Recent Advances in the Development of PET/SPECT Probes for Atherosclerosis Imaging

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Abstract The rupture of vulnerable atherosclerotic plaques and subsequent thrombus formation are the major causes of myocardial and cerebral infarction. Accordingly, the detection of vulnerable plaques is important for risk stratification and to provide appropriate treatment. Inflammation imaging using 2-deoxy-2-[¹⁸F]fluoro-D-glucose (¹⁸F-FDG) has been most extensively studied for detecting vulnerable atherosclerotic plaques. It is of great importance to develop PET/SPECT probes capable of specifically visualizing the biological molecules involved in atherosclerotic plaque formation and/or progression. In this article, we review recent advances in the development of PET/SPECT probes for visualizing atherosclerotic plaques and their application to therapy monitoring, mainly focusing on experimental studies.

Keywords Atherosclerosis · Nanocarrier (nanoparticle) · PET/SPECT probes · Radioimmunodetection · Therapy monitoring

Introduction

The rupture of vulnerable atherosclerotic plaques and subsequent thrombus formation are the major causes of

myocardial and cerebral infarction. Accordingly, the identification of vulnerable plaques is important for risk stratification and providing appropriate treatment. For these, various morphological and functional imaging methods are used. Intravascular ultrasound (IVUS) can sensitively classify lesions as soft, fibrous or calcified. Angioscopy can detect vulnerable plaques as yellow plaques. Computed tomography (CT) can noninvasively assess calcifications and fibrous tissue, in addition to important structural information, such as total plaque area, fibrous cap thickness, and the size of the lipid core. The soft-tissue-characterizing capabilities of magnetic resonance imaging (MRI) permit the depiction of various components of atherothrombotic plaques, including lipids, fibrous tissue, calcium, and thrombus formation. On the other hand, various biological molecules are involved in atherosclerotic plaque formation and/or progression, as shown in Fig. 1. It is of great importance to rationally evaluate these molecules to understand the pathological state of atherosclerosis plaques. For these, nuclear imaging techniques, such as positron emission tomography (PET) and single-photon emission computed tomography (SPECT), have several advantages, including their potential to quantify important determinants of plaque vulnerability on the basis of specific cellular and biochemical changes using appropriate molecular probes labeled with radioisotopes. Indeed, as shown in Table 1, extensive studies have been conducted to develop PET/SPECT probes capable of specifically visualizing biological molecules based on the biology of atherosclerosis (Fig. 1) [29–32]. In this article, we review recent advances in the development of PET/SPECT probes for visualizing atherosclerotic plaques and their application to therapy monitoring, mainly focusing on experimental studies.

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Fig. 1 Biological molecules involved in atherosclerotic plaque formation and/or progression. LDL, low-density lipoprotein; oxLDL, oxidized LDL; SR, scavenger receptor; LOX-1, Lectin-like oxidized low density lipoprotein receptor 1; GLUT, glucose transporter; MMP, matrix metalloproteinase; MT1-MMP, membrane type 1- MMP; SSTR, somatostatin receptor; TSPO, translocator protein; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor; MR, mannose receptor

PET/SPECT Probes for Imaging Inflammatory Cells

Inflammation imaging using 2-deoxy-2-[^{18}F]fluoro-D-glucose (^{18}F -FDG) has been most extensively studied for detecting vulnerable atherosclerotic plaques [32]. ^{18}F -FDG, a glucose analog, highly accumulates in cells with increased energy metabolism including inflammatory cells [10]. Accordingly, ^{18}F -FDG

PET enables us determine the infiltration of inflammatory cells, such as macrophages, on the basis of their glucose metabolism, and provides quantitative information on the inflammatory conditions in atherosclerotic plaque formation. Recent in vitro studies have revealed that ^{18}F -FDG uptake level into macrophages is increased during foam cell formation by acetylated low-density lipoprotein (LDL) loading, but the uptake

Table 1 Possible targets and probes for the detection and assessment of atherosclerotic plaques

