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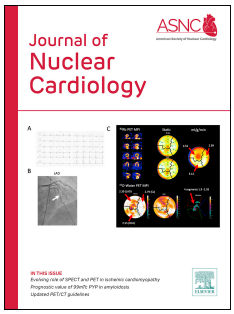

Cardiovascular PET imaging of fibroblast activation A review of the current literature

Krithika Loganath, Neil Craig, Anna Barton, Shruti Joshi, Constantinos Anagnostopoulos, Paola Anna Erba, Andor W.J.M. Glaudemans, Antti Saraste, Jan Bucerius, Mark Lubberink, Olivier Gheysens, Ronny R. Buechel, Gilbert Habib, Oliver Gaemperli, Alessia Gimelli, Fabien Hyafil, David E. Newby, Riemer H.J.A. Slart, Marc R. Dweck

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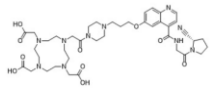
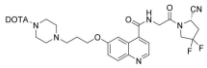
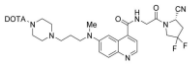
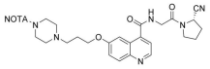
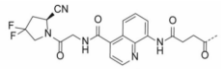
Name	Chelator	Healthy myocardial uptake and internalisation	Hepatobiliary excretion	Tissue retention
FAPI-02  Loktev et al, 2018 (34)	DOTA	Medium	High	Low
FAPI-04  Lindner et al, 2018 (29)	DOTA	Medium	Medium	Medium
FAPI-46  Loktev et al, 2019 (48) Meyer et al, 2020 (49)	DOTA	Medium	Medium	High
FAPI-74  Giesel et al, 2021 (32) Lindner et al, 2021 (113)	NOTA	High	Low	High
ONCOFAP  Millul et al, 2021 (114) Backhaus et al, 2022 (115)	NOTA and DOTA	Low	Low	High

Myocardial infarction					
Preclinical studies					
Title	**Model**	**Tracer**	**Imaging modality**	**Key results**	
Varasteh et al (59)	Murine	^{68}Ga-FAPI	PET-CT, autoradiography	• Peak radiotracer uptake observed at 6 days post MI with return to baseline by 14 days • Peri-infarct regions demonstrated more intense uptake than infarct regions • No remote myocardial uptake up to 14 days • FAP expression confirmed in areas on ^{68}Ga-FAPI uptake on immunofluorescence • FAP+ cells were observed 3 and 8 times more in the border zones than in infarct and remote myocardium, respectively	
Qiao et al, 2022 (60)	Rat	^{68}Ga-FAPI	PET-CT, Autoradiography, Immunofluorescenec, H&E staining	• Peak radiotracer uptake in infarcted myocardium with gradual decline until day 35, where uptake was similar to the sham-operated group • No significant remote ^{68}Ga-FAPI uptake was noted up to 35 days • Higher uptake demonstrated at the border zones on autoradiography	
Clinical studies					
Title	**Cohort and design**	**Tracer**	**Imaging modality**	**Key points**	**Correlation with imaging and serum biomarkers**

Diekmann et al (63)	12 STEMI patients, Prospective	^{68}Ga-FAPI	PET-CT, SPECT, CMR	• Significant ^{68}Ga-FAPI uptake in the infarct, extending into the peri-infarct zones	• ^{68}Ga-FAPI uptake areas exceeds infarct areas identified on SPECT and CMR LGE imaging
Kessler et al (65)	5 STEMI and 5 NSTEMI patients, Prospective	^{68}Ga-FAPI	PET-CT	• All patients had significant ^{68}Ga-FAPI uptake in the myocardium • A complete to partial concordance was observed of visual ^{68}Ga-FAPI myocardial uptake and areas supplied by the culprit vessel	• ^{68}Ga-FAPI uptake volume was positively correlated with peak CK level and negatively correlated with LVEF
Xie et al (62)	14 STEMI patients and 14 healthy volunteers, Prospective	^{68}Ga-FAPI	PET-CT and CMR	14 patients post STEMI • Significant ^{68}Ga-FAPI uptake was observed over infarct zones and beyond • Baseline TBR_{max} inversely correlated with LV function at 7 weeks	• Areas of ^{68}Ga-FAPI uptake extended beyond LGE on CMR
Zhang et al (64)	26 STEMI patients, Prospective	^{68}Ga-FAPI	PET-MR	• Increased ^{68}Ga-FAPI volumes and TBRmax at baseline correlated with reduced LVEF at 1 year • Patients who had late LV remodelling (increase in LVESV >10% from baseline) had a higher	• No significant correlation between ^{68}Ga-FAPI volumes and serum biomarkers such as NT-proBNP and serum troponin I. • Baseline ^{68}Ga-FAPI predicted late LV remodelling better

				baseline ^{68}Ga-FAPI volume	than LGE% and LGE volume on CMR

Myocardial diseases					
Preclinical studies					
Title	**Condition**	**Model**	**Tracer**	**Imaging Modality**	**Key points**
Wang et al (66)	Pressure-overload	Rat	^{68}Ga-FAPI	PET-CT, PET-MR, Echocardiography, Immunohistochemistry	• Areas of increased ^{68}Ga-FAPI uptake corresponded with areas of myocardial hypertrophy on CMR • Early ^{68}Ga-FAPI uptake at 4 weeks corresponded to a reduced LVEF at 8 weeks • Increased myocardial ^{68}Ga-FAPI uptake in areas corresponding FAP expression on immunohistochemistry
Sun et al (67)	HFpEF	Rat	^{18}F-AlF-FAPI	PET-CT, Immunohistochemistry, Masson staining	• Increased ^{18}F-AlF- FAPI uptake in the LV myocardium in rats corresponded to post-mortem areas of FAP+ cells and interstitial and vascular fibrosis
Song et al (68)	HFrEF	Murine	^{68}Ga-FAPI	PET-CT	• A positive correlation was seen between baseline LV myocardial ^{68}Ga-FAPI uptake and decline in LV contractility and increased LV dilatation at 28 days
Wei et al (69)	Anthracycline-induced cardiotoxicity	Rat	^{68}Ga-FAPI	PET-CT, Echocardiography, Immunohistochemistry, Masson staining	• ^{68}Ga-FAPI uptake was significantly higher in rats with cardiotoxicity compared to control rats at weeks 3 and 6 although no significant change in LVEF was seen between groups • Enalapril treatment for 3 weeks reduced the intensity of ^{68}Ga-FAPI uptake, preserved LVEF and reduced myocardial fibrosis on Masson staining

Clinical studies						
Title	**Condition**	**Cohort and design**	**Tracer**	**Imaging Modality**	**Key points**	**Correlation with imaging and serum biomarkers**
Wang et al (72)	Multiple non-ischaemic cardiomyopathies	29 patients, Prospective	^{68}Ga-FAPI	PET-CT, echocardiography	• 22 of the 29 scanned patients had heterogenous LV myocardial FAPI uptake • 10 patients had RV myocardial FAPI uptake across a spectrum of aetiologies	• SUV_{max} correlated significantly with LVEDD on echocardiography
Song et al (68)	HFrEF	7 patients , 20 participants without cardiac disease, Prospective	^{68}Ga-FAPI	PET-CT, ^{13}N-NH_3 perfusion scan	• Significantly elevated LV myocardial ^{68}Ga-FAPI uptake was seen in patients compared to healthy volunteers	• Areas of ^{68}Ga-FAPI uptake did not correspond with areas of reduced perfusion on ^{13}N- NH_3 myocardial perfusion scan
Wang et al (71)	HCM	50 patients with HCM vs 20 healthy volunteers, Prospective	^{18}F-AlF-FAPI	PET-CT, CMR (without gadolinium)	• Significantly higher LV myocardial ^{18}F-AlF-FAPI uptake in patients compared to healthy volunteers • 32 (64%) patients had increased ^{18}F-AlF-FAPI uptake in the RV myocardium and 4	• Increased ^{18}F-AlF-FAPI uptake was seen in all hypertrophied segments but also in non-hypertrophied segments in 84% of patients • ^{18}F-AlF-FAPI had a positive relationship

					(8%) had significant ^{18}F-AlF-FAPI uptake in the atria, 3 of whom had AF. • Increased ^{18}F-AlF-FAPI uptake positively correlated with the 5-year sudden cardiac death score and risk of malignant arrhythmia	with NT-proBNP and hs-cTnI and negative relationship with LVEF
Chen et al (74)	Chronic thromboembolic pulmonary hypertension	13 patients with CTEPH, prospectively recruited	^{68}Ga-FAPI	PET-CT, CMR, RHC	• 10 of the 13 patients demonstrated significant free RV wall ^{68}Ga-FAPI uptake • ^{68}Ga-FAPI uptake positively correlated with RV wall thickness and negatively correlated with RVEF	• Only 27% of significant RV ^{68}Ga-FAPI uptake corresponded to LGE on CMR • No correlation between ^{68}Ga-FAPI uptake and pulmonary haemodynamic parameters on RHC
Gu et al (75)	Pulmonary arterial hypertension	16 patients with PAH, prospectively recruited	^{68}Ga-FAPI	PET-CT, echocardiography, RHC	• 12 of the 16 patients had significant RV free wall and insertion point ^{68}Ga-FAPI uptake	• ^{68}Ga-FAPI uptake correlated with TAPSE on echocardiography • No significant correlation between ^{68}Ga-FAPI uptake and pulmonary haemodynamic parameters on RHC

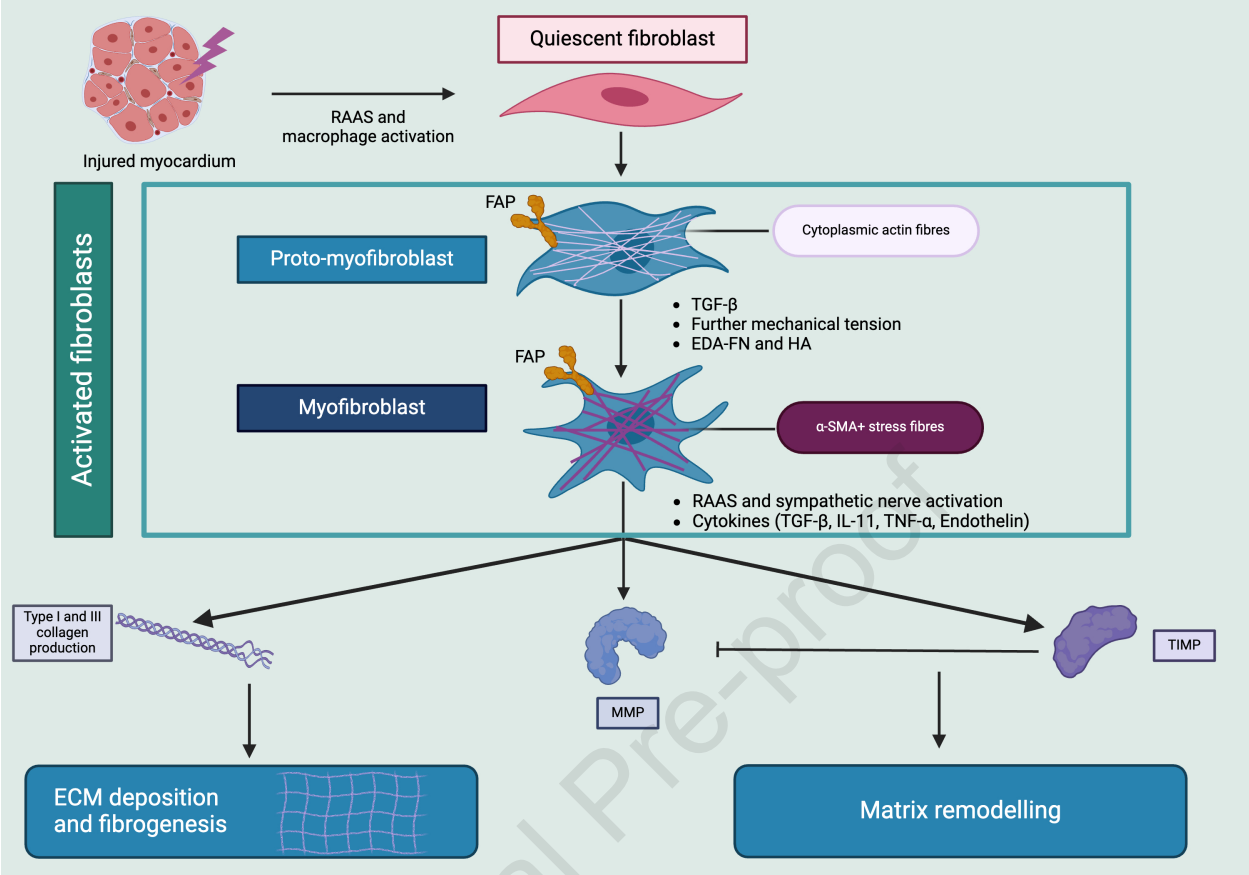
Finke et al (73)	Checkpoint-inhibitor (ICI) associated myocarditis	26 patients who received ICI, 3 with myocarditis, Retrospective analysis	^{68}Ga-FAPI	PET-CT, CMR	• ^{68}Ga-FAPI uptake significantly higher in ICI myocarditis patients	• 2/3 patients with ICI had MRI. 1 had globally elevated T1-mapping and another had basal LGE. T2 mapping was normal
Wang et al (70)	Cardiac AL-amyloidosis	30 patients with AL amyloidosis (27 with cardiac involvement and 3 without) , Prospective	^{68}Ga-FAPI	PET-CT, CMR, Echocardiography	• 80% of AL CA patients had increased intensity of myocardial ^{68}Ga-FAPI uptake	• 4 patients with LGE on CMR also had significant ^{68}Ga-FAPI uptake • FAPI uptake correlated with Mayo stage and NTpro-BNP levels, ECV percentage on CMR and negatively correlated with LVPW thickness and LVEF on CMR

