxy-5-pyridinamine), another TSPO tracer, showed a more specific vascular signal than ^{11}C -PK11195 in non-human primates. This tracer has been evaluated in a clinical trial (NCT00547976) and the results are awaited.

Further studies are required to test the feasibility of these second generation TSPO radioligands in a clinical setting and it remains to be seen whether the variable receptor binding affinity due to genetic polymorphisms will pose a challenge to the widespread use of these newer tracers.

Aluminium fluoride-18-labeled 1,4,7-triazacyclononane-1,4,7-triacetic acid conjugated folate (^{18}F -FOL)

^{18}F -FOL is a radioligand that binds to folate receptor- β (FR- β). FR- β is selectively expressed on macrophages making it a potential target for molecular probes aimed at studying inflammation in atherosclerosis. A study done using tissue samples from mice, rabbits and humans; demonstrated that ^{18}F -FOL co-localised to FR- β positive macrophages in atherosclerotic plaque. Furthermore, carotid endarterectomy samples from patients who experienced recent cardiovascular events showed increased ^{18}F -FOL uptake co-localised to areas of inflamed plaque (Silvola et al., 2018). Using PET/CT imaging in mice and rabbits the study demonstrated that ^{18}F -FOL accumulation in atherosclerotic plaque corresponded to areas of high macrophage density. Compared to ^{18}F -FDG, ^{18}F -FOL had considerably lower uptake in myocardial cells, potentially making it a radioligand that could be used to study coronary artery atherosclerosis.

2. PET imaging of microcalcification

Coronary artery macrocalcification as quantified by the Agatston score is a well validated marker of CVD burden and future cardiovascular risk. The presence of microcalcification (calcium deposits $<50\mu\text{m}$), which cannot be detected on CT or MRI, is a histological feature observed in early atherosclerotic lesions as well as high-risk rupture-prone plaque. The hydroxyapatite crystallisation on necrotic debris and matrix vesicles causes strain concentration patterns in the fibrous cap of the atherosclerotic plaque, potentially making it more prone to micro-fractures and rupture (New and Aikawa, 2011, Roijers et al., 2011).

^{18}F -sodium fluoride (^{18}F -NaF) is a PET radiotracer conventionally used to detect bony metastases in oncology patients. ^{18}F -NaF binds to hydroxyapatite crystals by exchanging hydroxyl ions to form fluorapatite. A recent study done using an *in vitro* 3D collagen platform, *ex-vivo* human endarterectomy specimens and a mouse model demonstrated that ^{18}F -NaF PET/CT was able to identify arterial microcalcification not detected by CT and these results were further validated using infra-red fluorescence imaging and histology (Creager et al., 2019). These results are in-keeping with findings of a previous study performed on carotid endarterectomy specimens that demonstrated ^{18}F -NaF had a high affinity, sensitivity and specificity for plaque microcalcification (Irkle et al., 2015).

Initial retrospective analyses of ^{18}F -NaF PET/CT data from oncology patients showed that measurement of arterial ^{18}F -NaF uptake was feasible, and that uptake was strongly associated with cardiovascular risk factors (Derlin et al., 2010). Similar results were also observed in another study where patients with higher coronary artery ^{18}F -NaF uptake had higher rates of prior cardiovascular events and higher Framingham risk scores (Dweck et al., 2012). Interestingly, a recent study demonstrated that only 15% of individuals diagnosed with diabetes had high ^{18}F -NaF uptake in the coronary arteries, potentially helping to better risk stratify this cohort of patients who have classically been thought of as being at higher risk of cardiovascular events (Raggi et al., 2019).

^{18}F -NaF is particularly suited for characterising microcalcification in coronary artery plaque due to relatively low tracer uptake by myocardial cells. A prospective study performed in patients who underwent PET/CT and invasive coronary angiography,

showed that ^{18}F -NaF uptake was higher in culprit coronary arteries than non-culprit arteries in 93% of patients diagnosed with a myocardial infarction (Joshi et al., 2014). In contrast coronary artery ^{18}F -FDG uptake could not be distinguished from patchy myocardial uptake in 52% of vessel territories, despite patients following a special diet prior to undergoing ^{18}F -FDG imaging to minimise myocardial uptake. Where coronary artery ^{18}F -FDG signals were interpretable, tracer uptake was not significantly different between culprit and non-culprit coronary arteries. Furthermore, in patients with stable angina whose coronary arteries had increased ^{18}F -NaF uptake, intravascular ultrasound demonstrated other high-risk plaque features such as a necrotic core and expansive remodeling. The highly selective nature of ^{18}F -NaF for intra-plaque microcalcification, coupled with its low uptake in myocardial cells, makes ^{18}F -NaF a promising imaging probe for studying vulnerable coronary artery plaque.