Process	Target	Probe
Inflammation	Macrophages	$^{99\text{m}}\text{Tc}$ -MCP-1 [1]
	Chemokine (C-C motif) receptor 2 (Ccr2)	^{11}C -choline [2]
	Choline metabolic activity	^{18}F -fluorocholine [3]
	Translocator protein (TSPO)	^{123}I -DPA-713 [4]
	Somatostatin receptor subtype 2	^{11}C -PK11195 [5]
	Phosphatidyl serine receptor	^{64}Cu -DOTATATE [6, 7] ^{68}Ga -DOTATATE [8]
	Foam cell, M1 macrophage	^{111}In -PS200 [9]
	Glucose metabolic activity	^{18}F -FDG [10]
	M2 macrophage	^{18}F -FDM [11]
	Mannose receptor	$^{99\text{m}}\text{Tc}$ -/ ^{123}I -IL-2 [12]
	Lymphocyte	
	Lipoprotein(OxLDL)	^{123}I -AHP [13]
Lipid core and fibrous cap formation	LOX-1 (scavenger receptor)	$^{99\text{m}}\text{Tc}$ -LOX-1-mAb [14]
	Fatty acid synthesis	^{11}C -Acetate [15]
Apoptosis	Phosphatidyl serine	$^{99\text{m}}\text{Tc}$ -annexin A5 [16]
	Caspase-3	^{18}F -isatin derivatives [17]
	Membrane alteration	^{18}F -ML-10 [18]
Angiogenesis	VEGF receptor	^{89}Zr -VEGF-mAb [19]
	Integrin- $\alpha_v\beta_3$	^{18}F -galacto-RGD [20]
		^{18}F -RGD-k5 [21]
Hypoxia	Hypoxia	^{18}F -FMISO [22]
Proteolysis	MMPs	$^{99\text{m}}\text{Tc}$ -/ ^{111}In -/ ^{123}I -/ ^{18}F -MMP inhibitors [23]
		$^{99\text{m}}\text{Tc}$ -MT1-MMP-mAb [24]
Thrombosis	Platelets	^{111}In -platelets [25]
	Tissue factor (TF)	$^{99\text{m}}\text{Tc}$ -TF-mAb [26]
Calcification	Mineral deposition/active calcification	^{18}F -NaF [27, 28]

is decreased to the control level after complete differentiation to foam cells [33]. It was also reported that pro-atherogenic (pro-inflammatory) M1 macrophages preferentially accumulate ^{18}F -FDG compared with M2 macrophages [34]. An important relationship was thus suggested between ^{18}F -FDG uptake and inflammatory conditions in atherosclerotic lesions. On the other hand, it is reported that glucose metabolism and ^{18}F -FDG uptake in macrophages are more strongly enhanced by hypoxic stimuli than by pro-inflammatory cytokines. Indeed, ^{18}F -FDG uptake was positively associated with hypoxic areas in atherosclerotic arteries induced by balloon injury in rabbits fed with a high-cholesterol diet [35]. However, high ^{18}F -FDG uptake was also observed in nonhypoxic macrophage-rich regions of the arteries.

Note that there are limitations in the imaging of atherosclerotic plaques with ^{18}F -FDG [32]. ^{18}F -FDG uptake in atherosclerotic lesions seems to be reduced in patients with a high blood glucose level, owing to the competition between glucose and ^{18}F -FDG for membrane transporters and hexokinase [32]. Recently, Ogawa et al. have demonstrated in a mouse model that ^{18}F -FDG uptake in atherosclerotic lesions is largely affected by blood glucose level [36]. Note also that cholesterol feeding can actually enhance the ^{18}F -FDG accumulation in the aorta without any visible atheroma [37]. In addition to these limitations, which are rather specific to ^{18}F -FDG, the deterioration of renal function and medications may affect the pharmacokinetics of ^{18}F -FDG. Our experimental study in mice showed elevated ^{18}F -FDG levels in blood and organs after angiotensin II receptor blocker (telmisartan) administration [38]. The deterioration of renal function likely causes a decline in the clearance of ^{18}F -FDG from the blood and other organs, indicating a potential increase in background activity on PET imaging.