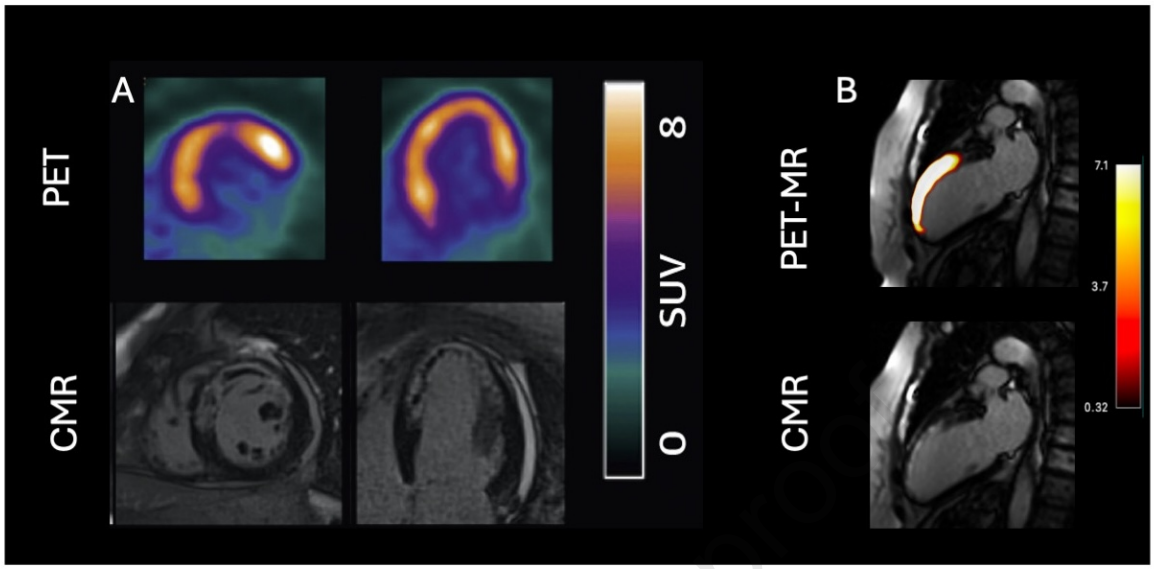
Receptor	Function	Name of drug	Preclinical data		Clinical data	
			Cohort and model	Key points	Cohort and design	Key points
CTGF Also known as CCN2	Mediates ECM production in pathological states	CTGF-mAb	Pressure-overload, mice(116)	• Improved LV systolic function • Reduced cardiomyocyte hypertrophy in murine models	Nil	
			DCM, mice (117)	• Improved LV systolic and diastolic dysfunction	Nil	
			MI, mice (118)	• Improved 7-day survival • Improved LV function • No difference in infarct size or 7 week survival	Nil	
Galectin-3	Interacts with aldosterone to promote macrophage infiltration and mediates cardiac fibroblast proliferation	Modified Citrus Protein (MCP)	HF, rats (119)	• Improved LV function • Reduced expression of collagen 1 and 3 genes	Nil	
			HF, mice (120)	• Improved LV function • Reduction in cardiac hypertrophy and fibrosis when treated in combination with an aldosterone antagonist		
MicroRNA- 132-3p (mIR-132)	Downregulates expression of Forkhead box O3 (FOXO3) which is anti-hypertrophic	Anti-mIR-132	Post-MI HFrEF, mice (121)	• Improved LVEF and NT pro-BNP levels • Reduction in myocardial interstitial fibrosis on in mice	Prospective randomised, double-blind, placebo-controlled trial of 28 patients	• No adverse reactions • Combined endpoint of NT pro-BNP reduction of >10% and LVEF increase

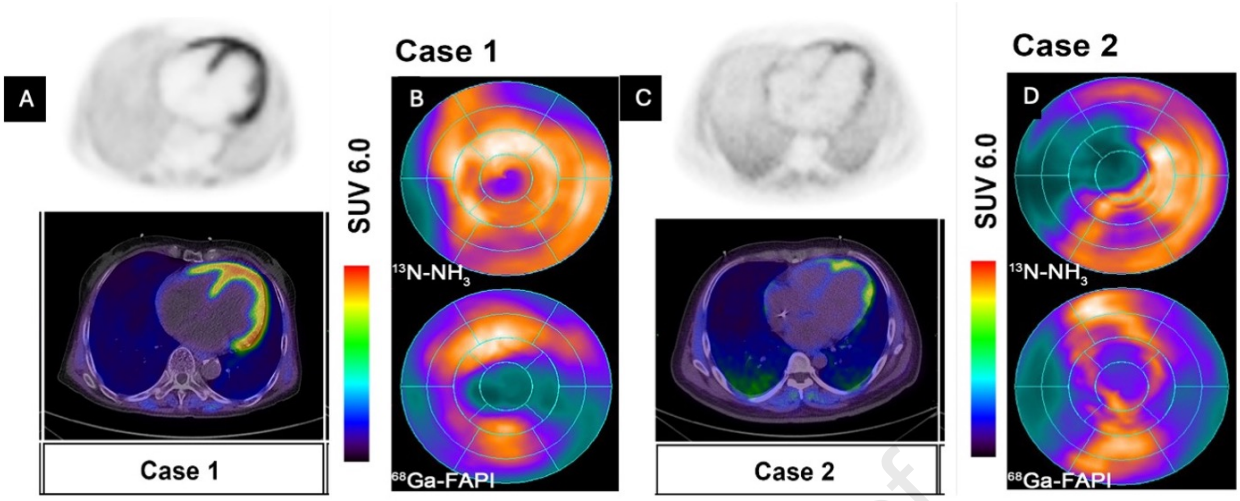
	and suppresses calcium handling and myocardial contractility				with HFrEF (LVEF 30-49%) (122)	of >2% was achieved in 79% in the pharmacodynamically active group vs 46% in the inactive group
Renin	Inhibits angiotensin II and promotes angiotensin II-independent reduction in TGFB-1 and collagen production	Aliskiren	Pressure-overload, mice (123)	• Improved LVEF, LVFS and collagen volume	Nil	
TGF-β	Inhibition of TGF-β reduces collagen deposition in the extracellular matrix	Pirfenidone	Pressure-overload, mice (124)		PIROUTTE trial-randomised, double-blind, placebo-controlled trial in 47 HFpEF patients (125)	• Reduction of ECV and LV mass on CMR • 26% of treated patients has side effects (nausea, insomnia, rash) although similar to placebo group
			MI (126)	• Lower total LV fibrosis • Higher LVEF		

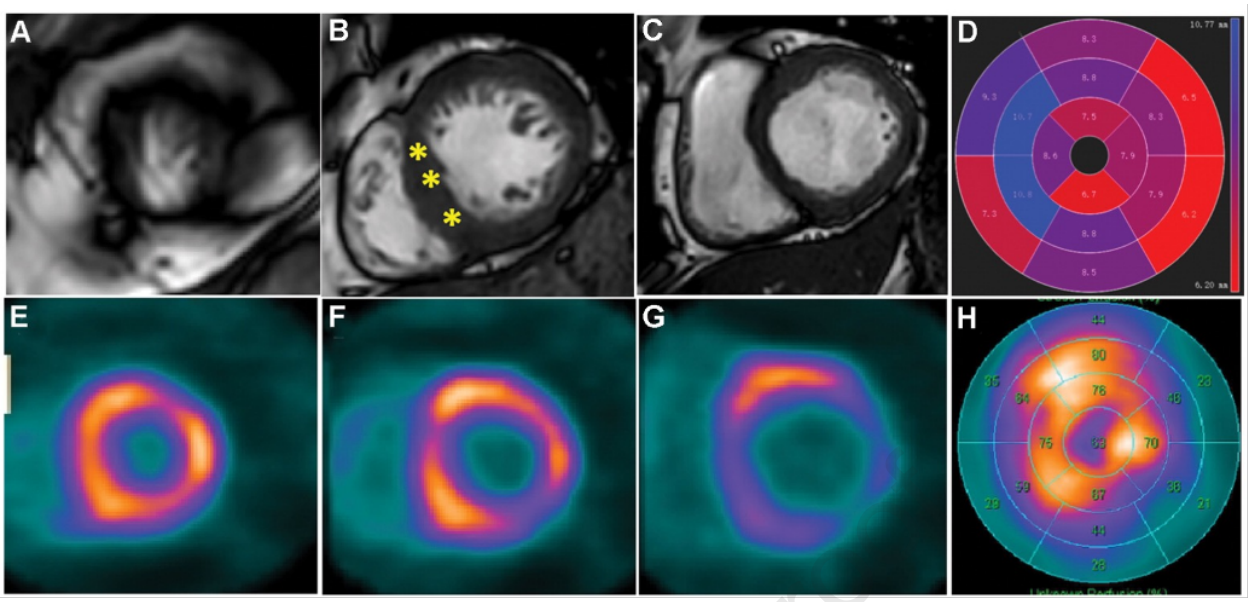
Phosphorylated Smad2 inhibitor	Phosphorylated Smad2, a downstream product of TGF-beta, increases ECM production	FT011	MI, rat (127)	• Improved LVEF • Reduced amount of cardiac fibrosis in non-infarct regions	Nil	
Matrix metalloproteinase (MMP) inhibitors	MMPs promote the degradation of ECM and adverse myocardial remodelling	PG-116800	Pressure-overload, mice (128)	• Lower LVEDV and intersitial fibrosis in MMP knockout (KO) mice • No difference in LVEF (128)	Randomised placebo-controlled double-blind trial of 253 post-STEMI HFrEF patients (129)	• No difference in mortality, cardiac admissions or LVEF • Higher incidence of gastrointestinal disturbance and joint stiffness in the treatment group
			MI, mice (130)	• MMP-29 KO mice had a lower 7-day survival mostly due to cardiac rupture. • LVESD and LVEDD were higher in KO mice than WT, while collagen I and III were lower in KO mice (130)		
Relaxin	Stimulates fibroblast differentiation and collagen deposition as well as promoting	Seralaxin	MI, mice (131)	• Lower LVEDP • Reduced collagen volume in infarct and border regions • No significant difference in LVEF	RELAX-AHF trial- a randomised placebo-controlled trial of Seralexin in 1161 acute	• Improved patient-reported dyspnoea but no difference in days alive out of hospital up to 60 days