A large study utilising ^{18}F -NaF imaging in patients diagnosed with myocardial infarction and multi-vessel coronary artery disease that aims to recruit up to 700 patients is currently underway (NCT02278211). The study aims to investigate the ability of ^{18}F -NaF PET/CT to predict plaque progression identified by CTCA, as well as longer-term clinical outcomes. Another study is planned in patients with a recent diagnosis of stroke, and aims to use transcranial doppler to further characterise ^{18}F -NaF positive atherosclerotic plaque lesions (NCT03215563).

^{18}F -NaF uptake is also being used in clinical trials to provide surrogate imaging endpoints in cardiovascular interventional trials. The results from two interventional studies investigating the effects of rosuvastatin (NCT03233243) and ticagrelor

(NCT0211033) on atherosclerotic plaque metabolism in the targeted treatment of individuals with high arterial ^{18}F -NaF uptake at baseline, are awaited.

Figure 2: PET imaging of Inflammation and microcalcification in atherosclerosis

3. PET imaging of hypoxia

Hypoxia is a feature of atherosclerotic plaque. Pre-clinical studies performed in rabbit atherosclerosis models have shown that ^{18}F -Fluoromisonadazole (FMISO) PET can be used to identify hypoxia as characterised by pimonidazole staining (Mateo et al., 2014). Hypoxia is thought to potentiate the inflammatory response via HIF-1 α expressed by macrophages in the plaque core, the most hypoxic area within the plaque. A clinical study conducted using both ^{18}F -FMISO and ^{18}F -FDG in patients with recent ischemic cerebrovascular events, showed that vascular uptake of ^{18}F -FMISO was higher in symptomatic carotid arteries compared to asymptomatic vessels (Joshi et al., 2017). This trial also demonstrated that plaque inflammation and hypoxia were closely correlated, with hypoxia underpinning about 40% of the measured ^{18}F -FDG signal.

^{18}F -2-(4-((2-nitro-1H-imidazol-1-yl)methyl)-1H-1,2,3-triazol-1-yl) propanolol (^{18}F -HX4) is another tracer that has been used in clinical studies to measure hypoxia in atherosclerosis. In a small study arterial ^{18}F -HX4 uptake was shown to localise to areas of atherosclerosis in patients who experienced a cardiovascular event in the preceding six months (van der Valk et al., 2015). More studies are needed to validate the role of this radiotracer in atherosclerosis.

Despite these impressive results demonstrating the relationship between hypoxia and inflammation within atherosclerotic plaque, low signal to noise ratios and high tracer costs have limited the widespread adoption of these radiotracers.

4. PET imaging of neoangiogenesis

Neoangiogenesis, which predisposes plaque to intra-plaque haemorrhage, is another feature of vulnerable plaque (Doyle and Caplice, 2007). Of the radiotracers used to image neoangiogenesis, the Arg–Gly–Asp (RGD) peptides targeting [αVβ3 integrin](#) have been the most widely studied in the context of atherosclerosis. αVβ3 integrin is expressed in a number of cells, notably endothelial cells and macrophages (Brooks et al., 1994). The expression of αVβ3 integrin is vital to endothelial cell sprouting in neoangiogenesis. This process has also been shown to be orchestrated to a degree by macrophages (Gurevich et al., 2018). Interestingly αVβ3 integrin expression on macrophages has also been implicated in their transition into foam cells.