Despite these limitations, ^{18}F -FDG is the most promising PET probe for imaging vulnerable atherosclerotic plaques [32]. On the other hand, several PET probes such as ^{11}C -choline have been evaluated to overcome the limitations of ^{18}F -FDG [32] (Table 1). Choline is a source for cell membrane lipids, and enhanced ^{11}C -choline uptake has been suggested in macrophages in the atherosclerotic plaques of mice [2]. Several clinical studies confirmed the increased uptake of ^{11}C -choline and ^{18}F -choline analogs in atherosclerotic plaques [32]. Note here that the effect of blood glucose level on ^{11}C -choline uptake was smaller than that on ^{18}F -FDG uptake [36]. ^{11}C -Choline may be used as an alternative to ^{18}F -FDG for examining diabetes patients, for example. PET/SPECT probes more specifically targeting important determinants of plaque vulnerability may solve the several limitations of ^{18}F -FDG owing to its nonspecific nature. In this regard, several PET/SPECT probes for the 18 kDa translocator protein (TSPO) and somatostatin receptor have been evaluated. TSPO is highly expressed on the mitochondrial membrane of activated

macrophages. The potential utility of radiolabeled TSPO ligands has been indicated in the ApoE $^{-/-}$ mouse model (^{125}I -DPA-713) [4] and in patients with carotid stenosis (^{11}C -PK11195) [5].

Recently, PET probes for somatostatin receptor subtype 2 (SSTR-2), which is highly expressed on macrophages, have attracted increasing interest in the evaluation of atherosclerotic plaques [32]. Rinne et al. [39] compared several PET probes targeting SSTR-2 in a mouse model and indicated the superior applicabilities of ^{68}Ga -DOTANOC and ^{68}Ga -DOTATATE in the detection of atherosclerotic plaques compared with ^{68}Ga -FDR-NOC. Several clinical studies have also indicated the potential roles of ^{68}Ga -DOTATATE and ^{64}Cu -DOTATATE in oncology patients and patients undergoing endarterectomy [6–8].

Integrin $\alpha_v\beta_3$, a well-known angiogenesis factor, is another target molecule for imaging inflammatory cells, as integrin $\alpha_v\beta_3$ is highly expressed in activated macrophages [40]. To detect integrin $\alpha_v\beta_3$, several probes such as ^{18}F -galacto-RGD [20] and ^{18}F -RGD-k5 [21] have been developed on the basis of arginyl-glycyl-aspartic acid (RGD)-tripeptide sequence which is implicated in cellular attachment via integrin. Laitinen et al. [20] showed that areas with high ^{18}F -galacto-RGD uptake levels colocalized with macrophage-rich areas in atherosclerotic plaques of ApoE $^{-/-}$ mice.

Given that ^{18}F -FDG preferentially accumulates in pro-atherogenic (pro-inflammatory) M1 macrophages [34], an attempt to develop a PET probe targeting anti-inflammatory M2 macrophages in atherosclerotic lesions has been reported [11]. M2 macrophages are also common in lesions with neovascularization and intraplaque hemorrhage. Tahara et al. [11] attempted to image M2 macrophages using ^{18}F -labeled mannose (2-deoxy-2- ^{18}F fluoro-D-mannose, ^{18}F -FDM) in a rabbit model, because mannose receptors are preferentially expressed on M2 macrophages in high-risk plaques. To date, the relationship between atherosclerotic lesion formation and macrophage polarization remains unclear. The development of PET/SPECT probes that can differentially image each subtype of macrophage (M1 and M2 macrophages) would largely contribute to clarifying the roles of each subtype in atherosclerotic lesion formation.

PET/SPECT Probes for Imaging Targets Other Than Inflammatory Cells

Among the PET/SPECT probes for imaging targets other than inflammatory cells, calcification imaging using ^{18}F -sodium fluoride (^{18}F -NaF) is of current interest for the detection and assessment of vulnerable plaques [32, 41]. Calcification is an important factor for atherosclerotic plaque formation and/or progression. High-risk plaques show microcalcification as well as inflammation (MΦ infiltration), leading to plaque

rupture and cardiovascular events. On the other hand, successful calcification (macrocalcification) stabilizes the plaques. While macrocalcification can be imaged using CT, it is difficult to image active calcification and microcalcification by CT. ^{18}F -NaF can be used to image new bone formation and remodeling by targeting hydroxyapatite, a mineral form of calcium apatite, which has been extensively used as a marker of bone turnover to study various bone-related diseases including malignant bone involvement. ^{18}F -NaF can also be applied to the assessment of microcalcification (active calcification) in atherosclerotic plaques [42]. In this regard, several clinical studies have been conducted in oncology patients, volunteers with/without aortic valve disease, and patients with myocardial infarction and stable angina [27, 32, 43, 44]. From the results of these studies, the authors suggested that ^{18}F -NaF provides new means for imaging microcalcification (active calcification) in atherosclerotic plaques, although future studies are required to clarify whether ^{18}F -NaF PET can identify ruptured and high-risk coronary plaques, and can improve the management and treatment of patients with atherosclerosis.