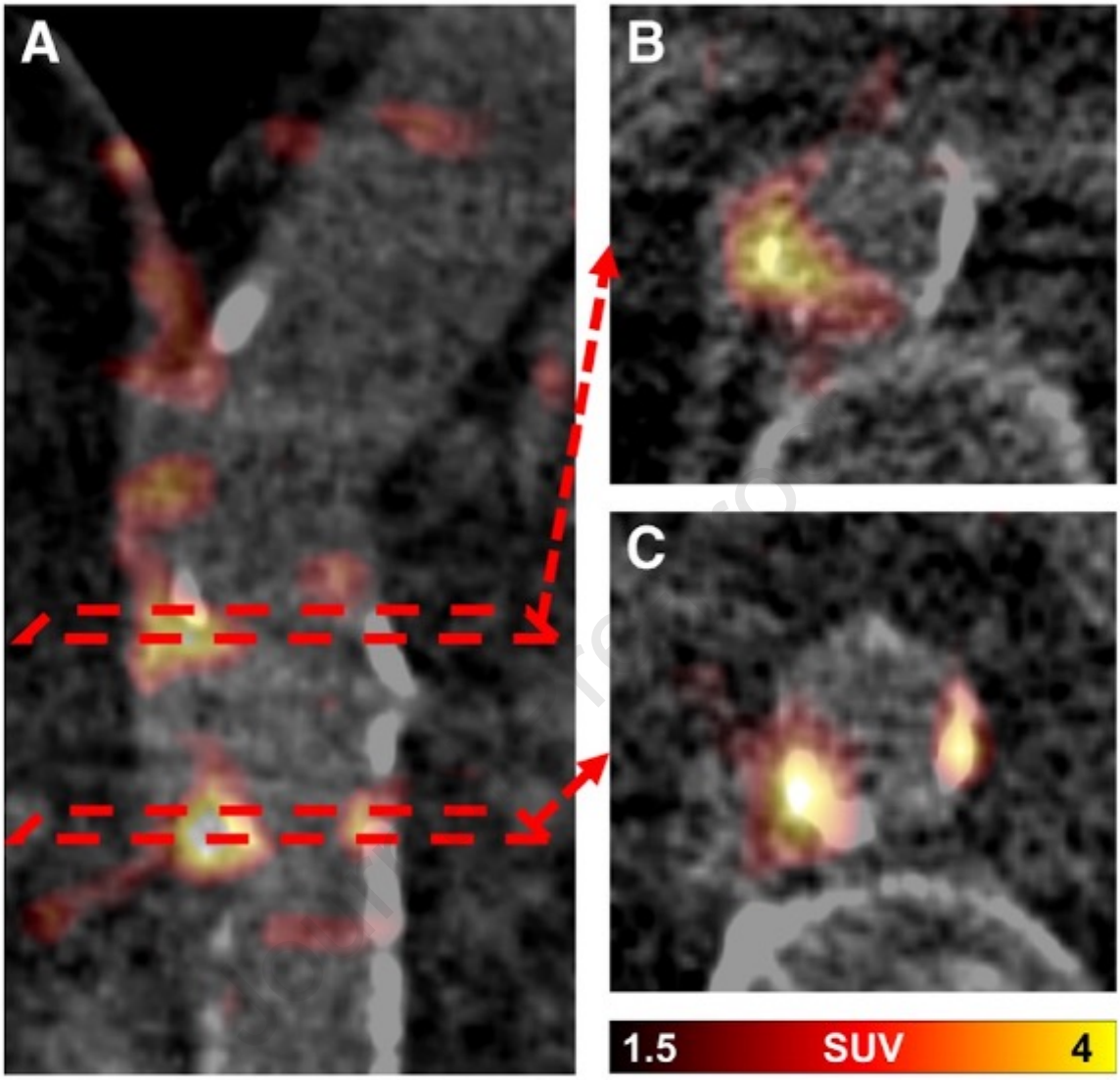
	MMP-induced ECM degradation		Pressure-overload, mice (133)	• No difference in LV mass, LVEF or collagen content	heart failure patients (132)	• Reduced number of cardiovascular deaths up to 180 days • No difference in cardiac-related hospitalisations







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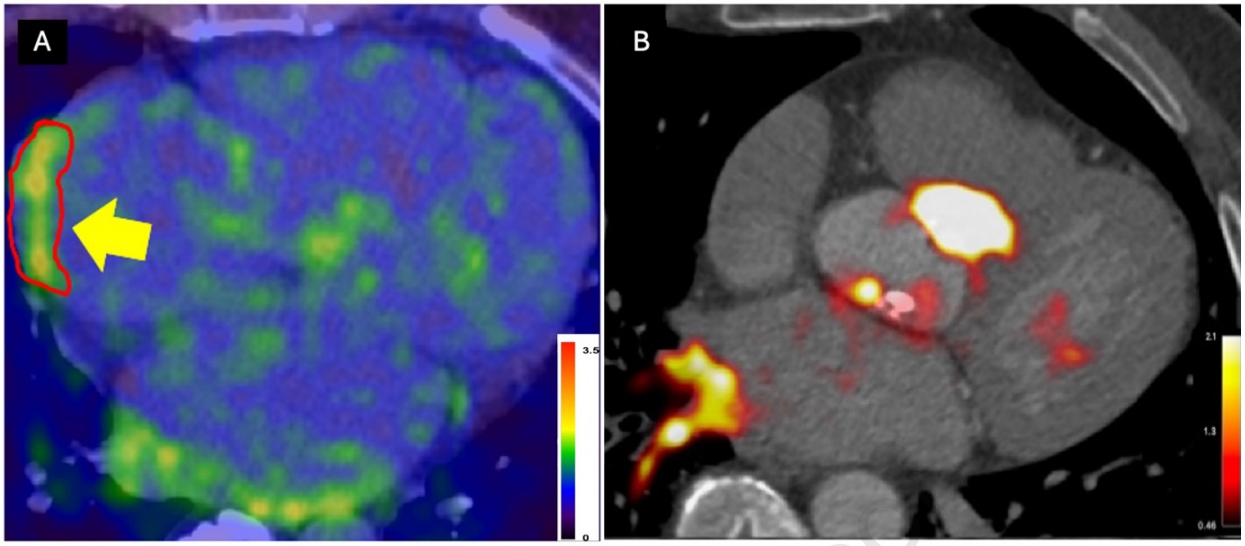

Declaration of interests

☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Paolo Anna Erba reports a relationship with SIEMENS that includes: consulting or advisory and speaking and lecture fees. Paolo Anna Erba reports a relationship with Novartis Pharmaceuticals Corporation that includes: consulting or advisory, funding grants, and speaking and lecture fees. Paolo Anna Erba reports a relationship with Springer Healthcare Limited that includes: equity or stocks. Paolo Anna Erba reports a relationship with Pfizer that includes: funding grants. Antti Saraste reports a relationship with AstraZeneca Pharmaceuticals LP that includes: consulting or advisory and speaking and lecture fees. Antti Saraste reports a relationship with Pfizer that includes: consulting or advisory and funding grants. Antti Saraste reports a relationship with Abbott that includes: speaking and lecture fees. Antti Sarate reports a relationship with Bristol Myers Squibb Co that includes: speaking and lecture fees. Antti Saraste reports a relationship with Janssen Pharmaceuticals Inc that includes: speaking and lecture fees. Jan Bucerius reports a relationship with International Atomic Energy Agency that includes: funding grants, non-financial support, and travel reimbursement. Jan Bucerius reports a relationship with European Association of Nuclear Medicine that includes: board membership. Olivier Gheysens reports a relationship with General Electric Company that includes: speaking and lecture fees. Ronny Buechel reports a relationship with General Electric Company that includes: speaking and lecture fees. Ronny Buechel reports a relationship with Pfizer Inc that includes: speaking and lecture fees. Ronny Buechel reports a relationship with Gilead Sciences Inc that includes: speaking and lecture fees. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Cardiovascular PET imaging of fibroblast activation

A review of the current literature

Krithika Loganath[1], Neil Craig[1], Anna Barton[1], Shruti Joshi[1], Constantinos Anagnostopoulos[2], Paola Anna Erba[3,4], Andor W.J.M. Glaudemans[4], Antti Saraste[5,6], Jan Bucerius[7], Mark Lubberink[8], Olivier Gheysens[9], Ronny R. Buechel[10], Gilbert Habib[11,12], Oliver Gaemperli[13], Alessia Gimelli[14], Fabien Hyafil[15,16], David E. Newby[1], Riemer H.J.A Slart[17,18], Marc R. Dweck[1]

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Abstract

Fibrosis is one of the key healing responses to injury, especially within the heart where it helps to maintain structural integrity following acute insults such as myocardial infarction. However, if it becomes dysregulated then fibrosis can become maladaptive leading to adverse remodelling, impaired cardiac function and heart failure. Fibroblast activation protein is exclusively expressed by activated fibroblasts, the key effector cells of fibrogenesis, and has a unique extracellular domain that is an ideal ligand for novel molecular imaging probes. Fibroblast activation protein inhibitor (FAPI) radiotracers have been developed for PET imaging, demonstrating high selectivity for activated fibroblasts across a range of different pathologies and disparate organ systems. In this review, we will summarize the role of fibroblast activation protein in cardiovascular disease and how FAPI radiotracers might improve the assessment and treatment of patients with cardiovascular diseases.

Key words: Fibroblast, Fibroblast activation protein inhibitor (FAPI), Positron Emission Tomography, Cardiac magnetic resonance, Cardiac Computed Tomography,

Introduction

Fibrosis- friend and foe

Fibrosis is one of the fundamental healing responses to injury and a highly conserved process across organ systems. In the short term, this process maintains tissue integrity and organ function. However, in the absence of regulation, excessive fibrosis can become harmful, leading to scarring, increased rigidity and progressive organ dysfunction (1). Indeed, myocardial fibrosis underlies almost every cardiomyopathic condition, making it a key target for imaging and therapeutic intervention. Yet the key to the success of such strategies will be targeting inappropriate and excessive fibrosis at an early stage whilst leaving the protective processes unscathed. The duality of fibrosis in the cardiovascular system is perhaps best illustrated by myocardial infarction (MI). In the acute phase, fibrosis is protective, providing structural integrity within the friable necrotic myocardium, and protecting against myocardial rupture. In contrast, later inappropriate fibrosis can lead to adverse remodelling, impaired diastolic and systolic function and the development of heart failure with the risk of fatal arrhythmias (2). An improved understanding of the timing of appropriate and inappropriate fibrotic responses after MI will be key in preventing the transition to heart failure and sudden cardiac death whilst leaving the protective scar intact.