⁶⁸Ga-1,4,7- triazacyclononane-1,4,7-triacetic acid (NOTA) -Arg–Gly–Asp (RGD) and ¹⁸F-Galacto-RGD PET tracers which bind to αVβ3 integrin have been studied in pre-clinical and to a limited extent in clinical atherosclerosis (Laitinen et al., 2009, Beer et al., 2006). In a study performed in patients undergoing imaging for solid tumours, arterial ¹⁸F-Galacto-RGD uptake correlated with tissue microvessel density and with αVβ3 integrin staining on histology (Beer et al., 2006). Furthermore, in 10 patients with high-grade carotid artery stenosis, ¹⁸F-Galacto-RGD accumulated in culprit atherosclerotic plaque and arterial ¹⁸F-Galacto-RGD uptake again correlated with

α V β 3 integrin expression (Beer et al., 2014). Dedicated studies are required to explore the scope of RGD PET tracers in imaging atherosclerosis. A study aiming to investigate arterial ^{18}F -FPP-RGD2 uptake in patients with significant carotid artery stenosis and advanced abdominal aortic aneurysms, is due to commence shortly (NCT02995642).

Another integrin PET tracer, ^{18}F -Fluciclatide, has been shown to correlate with atherosclerosis burden. A 46 participant study showed that ^{18}F -Fluciclatide uptake in the thoracic aorta was higher in patients with ischaemic heart disease and hypercholesterolaemia (Jenkins et al., 2015). However, studies have also shown that ^{18}F -Fluciclatide uptake correlates with fibrosis in tissue (Chen et al., 2017, Jenkins et al., 2017), thus more studies are required to characterise the role of ^{18}F -Fluciclatide in atherosclerosis imaging.

The α 7 nicotinic acetylcholine receptors (α 7nAChR), a subtype of nicotinic receptors, have also been implicated in angiogenesis. α 7nAChRs are highly expressed in endothelial cells as well as macrophages and the role of α 7nAChRs in atherosclerosis has been demonstrated in pre-clinical studies that have shown α 7nAChRs agonists accelerate atherosclerosis (Koga et al., 2014). Although, a number of radioactive tracers, both agonists (^{18}F -NS14490) and antagonists (^{18}F -ASEM), are available to target α 7nAChR (Boswijk et al., 2017) these have not yet been investigated in the context of atherosclerosis in clinical studies or clinical specimens.

The natriuretic peptide clearance receptor is another molecular target that appears to be upregulated in angiogenic lesions (with co-localisation in endothelial and smooth

muscle cells) in mouse models (Liu et al., 2011). A novel molecular nano-probe integrating ^{64}Cu -labeled C-type atrial natriuretic factor (CANF), that can be detected by PET/MRI, is currently under investigation in a feasibility study recruiting patients who are due to undergo carotid endarterectomy (NCT02417688).

Other novel PET tracers

^{68}Ga -pentixafor is a radiolabelled PET tracer selective for [CXC chemokine receptor 4](#) (CXCR4), these receptors are expressed on endothelial cells and macrophages, as well as a range of other immune cells. Pre-clinical studies suggest CXCR4 may have a role to play in endothelial progenitor recruitment and the transition of macrophages into foam cells (Yao et al., 2012). Retrospective studies in oncology patients have shown that ^{68}Ga -pentixafor uptake correlated with cardiovascular risk factors and atherosclerotic plaque burden (Li et al., 2018, Weiberg et al., 2018, Li et al., 2019). The first dedicated vascular study using ^{68}Ga -pentixafor was done in patients who underwent percutaneous coronary intervention following their first ST-elevation myocardial infarction (Derlin et al., 2018). ^{68}Ga -pentixafor uptake was highest in the culprit coronary artery with lower uptake in stented non-culprit arteries. The lowest ^{68}Ga -pentixafor uptake was observed in 'naïve' coronary segments. *Ex-vivo* studies done on coronary artery specimens showed that CXCR4 was overexpressed in atherosclerotic lesions and mainly co-localised with CB68+ cells.

^{18}F -Flutemetamol

^{18}F -[Flutemetamol](#) is a PET tracer with a high affinity for amyloid beta, extensively studied in patients with Alzheimer's disease. Amyloid beta is also associated with atherosclerosis (Gupta and Iadecola, 2015), with *in vitro* studies suggesting that a major part of this association is based on the ability of amyloid beta to trigger macrophage activation and plaque instability. Currently a feasibility study using PET/MRI is underway investigating arterial ^{18}F -Flutemetamol uptake in individuals who have recently had a stroke or TIA and have carotid artery stenosis (NCT03291093). This study will evaluate ^{18}F -Flutemetamol uptake in both carotid and coronary arteries.