The apoptosis of foam cells and macrophages significantly involved in the formation of lipid cores, and the apoptosis of smooth muscle cells contributes to the weakening of fibrous caps. Accordingly, apoptosis is considered to be an important factor to determine the vulnerability of atherosclerotic plaques. In the early stage of apoptosis, phosphatidylserine (PS), a membrane phospholipid normally restricted to the inner leaflet of the lipid bilayer, is translocated to the outer leaflet. A human protein, annexin A5 (annexin V) has a high affinity for phosphatidylserine, which may allow radiolabeled annexin A5 to image apoptosis noninvasively [45]. Several groups have investigated the possibility of imaging vulnerable plaques using $^{99\text{m}}\text{Tc}$ -annexin A5 in animals and patients [16, 46–49]. We also indicated the possibility of using $^{99\text{m}}\text{Tc}$ -annexin A5 for imaging apoptosis in macrophage-rich plaques and its superior potential for evaluating selectively plaque vulnerability in comparison with ^{18}F -FDG [16, 48, 49]. As for the PET/SPECT probes including ^{18}F -ML-10, 2-(5-fluoro-pentyl)-2-methylmalonic acid targeting apoptosis-specific membrane alterations [18] and ^{18}F -isatin derivatives targeting caspase-3 [17] have been evaluated. On the other hand, considering the fact that acellular necrotic cores are more abundant in advanced lesions, De Saint-Hubert et al. evaluated the potential of ^{124}I -Hypericin for imaging atherosclerotic plaques [50]. Consequently, they indicated that necrosis imaging can be feasible, although the pharmacokinetic characteristics of ^{124}I -Hypericin should be improved.

Hypoxia is an important microenvironmental factor affecting atherosclerotic plaque progression. Hypoxia induces foam cell formation, alterations in macrophage metabolism, and neovascularization in atherosclerotic plaques. Mateo et al. [22] evaluated the feasibility of ^{18}F -fluoronisomidazole

(^{18}F -FMISO), a hypoxia imaging PET probe, in a rabbit model to directly image hypoxic regions in atherosclerotic plaques. Consequently, they demonstrated that plaque hypoxia was enhanced with the progression of atherosclerosis, and the hypoxic regions were imaged by ^{18}F -FMISO PET. ^{18}F -FMISO, a nitroimidazole derivative, enters cells by diffusion and is reduced by nitroreductases. Under normal oxygenation conditions, ^{18}F -FMISO is rapidly reoxidized and leaves the cells. However, under hypoxic conditions, ^{18}F -FMISO is further reduced to a more reactive form, covalently binds to intracellular macromolecules or forms glutathione conjugate, and is trapped in the cells [22, 51, 52]. Thus, ^{18}F -FMISO can be used to image hypoxic microenvironments.

Lipid cores in atherosclerotic plaques largely consist of fatty acids. Fatty acid synthesis requires acetyl-CoA, which is produced from acetate. Derlin et al. [15] examined the topographic relationship between arterial ^{11}C -acetate uptake and vascular calcification in oncology patients, and indicated that ^{11}C -acetate uptake may represent the early stages of disease. Although there have been few studies on the use of ^{11}C -acetate for the imaging of atherosclerotic plaques, in our recent metabolome study in a rabbit model, markedly increased acetyl-CoA levels were found in macrophage-rich lesions [35]. Further studies, including comparative studies with ^{18}F -FDG, are required to clarify the usefulness of ^{11}C -acetate.