The non-invasive identification of fibrosis is important. Current cardiovascular magnetic resonance (CMR) methods are useful in identifying established fibrosis. In particular, T1 mapping techniques can identify reversible and diffuse interstitial fibrosis using native T1 values and extracellular volume percentage (ECV%). Late gadolinium enhancement (LGE) techniques can identify irreversible replacement fibrosis. These approaches are widely employed in clinical practice to aid the diagnosis and risk stratification of patients with various cardiac conditions but are limited to documenting established fibrosis in the myocardium without the spatial resolution to assess fibrosis in the valves and atherosclerotic plaques in vascular structures (3). Furthermore, they do not identify whether fibrogenesis remains active in the heart, being unable to distinguish between active and inactive or burnt-out disease: is it scarred or is it scarring (4)? Novel molecular positron emission tomography (PET) radiotracers, such as radiolabelled fibroblast activation protein inhibitors (FAPI), can address these shortcomings by identifying activated fibroblasts and active fibrosis, with the benefit of tracking fibroblast activity over time and and in response to treatment (5, 6). FAPI imaging is now being widely used to investigate fibroblast activation and fibrosis activity across organ systems, building upon the success of these tracers in imaging cancer-associated fibroblasts (7).

The Inflammatory, Infective, Infiltrative, and Innervation (4Is) Committee is a collaboration between the European Association of Cardiovascular Imaging (EACVI) and the European Association of Nuclear Medicine (EANM). Members of this committee identified FAPI PET imaging as one of the most promising new areas of molecular cardiovascular imaging and felt that the imaging community should be introduced to this novel application. Our aim is, therefore, to summarize the current status of this field as well as to outline what we consider to be the most exciting areas for future research with this novel imaging technique. This review will explore the imaging of cardiovascular fibrosis activity with FAPI PET. We will briefly discuss the pathophysiology of various cardiovascular

conditions, the role of activated fibroblasts in these and how FAPI is useful in targeting these processes. We will then review the existing FAPI literature in myocardial disease, before finally describing how this exciting area may develop in the future and improve both our understanding of cardiovascular fibrosis and in guiding patient care.

Fibrosis and Activated Fibroblasts

Myocardium

Cardiac fibrosis is a result of interstitial expansion from excess extracellular matrix (ECM) deposition within the myocardium, predominantly composed of fibrillar collagens. Cross-linking of these fibers reduces myocardial elasticity and contractile capacity, leading to diastolic and systolic dysfunction. Cardiac fibrosis is categorized into two forms, though overlap appears. Replacement fibrosis is irreversible scarring from intense myocardial injury and myocyte necrosis, such as MI or acute myocarditis. In contrast, diffuse interstitial fibrosis is reversible and associated with chronic disease processes like aortic stenosis and hypertension. Left unchecked, it can progress to replacement fibrosis (8).

Atherosclerosis

Atherosclerotic plaques form from low-density lipoprotein deposits in vessel walls. Immune cell infiltration triggers smooth muscle proliferation, forming early ‘fatty streaks’. (9) These evolve into fibrous plaques, causing luminal narrowing and creating sites for clot formation. Beneath the fibrous cap, cell apoptosis and cholesterol crystals form a necrotic core. (10, 11).

Activated fibroblasts

Fibroblasts are a group of cells in the connective tissue that synthesize collagen and other components of the extracellular matrix (ECM). They predominantly arise from epicardial cells that have undergone epithelial to mesenchymal transformation (EMT) (12, 13), but can also arise from endothelial cells within the interventricular septum (14), neural crest cells in the right atrium (15), adventitial fibroblasts (16) and local smooth muscle cell-to-fibroblast transition (17). In their quiescent form, fibroblasts are tasked with the role of maintaining tissue homeostasis, constantly monitoring the myocardium through its cycles of contraction and relaxation, and replenishing the ECM as required (18).

Activated fibroblasts are the key cells driving fibrogenesis in the myocardium and other organs of the body. In the myocardium they exist in two forms. They are first activated into an intermediate form called proto-myofibroblasts, which acquire proliferative and migratory properties, enabling them to move into areas of damage, secreting predominantly type I and type III collagens and cross-linking the ECM to generate wound healing (19). Further autocrine signalling and the action of fibroblast modulators (e.g extra domain A fibronectin and hyaluronan) result in the maturation of

proto-myofibroblasts into myofibroblasts. Myofibroblasts provide enhanced contractile strength with the aid of α-smooth muscle actin (α-SMA) stress fibres, which form stable adherence plaques between cell membranes and the ECM junction (20, 21) (Figure 1). In addition, activated fibroblasts have a key role in matrix remodelling, secreting matrix metalloproteinases (MMPs), which degrade components of the extracellular matrix, as well as their tissue inhibitors (TIMPs). The balance between collagen production and the activity of MMPs and TIMPS influences the nature and composition of the extracellular matrix. In atherosclerosis, metalloproteinase production by activated fibroblasts can weaken the fibrous cap overlying the necrotic core, predisposing it to rupture and myocardial infarction.

Fibroblast Activation Protein

Fibroblast activation protein (FAP) is a 760-amino acid transmembrane surface glycoprotein that is expressed almost exclusively on activated fibroblasts. It has a role in normal developmental processes during embryogenesis and organ formation but is not expressed by quiescent fibroblasts. FAP was first discovered in 1994 (22) and has both dipeptidyl transferase (DPP) and endopeptidase activity (23), the latter readily differentiating it from the more ubiquitous DPP IV receptors (CD26). The specificity of FAP for activated fibroblasts makes it a good marker of these cells and, therefore, of fibrosis activity (24). Indeed, fibroblasts expressing FAP have been identified in a range of conditions across different organ systems, including wound healing, liver, and lung fibrosis (25, 26). FAP is also a key protein in cancer-associated fibroblasts, where its protease activity is associated with cell migration and a poor prognosis explaining the strong interest in this molecular target in oncology (27).

Fibroblast Activation Protein Inhibitor Radiotracers

Quinoline-based FAP-specific inhibitors (28) bound to a DOTA chelator and then to a radioisotope: either gallium-68 (^{68}Ga) or aluminium fluorine-18 (^{18}F-AlF) have been developed (29). FAPI-02 and FAPI-04 were clinically promising initial radiotracers, demonstrating good stability in human serum, a strong affinity and high specificity for FAP+ cells, and high target-to-background ratio (TBR). However, hepatobiliary excretion was high, affecting image quality (29-31). This improved with the development of FAPI-46. Fluorinated tracers have the benefit of larger yields, lower radiation dosage and longer half-lives but they are incompatible with DOTA. As such, the smaller NOTA molecule is used for ^{18}F-AlF binding, resulting in the development of FAPI-74. ONCOFAP is an ultra-high affinity FAP ligand with superior specificity for FAP+ cells and excellent target-to-background ratios. It can be be attached to NOTA and DOTA molecules to bind reliably to ^{68}Ga, ^{18}F-AlF and even the larger ^{177}Lutetium for theranostic use (32, 33) (Table 1).

<u>Advantages of FAPI tracers</u>

FAPI tracers target the more exclusive endopeptidase activity of fibroblast activation protein, making them highly specific for this protein (34). Moreover, after binding FAP, FAPI becomes rapidly and almost completely internalised into FAP+ fibroblasts with minimal release into surrounding tissues

(34). These properties suggest excellent tracer specificity, and consistent with this, very little non-specific background uptake is observed when FAPI tracers are injected in vivo.

In oncology, FAPI tracers have demonstrated improved signal-to-noise in many solid organ tumours compared to ^{18}F-fluorodeoxyglucose (^{18}F-FDG) (35, 36). Increasing evidence is now demonstrating the value of FAPI PET imaging in detecting non-malignant fibrosis activity across organ systems, including the detection of lung, liver, and kidney fibrosis as well as systemic pro-fibrotic conditions (37-40).

Histological validation of the FAPI PET signal has been widely performed across an array of different conditions, demonstrating the co-localization and correlation of FAPI PET activity with FAP+ fibroblasts on immunohistochemistry. Blocking studies have confirmed that this uptake is specific to the FAP protein (41-45).

FAPI tracers demonstrate favourable pharmacokinetics with rapid renal clearance and reduced hepatobiliary excretion. Both the ^{68}Ga and ^{18}F-AlF-FAPI tracers have short half-lives (68 and 109 mins, respectively) and their administration is associated with low radiation doses (both <2 mSv per 100 MBq administered). Another benefit of FAPI radiotracers is their fast uptake time and blood clearance, resulting in good contrast to noise ratios as early as 10 minutes following injection, although optimal cardiovascular image quality appears to occur at 60 minutes (34, 46, 47). In addition, minimal uptake is seen in healthy myocardium with SUV_{mean} values ranging from 0.35 to 1.2 (29, 48, 49). The shorter positron range of fluoride tracers offers the benefit of improved image resolution (50). Finally, unlike ^{18}F-FDG imaging, FAPI PET does not require any dietary modification prior to imaging.

<u>Disadvantages of FAPI tracers</u>

Certain FAPI radioligands demonstrate hepatobiliary excretion resulting in uptake within the liver (29). In addition, moderate physiologic uptake is seen in the pancreas (51) and kidneys (52) with the highest physiologic uptake seen in the uterus (53) making it difficult to discern true pathological FAPI uptake in these and adjacent structures. With the current evidence, it remains uncertain as to which exact subpopulation of activated fibroblasts these FAPI radiotracers bind to.

Perhaps the main disadvantages of FAPI tracers relate to their availability and expense. FAPI is a new radiotracer that is not yet widely available commercially although the advent of fully automated processes will help achieve standardization (33, 54). Standard gallium generators can only provide 1 to 3 patient doses per production resulting in high per-dose production costs (55). Fluorinated alternatives such as ^{18}F-AlF-FAPI overcome this hurdle with a cyclotron able to generate more doses (32). More importantly, the feasibility and cost of FAPI imaging are likely to improve with its more widespread use. Indeed, the exciting preliminary results from FAPI imaging in cancer suggest that it may well replace a reasonable proportion of the ^{18}F-FDG PET imaging in future clinical practice (35). When one considers that more than 1 million ^{18}F-FDG PET scans are performed annually in the US alone, this is likely to drive wider availability and lower costs of FAPI tracers for use across various disease states.

FAPI Imaging in Cardiovascular Disease

The nascent literature supporting the role of FAPI radiotracers in cardiovascular disease is expanding (56). The first published report of a 67-year-old man with pancreatic ductal adenocarcinoma, a history of ischaemic heart disease, and a reduced left ventricular ejection fraction of 41%. Intense ^{68}Ga-FAPI uptake was noticed in his left ventricular (LV) myocardium in addition to his known tumour and metastases (57). It was hypothesized that cardiotoxicity following chemotherapy may have contributed to his cardiac dysfunction and intense FAPI uptake. Subsequently, in 185 oncology patients who had undergone ^{68}Ga-FAPI PET/CT imaging of their underlying cancer, increased focal FAPI uptake was identified in the myocardium of 42 patients. There was a positive correlation between this myocardial ^{68}Ga-FAPI uptake and cardiovascular risk factors, a history of MI, type 2 diabetes mellitus as well as the prior use of platinum-based chemotherapy agents and radiotherapy (58). Amongst a subgroup with concomitant echocardiography, ^{68}Ga-FAPI uptake was higher in patients with reduced compared to normal LVEF.

Myocardial Infarction

In small animal models, FAPI uptake occurs in areas of infarction peaking at day 6 after MI with higher uptake in the border regions and minimal uptake in the remote myocardium up to 35 days. These spatio-temporal patterns of uptake confirmed that ^{68}Ga-FAPI-PET provides different information to the detection of established myocardial fibrosis and scarring with CMR. Areas of uptake corresponded with autoradiography and immunofluorescence staining for FAP + fibroblasts (59, 60)(Figure 2).