^{11}C -acetate

Lipids play an important role in the development and progression of atherosclerotic plaque. Evidence from several studies has demonstrated that *de novo* fatty acid synthesis occurs within atherosclerotic lesions (Filipovic and Rutemöller, 1976, Derlin et al., 2011). Intra-plaque fatty acid synthesis is essential for macrophage-mediated inflammation, which in turn affects plaque stability. *De novo* fatty acid synthesis is mediated by the multifunctional enzyme fatty acid synthase (FAS) in arterial lesions. FAS requires acetyl-coenzyme-A, derived from acetate for the production of fatty acids. Thus ^{11}C -acetate could potentially be used to identify vulnerable plaque, although currently its main clinical use is in oncology. A ^{11}C -acetate PET/CT feasibility study undertaken in patients with oncological conditions showed vascular ^{11}C -acetate uptake could be quantified and that in 30% of cases ^{11}C -acetate uptake co-localised to areas of calcified plaque. A limitation of using this tracer is its short half-life and high uptake by myocardial cells (Derlin et al., 2011).

Dedicated studies are required to characterise its role in atherosclerotic plaque evaluation.

Table 3: PET tracers used in clinical research for atherosclerosis imaging

Conclusions and future directions.

The diverse molecular imaging targets that can be harnessed by PET imaging offers a wide range of tools by which to explore the pathophysiology of atherosclerosis and identify vulnerable plaque. The validation of more specific PET tracers will enable us to not only characterise vulnerable plaque better but also aid more accurate risk prediction in cardiovascular disease.

Methods for vascular imaging using hybrid PET/CT and PET/MRI scanners continue to advance (Robson et al., 2017a). The immediate advantages of PET/MRI over PET/CT scanning are lower radiation exposure allowing serial imaging for longitudinal studies, integrated cardiac and respiratory motion correction facilities built into the scanners, and improved soft tissue characterization of MRI compared to CT. While these new scanners also have improved detector sensitivity, as well as other technological advances important for vascular imaging, major challenges such as accurate attenuation correction remain (Silvera et al., 2009, Saito et al., 2013, Calcagno et al., 2013).

Furthermore, pre-clinical studies using PET/MRI have demonstrated proof of concept for using combinations of nanotracers for multi-parametric characterisation of atherosclerotic plaque (Senders et al., 2018). These however need further validation in clinical trials. Currently the commonest use of hybrid PET/MRI is in assessing myocardial function and viability post myocardial infarction. Other clinical applications of hybrid PET/MRI include the assessment of myocarditis and cardiomyopathies (Nensa et al., 2018).

Imaging coronary atherosclerosis using PET is particularly challenging due to motion artefacts that occur as a result of cardiac and respiratory motion. There is also a greater risk of misclassification of tracer uptake in the coronary arteries due to motion artefact as there is only a relatively small difference in tracer uptake between culprit and non-culprit coronary arteries (Rubeaux et al., 2016). Furthermore, due to the lack of anatomical landmarks general purpose motion correction methods are not particularly useful in the assessment of coronary arteries. However, a recent feasibility study done using motion corrected PET data reconstruction demonstrated a novel technique to reduce noise and increase TBR signals with ^{18}F -NaF PET/CT (Rubeaux et al., 2016).

In conclusion, PET imaging is a powerful tool that can provide important pathophysiological insights into the pathophysiology of atherosclerosis and enable more accurate characterisation of high-risk plaque, especially with the advent of hybrid imaging techniques and more specific tracers. PET imaging is also increasingly being used in cardiovascular interventional trials as a surrogate marker of

drug efficacy, and is proving to be an efficient and cost-effective method for bridging the translational divide that exists in drug development.

Competing Interests' Statement

No competing interests to declare.

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Figure 1: Molecular targets of PET tracers currently used in clinical studies for imaging atherosclerosis

Inflammation: ^{18}F -FDG is the most-widely used and validated tracer. ^{18}F CH, ^{18}F -FMCH, ^{68}Ga -DOTATATE, ^{18}F -FOL and ^{11}C -PK11195 are others used in clinical research.