Antibody or Peptide/Protein-Based PET/SPECT Probes

As mentioned above, various biological molecules are involved in atherosclerotic plaque formation and/or progression (Fig. 1) [53]. The imaging of these molecules enables us to understand precisely the pathological state of atherosclerotic plaques. In this regard, radioimmunodetection, an imaging technique that uses radiolabeled antibodies (immunoglobulin; IgG), is expected. The IgG-based PET/SPECT probes (radiolabeled antibodies) can provide high specificity and high affinity to target molecules utilizing antigen–antibody binding. In addition, the production and screening of IgGs that recognize specific target molecules as well as radiolabeling methods have been well established, which provide us with easy access to labeled antibodies that recognize specific target molecules. Using this strategy, we have evaluated several antibodies labeled with $^{99\text{m}}\text{Tc}$:

- 1) $^{99\text{m}}\text{Tc}$ -labeled monoclonal IgG which recognizes a scavenger receptor (LOX-1) [14]; LOX-1 is expressed on the cell membrane of macrophages and involved in ox-LDL uptake.
- 2) $^{99\text{m}}\text{Tc}$ -labeled monoclonal IgG which recognizes membrane type 1-matrix metalloproteinase (MT1-MMP) [24]; MT1-MMP is involved in the degradation of the extracellular matrix and the weakening of the fibrous cap.

- 3) ^{99m}Tc -labeled monoclonal IgG which recognizes tissue factor (TF) [26]; TF is an initiation factor of blood coagulation and involved in thrombus formation.

Radioimmunodetection using radiolabeled antibodies is useful for the validation of each biological molecule as a target molecule to assess the pathological state of atherosclerotic plaques. The slow blood clearance of IgG, owing to its nature being a macromolecule, causes a high background activity and requires a long waiting time for imaging. In addition, IgG, even without an affinity for any specific target molecule, accumulates in pro-inflammatory M1 macrophages probably via Fc γ receptors [54]. To overcome these limitations of a radiolabeled antibody (IgG), several attempts using a variety of antibody fragments have been conducted [55, 56]. In one of these attempts, Broisat et al. [57] evaluated ^{99m}Tc -anti-vascular cell adhesion molecule-1 (VCAM1) nanobodies for the SPECT imaging of atherosclerotic lesions. A nanobody, naturally present in camelids, is an antibody fragment (10–15 kDa) consisting of a single monomeric variable antibody domain. Consequently, atherosclerotic lesions in the aortic arch of ApoE $^{-/-}$ mice were successfully identified with a ^{99m}Tc -anti-VCAM1 nanobody. These results indicate the potential use of radiolabeled nanobodies in the evaluation of atherosclerotic plaques.

Radiolabeled peptides and proteins have been widely applied as PET/SPECT probes. Previously mentioned PET probes targeting SSTR-2 (^{68}Ga -DOTATATE, ^{64}Cu -DOTATATE) are somatostatin analogs and have peptide structures. Radiolabeled annexin A5 utilizes the high-affinity binding of annexin A5, a 36 kD human protein, to phosphatidylserine. Jiang et al. [58] reported another approach using a cysteine knot peptide (knottin), a polypeptide (ca. 30 amino residues) containing multiple disulfide bonds. They used a ^{64}Cu -labeled divalent knottin miniprotein targeting integrin $\alpha_v\beta_3$ (^{64}Cu -NOTA-3-4A) and evaluated its potential for imaging carotid atherosclerotic plaques in a mouse model. ^{64}Cu -NOTA-3-4A demonstrated specific accumulation in carotid atherosclerotic plaques. This technique using knottin can be applied to the development of PET/SPECT probes targeting various biological molecules, which should allow us to understand the pathological state of the atherosclerosis plaque. It is also advantageous that the production and screening of knottins have been well established, as in the case of IgG, although further studies on toxicity and adverse effects, including the immunogenicity of knottins, are required before clinical applications.

Nanocarrier-Type PET/SPECT Probes

Nanocarriers such as liposomes have been used to facilitate drug delivery into target tissues/organs particularly into tumor

tissues. This technique has also been applied to the development of imaging agents. Nahrendorf et al. [59] used ^{64}Cu -TNP, a ^{64}Cu -labeled nanoparticle (iron oxide-dextran complex), to image inflammatory atherosclerosis in ApoE $^{-/-}$ mice. High accumulation level of ^{64}Cu -TNP was observed in macrophage-rich atherosclerotic lesions, probably due to the ingestion of ^{64}Cu -TNP by phagocytic cells. Recently, Ogawa et al. [9] have prepared PS-modified liposomes and have labeled them with ^{111}In for SPECT imaging (^{111}In -PS200). As mentioned above, PS is externalized to the outer leaflet of the cell membrane at the early stage of apoptosis and recognized as an “eat-me signal” by phagocytic cells including macrophages. Accordingly, ^{111}In -PS200 can be used to image macrophage infiltration in atherosclerotic lesions. Indeed, atherosclerotic lesions were detected by ^{111}In -PS200 in ApoE $^{-/-}$ mice and Watanabe heritable hyperlipidemic rabbits *in vivo*.