Clinical studies similarly demonstrated good concordance between FAPI uptake and infarcted myocardium. In a study of 35 people up to 11 days post myocardial infarction, the volume of fibroblast activation protein uptake positively correlated with LGE on CMR and exceeded the area of perfusion defect on myocardial perfusion scanning. Indeed, 14% of segments demonstrating FAPI uptake exhibited no LGE and normal T1 and T2 levels. The volume of FAPI uptake correlated with a fall in LVEF at a median of 140 days post myocardial infarction in 14 of the 35 patients (61). Similar results were seen in smaller studies where FAPI uptake extended beyond the infarct zone (62, 63) and correlated with late LV remodelling (64) and LVEF (62, 65) (Table 2). PET-MR benefits from combining both LGE-based fibrosis imaging with FAPI uptake. Initial studies have demonstrated that FAPI volumes correlate with LGE and serum biomarkers (64, 66), with more research underway (ClinicalTrials.gov Identifier: NCT04723953). Aside from PET imaging, ^{99m}Tc-labeled fibroblast activation protein inhibitor (^{99m}Tc-HFAPi) SPECT imaging has similarly demonstrated increased FAP activity in patients post acute MI which exceed perfusion defects and inversely correlated with LVEF (67).

Myocardial diseases

Myocardial FAPI uptake has been investigated in a variety of cardiomyopathies and conditions that can lead to heart failure (Table 3). In animal models, FAPI uptake is increased in pressure-overload conditions, heart failure with reduced ejection fraction (HFrEF), heart failure with preserved ejection fraction (HFpEF) (68-70) and anthracycline-induced cardiotoxicity (71). Similar results have been

found in clinical studies with increased LV myocardial FAPI uptake across a range of cardiomyopathies (69, 70, 72-77)

Interestingly, FAPI uptake did not necessarily correspond to areas of reduced perfusion on ^{13}N-NH_3 imaging in non-ischaemic cardiomyopathy (70) (Figure 3) or with established scarring in LGE in chronic thromboembolic pulmonary hypertension (76) and hypertrophic cardiomyopathy (73). FAPI uptake did, however, correlate positively with the 5-year sudden cardiac death score in hypertrophic cardiomyopathy, demonstrating a potential prognostic role in this group (73) (Figure 4).

Myocardial FAPI uptake is increased in patients with severe aortic stenosis about to undergo transcatheter aortic valve implantation. FAPI volume correlates with markers of heart failure such as N-terminal pro b-type natriuretic peptide (NT-proBNP) and left ventricular ejection fraction, indicating a potential for its use in prognostication. As with other cardiovascular conditions, FAPI uptake did not necessarily correspond to areas of fibrosis identified on CMR (78). Studies are currently in progress to investigate these further (ClinicalTrials.gov Identifier: NCT06047561). (Figure 5)

Finally, numerous case reports have now described increased myocardial ^{68}Ga-FAPI uptake across a range of cardiovascular conditions, including anthracycline-induced cardiotoxicity (57), hypertensive heart disease (79), cardiac sarcoidosis (80) and Fabry's disease (81). Trials are ongoing at the moment investigating further cardiomyopathies (ClinicalTrials.gov Identifier: NCT04555642, NCT05756608).

<u>Atherosclerosis</u>

Activated fibroblasts have been implicated across the spectrum of atherosclerosis, from initial plaque formation in response to injury and MMP production to constrictive vascular remodelling and the formation of stable plaques as the cycle of inflammation and injury perpetuates (82, 83). Two retrospective studies in oncology and IgG4-related disease populations have assessed FAPI imaging in vivo. FAPI uptake was seen in only about half of calcified arterial lesions (Figure 5) (84), with the intensity of FAPI uptake correlating inversely with the extent of calcification (85). An elevated body mass index was the only individual cardiovascular risk factor that significantly correlated with FAPI uptake, although Wu and colleagues found that the presence of 4 or more risk factors positively correlated with the number of FAPI + lesions and TBR_{mean}(85), indicating that FAPI imaging may be useful in high-risk populations. These were non-cardiac gated scans and both groups reported significant noise and partial-volume effects, so prospective data with dedicated vascular scanning protocols would be warranted to investigate this further. No study has yet reported FAPI imaging in the coronary arteries.

<u>Inflammatory vascular diseases</u>

Vascular uptake has been demonstrated in patients with Takayasu's arteritis, giant cell arteritis and IgG4-related disease (35, 86, 87). A case report of a patient with Takayasu's arteritis reported that ^{68}Ga-FAPI uptake was detected in the thickened thoraco-abdominal aortic wall as well as in the carotid and subclavian arteries where ^{18}F-FDG uptake was absent (86). ^{68}Ga-FAPI uptake has also been documented in IgG4-related disease, with potentially increased sensitivity in organs (pancreas and bile duct) compared to ^{18}F-FDG (38, 87).

Arrhythmia

Atrial fibrillation is the commonest sustained arrhythmia and is associated with changes in atrial structure and atrial fibrosis (88, 89). Increased ^{18}F-AlF-FAPI uptake was seen in the atria of beagle-models of AF which corresponded to autoradiography and FAP + fibroblasts on histology, with similar results seen in patients with AF (90) (Figure 6). Focal ^{68}Ga-FAPI uptake is commonly seen following pulmonary vein isolation, especially following cryoballoon ablation (91). As with other conditions, further studies are warranted to investigate the role of activated fibroblast in the pathology of AF and atrial cardiomyopathy as well as its recurrence following pulmonary vein isolation.

Other fibrosis radiotracers

Other fibrosis radiotracers are currently being investigated with promising results. ^{18}F-Fluciclatide binds to the $\alpha_v\beta_3$ integrin transmembrane receptor responsible for ECM production and angiogenesis with increased tracer activity observed in acutely infarcted myocardium that demonstrated a positive correlation with improved subsequent myocardial recovery (92). Proline is a pre-cursor of collagen, which gets incorporated into developing collagen and might therefore serve as a marker of fibrosis formation (93). However, the presence of various isomeric forms, each with its different radiotracer properties, makes standardisation and manufacture a challenge. Thus far, fluorinated proline (^{18}F-Proline) has had mixed results in extra-cardiac preclinical and clinical studies (94, 95).

The future of FAPI radiotracers

Whilst activated fibroblast imaging in cardiovascular disease has a promising future, it remains in its infancy. Considerable work is required before we can fully appreciate how this technique may aid our understanding of activated fibroblasts and fibrosis activity in cardiovascular disease, let alone appreciate whether it might fulfil a useful clinical role. Based upon the information currently available from both cardiovascular and non-cardiovascular diseases, we believe it is helpful to speculate about how the field may evolve, to outline what we consider the most exciting areas for research, and to discuss potential future applications of FAPI-PET in patients with cardiovascular disease. We hope that this will stimulate discussion and ideas for future studies in this field.

Detection of early disease

Molecular PET imaging provides unrivalled sensitivity in the detection of disease and can potentially detect the activity of disease processes before they are apparent with other modalities. An excellent example is ^{18}F-NaF PET, which can detect calcification activity before calcium is evident on CT (96). FAPI PET might provide similar sensitivity for myocardial fibrosis allowing the detection of activated fibroblasts prior to the deposition of collagen and ECM – the changes detectable on CMR. This may prove of particular value in patients with chemotherapy-induced cardiotoxicity, where the early identification of myocardial injury could help guide the use and dose of chemotherapy regimens and the need for cardioprotective medication, especially as its utility in cancer care continues to expand. Other potential clinical uses related to early detection include the prompt identification of left ventricular decompensation in patients with aortic stenosis and the differentiation of cardiomyopathy from athlete's heart. Importantly, not all FAP+ fibroblasts will result in fibrosis, as seen in studies of acute MI patients where areas of FAPI uptake extend beyond that of LGE, even at follow-up (61). Investigation of how FAP+ fibroblasts behave with disease progression and how they can be modulated to avoid fibrosis, warrants further exploration but suggests that FAPI imaging might identify a reversible stage of the disease.

Detecting fibrosis in thin-walled structures

The high sensitivity of FAPI PET can be used to identify fibroblast activation in thin-walled structures where reliable fibrosis imaging has previously been challeging, particularly in the right ventricle and cardiac atria. Detection of right ventricular fibroblast activity might be of particular value in patients with arrhythmogenic cardiomyopathy, pulmonary hypertension, and congenital heart disease. The role that fibrosis plays in these conditions might be elucidated, and patient diagnosis, risk stratification, and difficult management decisions might be aided by such techniques (58, 76, 77). Similarly, with the atria, FAPI PET holds promise in improving our understanding of atrial cardiomyopathy and the triggers to atrial fibrillation. We know that atrial fibrosis is implicated in the development of atrial fibrillation and is positively linked to the development of atrial thrombus and subsequent stroke risk (97-99) but have previously not been able to image it reliably. Indeed, detection of atrial LGE with CMR remains a challenge and is limited to a handful of expert centres.

In larger arterial vessels such as the aorta and femoral arteries , FAPI has been useful to characterise plaques with thin fibrous caps that may be prone to rupture compared to their stable calcific equivalent (84, 85). Smaller calibre vessels such the carotids and coronary arteries have not yet been investigated although the ability to identify at-risk plaques would be useful to target primary and secondary prevention therapy in these patients. As with established CMR methods, partial volume issues are a concern and further research is required to optimise PET imaging in these thin walled structures.

Active versus established disease

Molecular imaging is already used clinically to differentiate active from inactive or burnt-out disease states and to guide therapy. A good example is cardiac sarcoidosis, where ^{18}F-FDG is used to identify

ongoing myocardial inflammation, providing complementary information to CMR, that is used to guide the need for immunosuppressive agents (100). Given the central pathological role of fibrosis, FAPI PET might fulfil a similar role across a wide range of cardiomyopathic processes and is likely to lead to important insights into the spatiotemporal activation of fibroblasts in different cardiovascular conditions. For the evaluation of systemic diseases such as atherosclerosis, vasculitis, sarcoidosis and amyloidosis, total body PET may prove of additional value. Dynamic quantification with whole-body coverage allows assessment of disease activity across the body and can provide optimal quantification while evaluating cross-over interactions between different organs and tissue in the same subject (101, 102).

Important questions regarding fibroblast activation can be addressed for the first time in humans. For example, when does fibroblast activity start and stop following MI? Is it the same in larger versus smaller infarcts and the same in the infarct zone versus the remote myocardium? Once the normal pattern of fibrosis activity has been established following a particular insult, then FAPI PET may help to differentiate protective forms of fibrosis from maladaptive patterns. This would allow us to identify the patients most likely to benefit from the raft of novel anti-fibrotic medication currently under development (Table 4) (103, 104).

<u>Predicting disease progression and monitoring response to treatment</u>

Promising initial data suggest that FAPI PET can track changes in fibroblast activation in response to antifibrotic treatments in systemic sclerosis-related interstitial lung disease and IgG4 -related disease (38, 105). An exciting possibility is that FAPI PET could be used similarly in patients with cardiovascular disease to identify with an adverse pattern of fibroblast activation who can then be targered with therapies at a potentially reversible stage of their disease process. FAPI PET could then be used to confirm treatment response and to identify patients in need of more aggressive management strategies. In addition, FAPI PET could then be considered to assess long-term treatment response to such therapies and to identify the time point at which such therapies could be stopped. This would allow a truly personalized approach to the treatment of cardiovascular disease and direct the right treatment to the right patient at the right time.

FAPI PET might also prove of value in accelerating the development of novel anti-fibrotic therapies. The pervasiveness of fibrosis across various disease processes underlines the urgent need to develop such treatment. Whilst some existing antifibrotic therapies have been shown to be effective in non-malignant diseases such as intersitial pulmonary fibrosis (106, 107) these frequently come at the cost of side effects such as nausea, vomiting and photosensitivity. Alternative strategies are therefore required. FAPI imaging might prove a useful tool in enriching study populations with patients that have a reversible disease state in whom benefit might be most likely to develop. Moreover, FAPI PET could then be used to track response to therapy as a marker of efficacy. Such changes in fibroblast activation could be detected earlier and with greater sensitivity utilising this technique, compared to existing approaches which have to wait until overt changes in myocardial fibrosis burden or systolic function become apparent (Table 4). In that way FAPI PET might accelerate and reduce the cost of phase 2 clinical trials.