Hypoxia: FMISO and ^{18}F -HX4 are tracers currently being trialed for imaging hypoxia in atherosclerosis

Neovascularization: These tracers target integrin found on vascular smooth muscle cells and on macrophages. RGD PET tracers such as ^{18}F -Galacto-RGD, and ^{18}F -Fluciclatide are currently being evaluated for imaging atherosclerosis.

Microcalcification: ^{18}F -NaF is increasingly being used to image vulnerable plaque in atherosclerosis by targeting microcalcification.

Other: ^{68}Ga -pentixafor is a radiolabelled PET tracer selective for CXCR4 found on macrophages and endothelial cells. ^{18}F -Flutemetamol is a PET tracer with high affinity for amyloid beta (Ab), which has been associated with atherosclerosis. ^{11}C -acetate is implicated in fatty acid synthesis.

Abbreviations: ^{18}F -FDG, ^{18}F -fluorodeoxyglucose; ^{18}F CH, ^{18}F -Fluorocholine; ^{18}F -FMCH, ^{18}F -methylcholine; DOTATATE, [1,4,7,10-tetraazacyclododecane-N,N',N'',N'''- tetraacetic acid]-D-Phe1,Tyr3-octreotate; ^{18}F -FOL, Aluminum fluoride-18-labeled 1,4,7-triazacyclononane-1,4,7-triacetic acid conjugated folate; FMISO, ^{18}F -Fluoromisonadazole; ^{18}F -HX4, ^{18}F -2-(4-((2-nitro-1H-imidazol-1-yl)methyl)-1H-1,2,3-triazol-1-yl) propranolol; RGD, Arg-Gly-Asp; ^{18}F -NaF, ^{18}F -Sodium fluoride; CXCR4, CXC chemokine receptor 4; SSTR2, somatostatin receptor type 2; TSPO, translocator protein.

Figure 2: PET imaging of Inflammation and microcalcification in atherosclerosis

A) Coronary artery inflammation as visualised by ^{18}F -FDG/CT (B) Carotid artery inflammation as visualised by ^{18}F -FDG/CT (C) ^{18}F -NaF PET/CT image of symptomatic right carotid artery (D) Coronary artery inflammation visualized by ^{68}Ga -DOTATATE (E) Carotid artery inflammation visualised by ^{68}Ga -DOTATATE (F) ^{18}F -NaF PET/CT image of symptomatic coronary artery

B, C, E, F Modified from Tarkin et al, 2017 with permission from the copyright holder; C courtesy of Nick Evans; F modified from Joshi et al, 2014 with permission from the copyright holder)

Study	N	Radiotracer	Result
Prospective observational study done in oncology Patients (Rominger et al., 2009)	932	¹⁸ F-FDG	Arterial meanTBR > 1.7 is an independent predictor for (hazard ratio =14; <i>P</i> < 0.001).
Prospective observational study in oncology patients with stable disease (Paulmier et al., 2008)	101	¹⁸ F-FDG	Individuals in the high arterial ¹⁸ F-FDG uptake group had cardiovascular events in the six months after the scan compared to Vs 1.8 %, <i>p</i> = 0.0002).
Prospective observational study done in cancer free patients who underwent PET/CT for oncological indications (Figuerola et al., 2013)	513	¹⁸ F-FDG	Aortic TBR values strongly predicted future CVD even after adjustment for traditional risk factors (hazard ratio = 4.17, <i>p</i> =0.004). ¹⁸ F-FDG uptake inversely proportional to CVD risk.
Prospective observational study in patients with stroke, TIAs, retinal emboli (Marnane et al., 2012)	60	¹⁸ F-FDG	80% of those with higher arterial ¹⁸ F-FDG (SUV _{mean} > 2.0) had cerebrovascular events in the 30 days compared to those with low uptake (<i>p</i> <0.0001).
Prospective observational cohort study in patients who underwent angioplasty for peripheral vascular disease (Chowdhury et al., 2019)	50	¹⁸ F-FDG ¹⁸ F-NaF	Arterial ¹⁸ F-FDG (TBR _{max} > 1.98) and ¹⁸ F-NaF (TBR _{max} > 1.98) were independent predictors of vessel restenosis (<i>p</i> < 0.001).

Table 1: PET imaging and cardiovascular risk prediction.