Nanocarriers such as liposomes can contain therapeutic drugs as well as contrast agents and/or fluorescent compounds for other imaging modalities, in addition to radiolabeling for PET/SPECT imaging. Accordingly, the nanocarrier technique may be able to provide a novel means of multimodality molecular imaging and for theranostics, which is the integration of therapeutics and diagnostics.

Application of PET/SPECT Imaging for Monitoring of Therapeutic Efficacy

Not only for evaluating the pathological states of atherosclerosis plaques, PET/SPECT imaging techniques have also been applied to the monitoring of therapeutic efficacy. Several studies demonstrated the potential of ^{18}F -FDG for precisely evaluating the therapeutic efficacy of atorvastatin [60] and probucol [61] in animal models and simvastatin in patients with atherosclerosis [62]. Recently, we have evaluated the effects of irbesartan, an angiotensin II AT1 receptor blocker (ARB), in mouse and rabbit atherosclerosis models [63, 64]. Consequently, remission of inflammation as the potential therapeutic effect of irbesartan on atherosclerosis was observed with decreased ^{18}F -FDG accumulation level in the lesion (Fig. 2) [64]. These results provide visible evidence of the efficacy of irbesartan in suppressing the progression of atherosclerosis. Thus, ^{18}F -FDG PET provides a non-invasive means of monitoring the therapeutic efficacy of atherosclerosis by imaging changes in inflammation in the lesions.

Several PET/SPECT probes other than ^{18}F -FDG have also been applied to monitoring therapeutic efficacy in animal models of atherosclerosis. Hartung et al. [65] evaluated the potentials of ^{99m}Tc -annexin A5 to monitor the effects of simvastatin treatment in a rabbit model. We also used ^{99m}Tc -annexin A5 to monitor the effects of ARBs, telmisartan, and irbesartan in a mouse model [63, 66].

Fig. 2 Representative ^{18}F -FDG PET images in Watanabe heritable hyperlipidemic rabbits. The thoracic lesions of the control rabbit were clearly visualized, whereas a significant decrease in ^{18}F -FDG uptake level was observed in the lesions of the irbesartan-treated rabbit. Black arrows highlight the thoracic aorta

ARB treatment significantly decreased the plaque size, macrophage infiltration level, lipid deposition level, apoptotic cell number, and $^{99\text{m}}\text{Tc}$ -annexin A5 accumulation level. The $^{99\text{m}}\text{Tc}$ -annexin A5 accumulation level positively correlated with the macrophage infiltration level. Saraste et al. [67] prepared ^{18}F -galacto-RGD, a PET probe targeting integrin $\alpha_v\beta_3$, and examined whether diet intervention reduces the uptake of ^{18}F -galacto-RGD in mouse atherosclerotic plaques. They showed that the uptake of ^{18}F -galacto-RGD in mouse atherosclerotic lesions was reduced by lipid-lowering diet intervention, indicating the potentials of this probe for monitoring therapeutic efficacy.

Conclusions

In this review, we described recent advances in the development of PET/SPECT probes for visualizing atherosclerotic plaques and their application to therapy monitoring, mainly focusing on experimental studies. By expanding the results of these studies, the serial and spatial determination of important determinants involved in atherosclerotic plaque formation and/or progression by PET/SPECT imaging should be realized. These efforts would help PET/SPECT techniques become important clinical tools for evaluating precisely the pathological state of atherosclerosis, leading to the proper selection of therapeutic strategies. In addition, PET/SPECT techniques would contribute to drug discovery and development, as the noninvasive nature of PET/SPECT enables the serial and repetitive monitoring of therapeutic effects in the same animal, which provides results with higher accuracy in a time- and cost-saving manner.

Compliance with Ethical Standards

Conflict of Interest Yoichi Simizu and Yuji Kuge declare that they have no conflict of interest.

Ethical Statement This review article does not contain any studies with human participants or animals performed by any of the authors.

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