FAP as a therapeutic marker and target

There is considerable interest in FAP+ cells as a therapeutic target. Whilst genetic deletion of FAP does not greatly affect cardiac fibrosis, targeting of FAP+ fibroblasts holds substantial potential. CAR-T cell therapy has resulted in major advances in the treatment of cancer over recent years. Cytotoxic T cells are engineered to target cancer cells and eliminate them with little collateral damage. Recently, several preclinical studies have investigated CAR-T cell therapy targeting FAP+ fibroblasts. Lee and colleagues demonstrated promising results in a rodent model of mesothelioma and used ^{18}F-AlF-FAPI to track this treatment response (45).In a mouse model of heart failure, CAR-T cell therapy targeting FAP+ fibroblasts reduced cardiac fibrosis and led to improvements in LV systolic and diastolic function (108) with more work ongoing to ensure that this is a feasible and safe treatment in cardiovascular conditions (109, 110)

Beyond CAR-T cell therapy, an alternative approach is to modify FAPI radiotracers so that they become theranostic agents. This can be accomplished by binding the tracers to a therapeutic radioligand (e.g. ^{131}I or ^{177}Lu) that emits high-energy beta or alpha particles, which subsequently kill nearby cells. This form of therapy provides temporal control so that FAP+ fibroblasts can be targeted only at those time points when they are inappropriately activated and not indefinitely (111). Theranostic FAPI approaches have been explored in several oncological research studies (43, 112, 113), but its use in cardiovascular diseases has not been investigated.

Conclusion

FAPI-PET is a novel molecular imaging technique that, for the first time, allows the assessment of fibroblast activation and fibrosis activity in patients with cardiovascular disease. Whilst in its infancy, initial reports are highly promising, and this approach looks set to dramatically improve our understanding of fibrosis activity across a wide range of cardiovascular disease states with important potential to accelerate novel therapeutic strategies and improve patient assessment and outcomes.

Figures

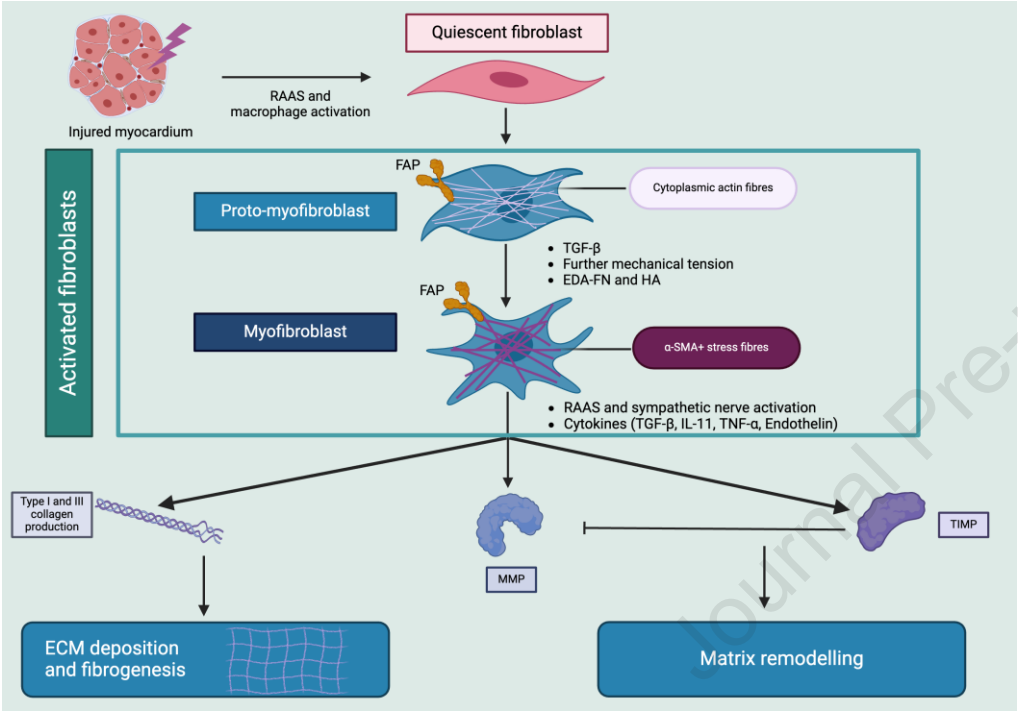


Figure 1: Cascade of myocardial fibrosis formation regulated by activated fibroblasts.

Following myocardial injury, RAAS and macrophage activation prompt the activation of the quiescent fibroblast to the intermediate proto-myofibroblast which rearranges membrane-associated actin into cytoplasmic filamentous actin fibres that form adhesion sites at membrane–ECM junctions. Further cytokine activity and synthesis of modulators that maintain the myofibroblast such as EDA-FN and HA result in maturation of the proto-myofibroblast into the myofibroblast. Myofibroblasts are characterised by the presence of thick, interconnected actin fibres that are α-SMA positive and result in the formation of mature, stable adhesion plaques at membrane-ECM junctions and, hence, increased contractile strength.

RAAS: Renin-angiotensin-aldosterone- system; TGF- β: Transforming growth factor- Beta; IL-11: Interleukin-11; TNF-α: Tumour Necrosis Factor-α; ECM: Extracellular matrix; MMP: Matrix metalloproteinases; TIMP: Tissue inhibitor of matrix metalloproteinases; α- SMA: Alpha smooth muscle actin; HA: Hyaluronan; EDA-FN : extra domain A fibronectin

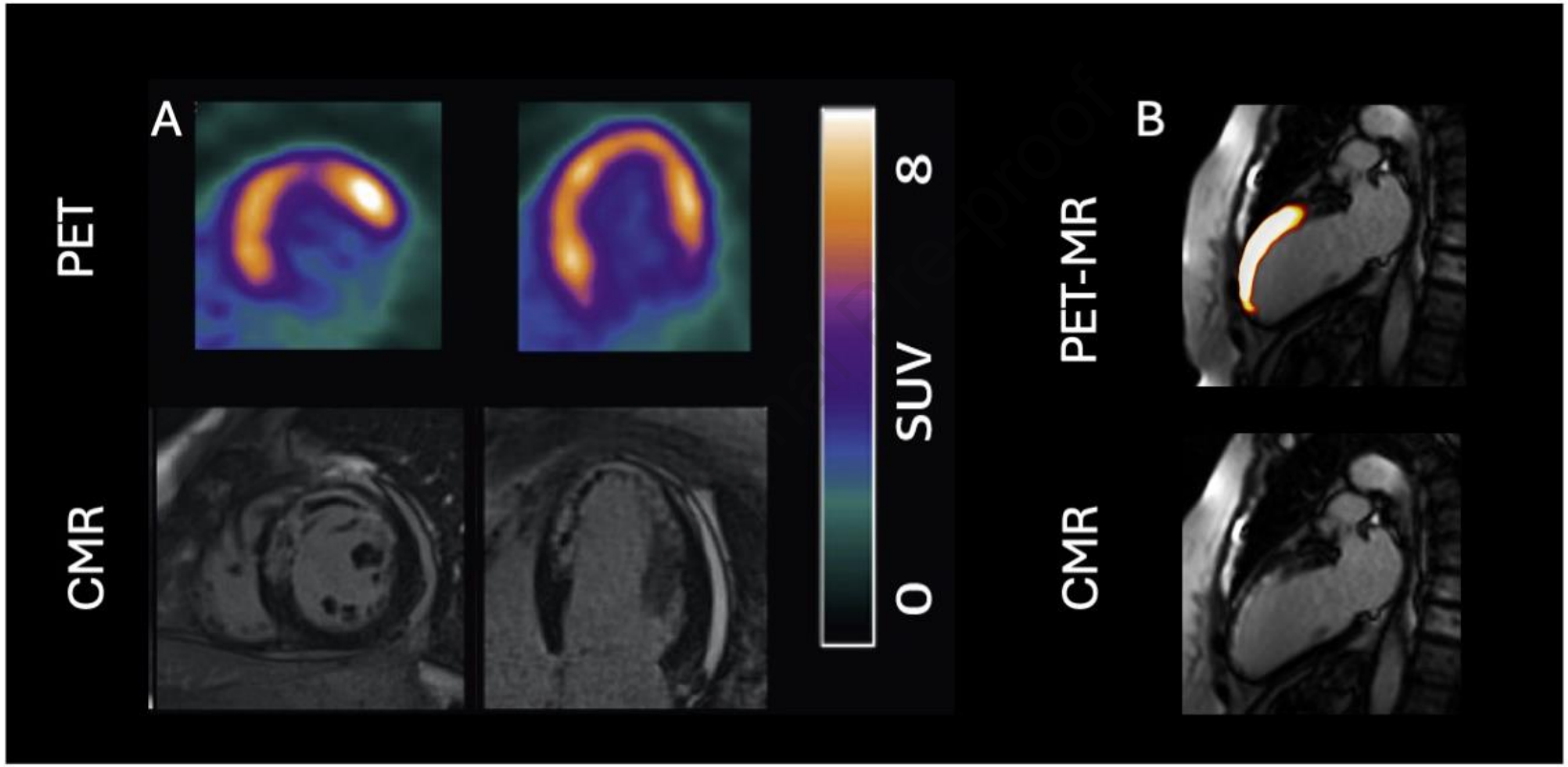


Figure 2: Examples of ^{68}Ga-FAPI uptake in acute myocardial infarction

A: An example of ^{68}Ga-FAPI uptake in a patient with an anterior acute myocardial infarction (top) with corresponding contrast-enhanced CMR images (bottom). This image was adapted from research published in *JNM*. Diekmann et.al. Cardiac Fibroblast Activation in Patients Early After Acute Myocardial Infarction: Integration with MR Tissue Characterization and Subsequent Functional Outcome. J Nucl Med. 2022. 63 (9) 1415-1423. © SNMMI.

B: Example of ^{68}Ga-FAPI uptake on hybrid PET-MR (Top) using FusionQuant software (Cedars- Sinai, Los Angeles) which provides automated motion correction and smooth image processing that reduces background noise.