TIA – transient ischaemic attacks, ¹⁸F-FDG - ¹⁸F-fluorodeoxyglucose, ¹⁸F-NaF - ¹⁸F-sodium fluoride, TBR- Target to background ratio, PET - positron emission tomography, CT- computed tomography, CVD – cardiovascular disease.

Study population	N	Study Design	Drug	Imaging modality	Result
Statin naïve patients with established CVD (Tawakol et al., 2013)	83	Double-blind randomised multicentre trial	Atorvastatin 10mg/day or atorvastatin 80mg/day	¹⁸ F-FDG PET/CT (3 scans – baseline, 4 weeks, 12 weeks)	Rapid dose-dependent increase in ¹⁸ F-FDG uptake (p<0.001).
Statin naïve patients undergoing scanning for cancer screening (Tahara et al., 2006)	43	Randomised controlled study	Simvastatin (5-20mg/day) + dietary management or only dietary management	¹⁸ F-FDG PET/CT (2 scans – baseline and 3 months)	A reduction in cardiovascular events with statin treatment (P<0.01).
Statin naïve patients with established CVD	43	Open-label single centre	Atorvastatin 40mg/day	¹⁸ F-FDG PET/CT (2 scans – baseline and 12 weeks)	A reduction in cardiovascular events over 12 months of treatment.

(Wu et al., 2012)		study			
Statin naïve patients with angina due to undergo percutaneous coronary intervention (Ishii et al., 2010)	30	Randomised, investigator blinded, open label study	5mg/day or 20mg/day of atorvastatin once a day	¹⁸ F-FDG PET/CT (2 scans – baseline and 6 months)	A reduction in months of 20mg treatment P=0.007, f
Patients with known CVD or risk factors not on high dose statins (Subramanian et al., 2013)	83	Randomised, double blind, multicentre study	10mg/day or 80mg/day atorvastatin	¹⁸ F-FDG PET/CT (3 scans - baseline, 4 weeks and 12 weeks)	A reduction in uptake at 12 weeks of atorvastati
Patients with dyslipidaemia (Stiekema et al., 2018)	129	Randomised, double-blind, placebo controlled	Evolocumab 420mg/month or placebo	¹⁸ F-FDG PET/CT (2 scans - baseline and 16 weeks)	¹⁸ F-FDG uptake reduced by
Patients with atherosclerosis and on stable statin therapy (Elkhawad et al., 2012)	99	Randomised, double-blind, placebo-controlled study	Losmapimod 7.5mg/day, Losmapimod 7.5mg twice a day or placebo	¹⁸ F-FDG PET/CT (2 scans - baseline and 84 days)	¹⁸ F-FDG uptake reduced by
Patient with known atherosclerosis or risk factors and on stable lipid lowering therapy (Fayad et al., 2011)	130	Randomised, double-blind, placebo-controlled study	Dalcetrapid 600mg/day or placebo	¹⁸ F-FDG PET/CT (2 scans - baseline and 24 months)	¹⁸ F-FDG uptake reduced by
Patients with stable cardiovascular disease and on stable statin therapy (Tawakol et al., 2014)	83	Randomised, double-blind, placebo-controlled study	Rilapladipl 250mg/day or placebo	¹⁸ F-FDG PET/CT (2 scans - baseline and 84 days)	¹⁸ F-FDG uptake reduced by
Patients with atherosclerosis with impaired glucose tolerance or T2DM (Nitta et al., 2013)	50	Randomised, active comparator-controlled, single centre trial	Pioglitazone 15-30mg/ day or glimpiride 0.5-4mg/day	¹⁸ F-FDG PET/CT (2 scans - baseline and 16 weeks)	¹⁸ F-FDG uptake in the left coronary artery Pioglitazone or glimpiride
Statin naïve patients with stable CAD who underwent percutaneous coronary intervention and had carotid atherosclerosis (Choo et al., 2018).	41	Open-labelled, randomised, controlled single centre study	Atrovastatin 30mg/day or Atorvastatin 30mg/day + pioglitazone 30mg/day	¹⁸ F-FDG PET/CT (2 scans - baseline and 3 months)	¹⁸ F-FDG uptake reduced in group compared (p=0.58). In patients with uptake above significant inflammatory pioglitazone
Patients with stable atherosclerosis (Emami et al., 2015)	72	Randomised, placebo controlled	BMS-582949 (100mg once/day),	¹⁸ F-FDG PET/CT (2 scans - baseline and 12 weeks)	¹⁸ F-FDG uptake reduced by