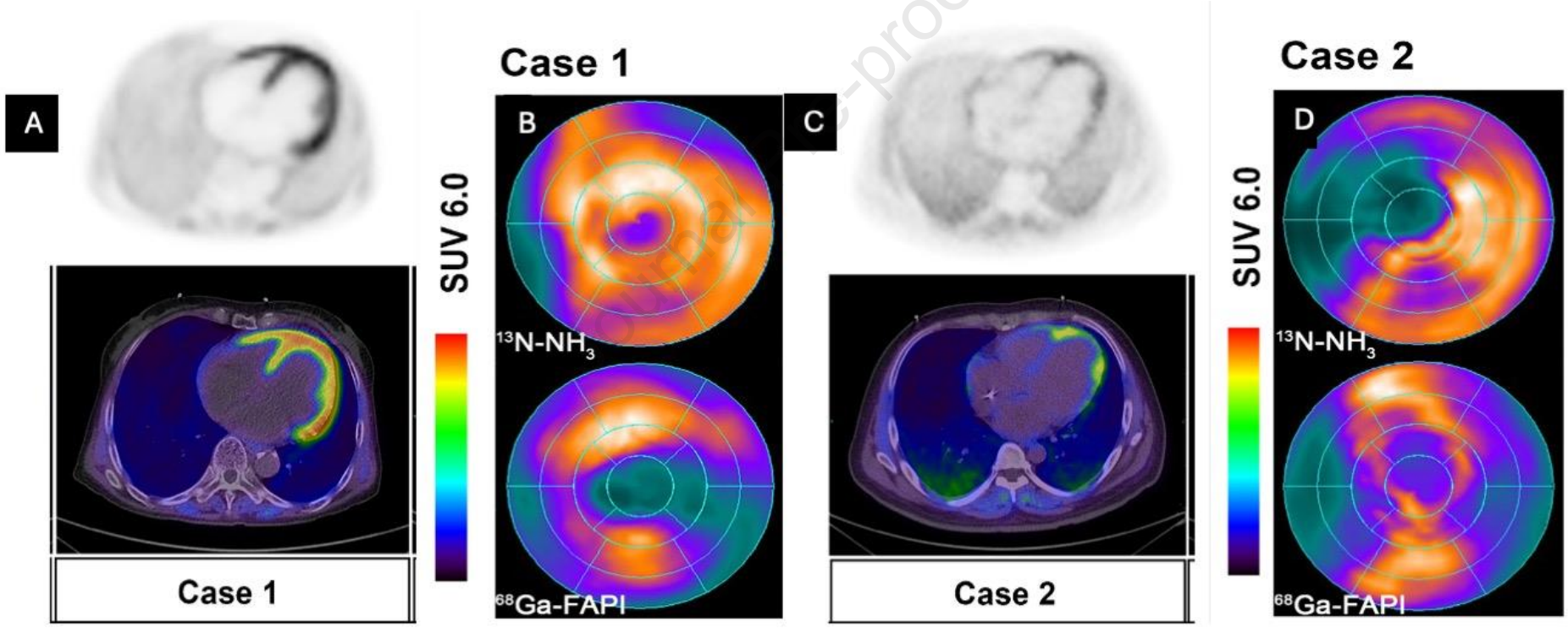


Figure 3: Examples of ^{68}Ga-FAPI PET-CT uptake in patients with heart failure and reduced ejection fraction compared to polar maps of ^{13}N-NH$_3$ perfusion scanning showing little correlation in patterns between the two.

A: axial view of ^{68}Ga- FAPI uptake in a patient with dilated cardiomyopathy. B (top) myocardial perfusion scanning demonstrating heterogenous reduction in perfusion while ^{68}Ga-FAPI (bottom) uptake shows more anterior and inferior fibroblast activation

C: Axial view of ^{68}Ga-FAPI uptake in a patient with severe left ventricular systolic dysfunction and a history of ischaemic heart disease. D (top) shows a large perfusion defect in the septum while 68Ga-FAPI (bottom) demonstrated fibroblast activation in the anterior and inferior walls.

Adapted from research originally published in EJNMMI. Song et.al. ^{68}Ga-FAPI PET visualize heart failure: from mechanism to clinic. *Eur J Nucl Med Mol Imaging.* 2023. 50 (2), 475–485 .© Springer Nature.

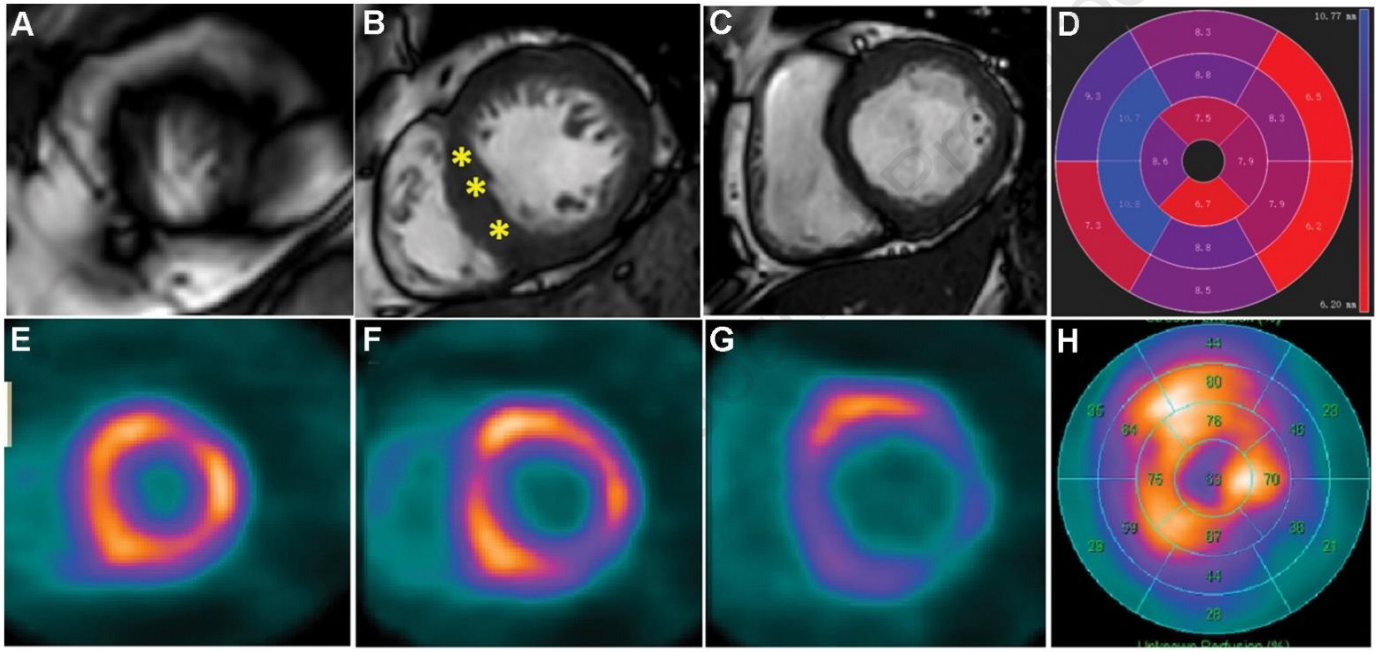


Figure 4: Example of ^{68}Ga-FAPI uptake in hypertrophic cardiomyopathy

(A–D): Image of a patient with HCM demonstrating that involved myocardium extended beyond areas of hypertrophy. The selected short-axis images from cardiac MRI (A–C) showed hypertrophic midseptum (15 mm, *), which is presented with a polar plot (D).

E–H : corresponding short-axis images of ^{18}F-FAPI (E–G) and polar plot (H) indicated the larger area of cardiac fibroblast activation beyond the hypertrophic region.

This research was originally published in *Radiology*. Wang et.al. Myocardial Activity at 18F-FAPI PET/CT and Risk for Sudden Cardiac Death in Hypertrophic Cardiomyopathy. Radiology. 2022. 11;306(2):e221052.© RSNA.

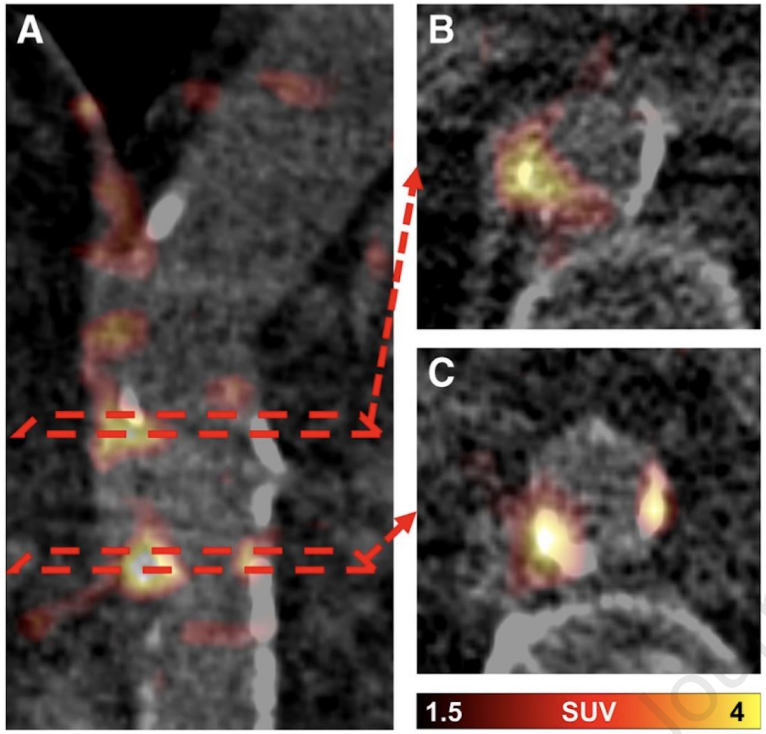


Figure 5: Example of atherosclerotic plaque FAPI uptake This research was originally published in *EJNMMI.* Kosmala et.al. Molecular imaging of arterial fibroblast activation protein: association with calcified plaque burden and cardiovascular risk factors Eur J Nucl Med Mol Imaging. 2023. 50(10); 3011-3021(84).

Fused ^{68}Ga-FAPI PET CT images of a patients with aortic valve atherosclerosis

A: Coronal PET-CT image demonstrating 2 major foci of ^{68}Ga-FAPI uptake with corresponding axial images (B and C).

B: ^{68}Ga-FAPI uptake exceeds the vessel wall calcification while a further calcified lesions shows no uptake.

C: ^{68}Ga-FAPI uptake co-localized well with vessel wall calcifications

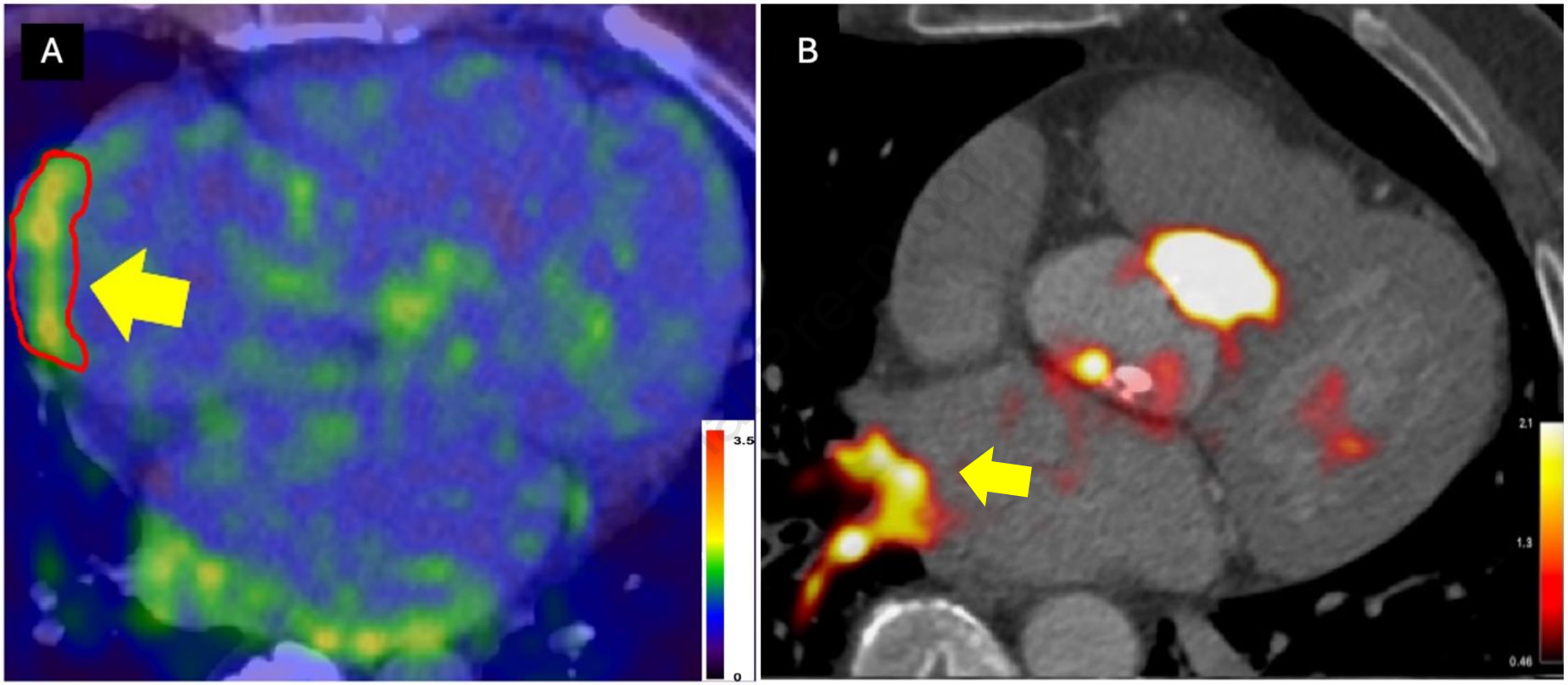
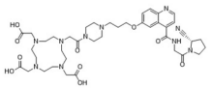


Figure 6: FAPI uptake in atrial fibrillation

A: An example of increased ^{18}F-AlF-FAPI uptake in the right atrial wall (TBRmax 2.5) in a fused PET-CT . Adapted from research originally published in *JNC.* Li et.al. Li, Lina, et al. Fibroblast activation protein imaging in atrial fibrillation: a proof-of-concept study. *J. Nucl. Cardiol.* 2023. 30(6); 2712-2720(90)^{68}Ga-FAPI uptake at the ostium of the left inferior pulmonary vein (A) and posterior wall of the left atrium (B)in a patient who also demonstrates ^{68}Ga-FAPI in their stenotic aortic valve.