			placebo, or atorvastatin (80mg once/day)		
Patients with severe psoriasis without a history of CVD (NCT01522742)	29	Open-labelled, Single centre prospective observational	Etanercept 50mg once or twice weekly	¹⁸ F-FDG PET/CT and coronary angiography (2 - baseline and 3 months)	Study term
Patients with stable coronary artery disease (NCT02162303)	106	Randomised, double-blind, placebo controlled	Colchicine 0.6mg once/day or placebo	¹⁸ F-FDG PET/CT (2 scans - baseline and 6 months)	Study cor
Patients with multivessel stable coronary artery disease (NCT0211033)	220	Randomised, placebo controlled, quadruple blinded multicentre study	Ticagrelor 90mg BD or Placebo	¹⁸ F-NaF PET/CT (2 scans - baseline and 12 months)	Study cor
Patients with stable cardiovascular disease (NCT03355027)	60	Randomised, open-label, single centre	Alicurumab (150mg) + statin (simvastatin 80mg or rosuvastatin 20-40mg or atorvastatin 40-80mg/day) or Ezitimibe 10mg + statin (simvastatin 80mg or rosuvastatin 20-40mg or atorvastatin 40-80mg/day)	¹⁸ F-FDG PET/CT (2 scans - baseline and 8 weeks)	This study
Patients with cardiovascular risk factors (NCT032332430)	43	Open-labelled, single centre trial	Rosuvastatin 20-40mg once/day	¹⁸ F-NaF PET/CT (2 scans - baseline and 6 months)	This study

Table 2: PET imaging in cardiovascular interventional trials.

CVD – cardiovascular disease, CAD- coronary artery disease, T2DM – type 2 diabetes mellitus, ^{18}F -FDG - ^{18}F -fluorodeoxyglucose, ^{18}F -NaF - ^{18}F -sodium fluoride, PET -positron emission tomography, CT- computed tomography.

Tracer	Target	Comments	Ongoing clinical studies
^{18}F -FDG	Metabolic activity/inflammation	<ul style="list-style-type: none"> Widely used and validated in atherosclerosis Non-specific uptake, high myocardial signal spill over 	<ol style="list-style-type: none"> PPG Project 3 – aiming to recruit 240 patients with PTSD, without PTSD but a background of severe trauma and matched controls; without a history of atherosclerosis. The study aims to compare activity of stress centres of the brain with arterial ^{18}F-FDG uptake (NCT00756379) 159 participant study due to recruit patients with a diagnosis of coronary artery disease. Comparing DCE-MRI, PET/CT and

			<p>PET/MRI (NCT01418313)</p> <p>3. INTENSITY-HIGH – A 60 patient study aiming to investigate the lowest threshold of vascular benefits from LDL cholesterol lowering in patients with stable cardiovascular disease. (NCT03355027)</p>
⁶⁸ Ga-DOTATATE	Activated macrophages	<ul style="list-style-type: none"> • More specific for inflammation • Low myocardial signal spillover, enabling analysis of coronary artery disease 	None
¹¹ CPK11195 and TPSO radioligands	Activated macrophages	<ul style="list-style-type: none"> • Short half life of ~20mins • Non-specific binding • Low signal to noise ratio 	None
¹⁸ F-FOL	Activated macrophages	<ul style="list-style-type: none"> • Low myocardial signal 	None

		<ul style="list-style-type: none"> spillover Higher cost 	
¹⁸ F-FCH	Activated macrophages	<ul style="list-style-type: none"> Murine models showed better identification of atherosclerotic plaque when compared to ¹⁸F-FDG Low myocardial uptake 	<ol style="list-style-type: none"> PET/MRI study aiming to recruit 35 participants diagnosed with NSTEMI or STEMI as well as patients with multivessel disease due to undergo a second procedure (during which an OCT will be done to investigate if intravascular findings of high risk plaque correlate with ¹⁸F-FCH uptake (NCT03252990)) PET/MRI study of 14 participants with symptomatic carotid artery stenosis who are due to undergo endarterectomy or conservative treatment. In patients undergoing endarterectomy ¹⁸F-FCH uptake will be correlated to CD68 positivity on histology (NCT02640313)
¹⁸ F-NaF	Microcalcification	<ul style="list-style-type: none"> Highly 	<ol style="list-style-type: none"> PET/MRI study

		<p>selective</p> <ul style="list-style-type: none"> • Low myocardial uptake 	<p>aiming to recruit 40 patients with recent cerebrovascular symptoms (stroke, TIA or amaurosis fugax) who are due to undergo carotid endarterectomy. (NCT03215550)</p> <p>2. PET/MRI study aiming to recruit 80 patients with recent cerebrovascular symptoms (stroke, TIA or amaurosis fugax) who have non-significant carotid artery stenosis, have declined surgery or have atherosclerosis of the aorta and its major branches excluding the internal carotid artery (NCT03215563)</p> <p>3. PET/CT study aiming to recruit 700 patients with recent MIs to study the ability of ^{18}F-NaF to detect symptomatic coronary artery plaque</p>
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			<p>(NCT02278211)</p> <p>4. PET/CT study aiming to recruit 43 patients with cardiovascular risk factors on Rosuvastatin 20-40mg once/day comparing baseline to 6 months of treatment (NCT032332430).</p>
FMISO/ ¹⁸ F-HX4	Hypoxia	<ul style="list-style-type: none"> • Low signal to noise ratio • Higher cost 	None
Arg–Gly–Asp (RGD) peptides	Neoangiogenesis	<ul style="list-style-type: none"> • Cost • Not well validated in clinical atherosclerosis 	<p>1. ¹⁸F-FPPRGD2 PET/CT or ¹⁸F-FPPRGD2 PET/MRI study in 20 patients with carotid artery stenosis or advanced abdominal aortic aneurysms in whom a surgical procedure is planned (NCT02995642)</p>
¹⁸ F-Fluciclatide	Neoangiogenesis	<ul style="list-style-type: none"> • Highly selective radioligand for αVβ3 and αVβ5 	None
⁶⁴ Cu-labeled C-type atrial natriuretic factor	Neoangiogenesis	<ul style="list-style-type: none"> • Cost • Limited clinical studies 	<p>1. ⁶⁴Cu-25%-CANF-Comb PET/MRI study</p>

(CANF)			in 22 patients with significant carotid artery stenosis due to undergo carotid endarterectomy (NCT02417688)
⁶⁸ Ga-pentixafor	Inflammation/endothelial progenitor recruitment	<ul style="list-style-type: none"> • Cost • Limited clinical studies 	None
¹⁸ F-Flutemetamol	Macrophage activation	<ul style="list-style-type: none"> • Cost • Limited clinical studies 	1. ¹⁸ F-Flutemetamol PET/MRI study in 25 patients with a recent diagnosis of stroke or TIA, and carotid artery stenosis of >30% will undergo carotid and coronary artery imaging
¹¹ C-acetate	Intraplaque fatty acid synthesis	<ul style="list-style-type: none"> • Short half life • High uptake by myocardial cells 	None

Table 3: PET tracers used in clinical research for atherosclerosis imaging

CANF - C-type atrial natriuretic factor, CT - computed tomography, CD - cluster of differentiation, ⁶⁸Ga-DOTATATE - ⁶⁸Ga-[1,4,7,10-tetraazacyclododecane-*N,N',N'',N'''*-tetraacetic acid]-D-Phe¹, Tyr³-octreotate, ¹⁸F-FDG - ¹⁸F-fluorodeoxyglucose, ¹⁸F-FCH - ¹⁸F-Flourocholine, ¹⁸F-FOL - ¹⁸F-fluoride, 1,4,7-triazacyclononane-1,4,7-triacetic acid conjugated folate, FMSO - ¹⁸F-Fluoromisonadazole, ¹⁸F-NaF - ¹⁸F-sodium fluoride, ¹⁸F-HX4 - ¹⁸F-2-(4-((2-nitro-1H-imidazol-1-yl)methyl)-1H-1,2,3-triazol-1-yl) propranolol, MI – myocardial infarctions, MRI - magnetic resonance imaging, NSTEMI - non-ST elevation myocardial infarction, PET - positron emission tomography, STEMI - ST elevation myocardial infarction, TIA - transient ischaemic attack, TPSO- translocator protein (18KDa).