Tables

Name	Chelator	Healthy myocardial uptake and internalisation	Hepatobiliary excretion	Tissue retention
FAPI-02  Loktev et al, 2018 (34)	DOTA	Medium	High	Low

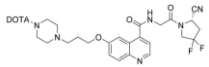
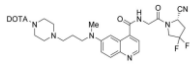
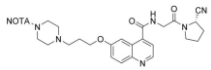
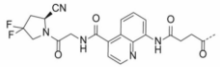
FAPI-04  Lindner et al, 2018 (29)	DOTA	Medium	Medium	Medium
FAPI-46  Loktev et al, 2019 (48) Meyer et al, 2020 (49)	DOTA	Medium	Medium	High
FAPI-74  Giesel et al, 2021 (32) Lindner et al, 2021 (114)	NOTA	High	Low	High
ONCOFAP  Millul et al, 2021 (115) Backhaus et al, 2022 (116)	NOTA and DOTA	Low	Low	High

Table 1: Properties of various FAPI radiologands based on dosimetry and biodistribution data across various organs.

Healthy myocardial uptake: SUVmeans at 1 hour post injection range from 0.32 to 1.2

Hepatobiliary excretion: SUVmeans at 1 hour post injection range from 0.5 – 1.6

Tissue retention: measured by the percentage drop in SUVmean in tumours in 3 hours post injection, ranging from 12-75%

Table 2: Key studies investigating FAPI imaging in Myocardial Infarction

Myocardial infarction				
Preclinical studies				
Title	**Model**	**Tracer**	**Imaging modality**	**Key results**
Varasteh et al (59)	Murine	^{68}Ga-FAPI	PET-CT, autoradiography	• Peak radiotracer uptake observed at 6 days post MI with return to baseline by 14 days • Peri-infarct regions demonstrated more intense uptake than infarct regions • No remote myocardial uptake up to 14 days • FAP expression confirmed in areas on ^{68}Ga-FAPI uptake on immunofluorescence • FAP+ cells were observed 3 and 8 times more in the border zones than in infarct and remote myocardium, respectively
Qiao et al, 2022 (60)	Rat	^{68}Ga-FAPI	PET-CT, Autoradiography, Immunofluorescenec, H&E staining	• Peak radiotracer uptake in infarcted myocardium with gradual decline until day 35, where uptake was similar to the sham-operated group • No significant remote ^{68}Ga-FAPI uptake was noted up to 35 days

				• Higher uptake demonstrated at the border zones on autoradiography	
Clinical studies					
Title	**Cohort and design**	**Tracer**	**Imaging modality**	**Key points**	**Correlation with imaging and serum biomarkers**
Diekmann et al (61)	35 STEMI patients, prospective	^{68}Ga-FAPI	PET-CT, SPECT, CMR	• Volume of ^{68}Ga-FAPI uptake correlated with a fall in LVEF at 140 days post infarct in 14 of the 35 patients • Segmental SUV_{mean} inversely correlated with CMR wall thickening	• ^{68}Ga-FAPI uptake areas exceeed areas of fibrosis as idenfitied by LGE, T1 and T2 levels • Positive correlation observed between ^{68}Ga-FAPI volume and maximum serum creatine kinase and C-reactive protein
Diekmann et al (63)	12 STEMI patients, Prospective	^{68}Ga-FAPI	PET-CT, SPECT, CMR	• Significant ^{68}Ga-FAPI uptake in the infarct, extending into the peri-infarct zones	• ^{68}Ga-FAPI uptake areas exceeds infarct areas identified on SPECT and CMR LGE imaging
Kessler et al (65)	5 STEMI and 5 NSTEMI patients, Prospective	^{68}Ga-FAPI	PET-CT	• All patients had significant ^{68}Ga-FAPI uptake in the myocardium • A complete to partial concordance was observed of visual ^{68}Ga-FAPI myocardial uptake and areas supplied by the culprit vessel	• ^{68}Ga-FAPI uptake volume was positively correlated with peak CK level and negatively correlated with LVEF

Xie et al (62)	14 STEMI patients and 14 healthy volunteers, Prospective	^{68}Ga-FAPI	PET-CT and CMR	14 patients post STEMI • Significant ^{68}Ga-FAPI uptake was observed over infarct zones and beyond • Baseline TBR_{max} inversely correlated with LV function at 7 weeks	• Areas of ^{68}Ga-FAPI uptake extended beyond LGE on CMR
Zhang et al (64)	26 STEMI patients, Prospective	^{68}Ga-FAPI	PET-MR	• Increased ^{68}Ga-FAPI volumes and TBRmax at baseline correlated with reduced LVEF at 1 year • Patients who had late LV remodelling (increase in LVESV >10% from baseline) had a higher baseline ^{68}Ga-FAPI volume	• No significant correlation between ^{68}Ga-FAPI volumes and serum biomarkers such as NT-proBNP and serum troponin I. • Baseline ^{68}Ga-FAPI predicted late LV remodelling better than LGE% and LGE volume on CMR
Kupusovic et al (66)	4 NSTEMI and 7 STEMI patients, prospective	^{68}Ga-FAPI	PET-MR	• Higher 68Ga-FAPI SUVmax was observed in STEMI than NSTEMI patients although not statistrically significant	• Mean ^{68}Ga-FAPI uptake volume exceeded estimated MRI infarct size but showed a strong positive correlation • ^{68}Ga-FAPI uptake volume had a strong correlation with LDH and peak creatine kinase

PET: Positron Emission Tomography; CT: Computed Tomography; 68Ga-FAPI: Gallium-labelled fibroblast activation protein inhibitor; MI: Myocardial infarction; FAP: Fibroblast activation protein; H&E: hematoxylin and eosin; STEMI: ST-elevation myocardial infarction; NSTEMI: Non ST-elevation myocardial infarction; CK: Creatine kinase; SPECT: Single-photon emission computed tomography; CMR: Cardiac magnetic resonance imaging; LGE: Late gadolinium enhancement; TBR_{max}: Maximum tissue-to-background ratio; LV: Left ventricle; LVEF: Left ventricular ejection fraction; NT-proBNP: N-terminal pro b-type natriuretic peptide

Table 3: Key studies of FAPI imaging in cardiomyopathies

Myocardial diseases					
Preclinical studies					
Title	**Condition**	**Model**	**Tracer**	**Imaging Modality**	**Key points**
Wang et al (68)	Pressure-overload	Rat	^{68}Ga-FAPI	PET-CT, PET-MR, Echocardiography, Immunohistochemistry	• Areas of increased ^{68}Ga-FAPI uptake corresponded with areas of myocardial hypertrophy on CMR • Early ^{68}Ga-FAPI uptake at 4 weeks corresponded to a reduced LVEF at 8 weeks • Increased myocardial ^{68}Ga-FAPI uptake in areas corresponding FAP expression on immunohistochemistry
Sun et al (69)	HFpEF	Rat	^{18}F-AlF-FAPI	PET-CT, Immunohistochemistry, Masson staining	• Increased ^{18}F-AlF- FAPI uptake in the LV myocardium in rats corresponded to post-mortem areas of FAP+ cells and interstitial and vascular fibrosis
Song et al (70)	HFrEF	Murine	^{68}Ga-FAPI	PET-CT	• A positive correlation was seen between baseline LV myocardial ^{68}Ga-FAPI uptake and decline in LV contractility and increased LV dilatation at 28 days

Wei et al (71)	Anthracycline-induced cardiotoxicity	Rat	^{68}Ga-FAPI	PET-CT, Echocardiography, Immunohistochemistry, Masson staining	• ^{68}Ga-FAPI uptake was significantly higher in rats with cardiotoxicity compared to control rats at weeks 3 and 6 although no significant change in LVEF was seen between groups • Enalapril treatment for 3 weeks reduced the intensity of ^{68}Ga-FAPI uptake, preserved LVEF and reduced myocardial fibrosis on Masson staining	
Clinical studies						
Title	**Condition**	**Cohort and design**	**Tracer**	**Imaging Modality**	**Key points**	**Correlation with imaging and serum biomarkers**
Wang et al (74)	Multiple non-ischaemic cardiomyopathies	29 patients, Prospective	^{68}Ga-FAPI	PET-CT, echocardiography	• 22 of the 29 scanned patients had heterogenous LV myocardial FAPI uptake • 10 patients had RV myocardial FAPI uptake across a spectrum of aetiologies	• SUV_{max} correlated significantly with LVEDD on echocardiography
Song et al (70)	HFrEF	7 patients , 20 participants without cardiac disease, Prospective	^{68}Ga-FAPI	PET-CT, ^{13}N-NH_3 perfusion scan	• Significantly elevated LV myocardial ^{68}Ga-FAPI uptake was seen in patients compared to healthy volunteers	• Areas of ^{68}Ga-FAPI uptake did not correspond with areas of reduced perfusion on ^{13}N- NH_3 myocardial perfusion scan

Wang et al (73)	HCM	50 patients with HCM vs 20 healthy volunteers, Prospective	^{18}F-AlF-FAPI	PET-CT, CMR (without gadolinium)	• Significantly higher LV myocardial ^{18}F-AlF-FAPI uptake in patients compared to healthy volunteers • 32 (64%) patients had increased ^{18}F-AlF-FAPI uptake in the RV myocardium and 4 (8%) had significant ^{18}F-AlF-FAPI uptake in the atria, 3 of whom had AF. • Increased ^{18}F-AlF-FAPI uptake positively correlated with the 5-year sudden cardiac death score and risk of malignant arrhythmia	• Increased ^{18}F-AlF-FAPI uptake was seen in all hypertrophied segments but also in non-hypertrophied segments in 84% of patients • ^{18}F-AlF-FAPI had a positive relationship with NT-proBNP and hs-cTnI and negative relationship with LVEF
Chen et al (76)	Chronic thromboembolic pulmonary hypertension	13 patients with CTEPH, prospectively recruited	^{68}Ga-FAPI	PET-CT, CMR, RHC	• 10 of the 13 patients demonstrated significant free RV wall ^{68}Ga-FAPI uptake • ^{68}Ga-FAPI uptake positively correlated with RV wall thickness and negatively correlated with RVEF	• Only 27% of significant RV ^{68}Ga-FAPI uptake corresponded to LGE on CMR • No correlation between ^{68}Ga-FAPI uptake and pulmonary haemodynamic parameters on RHC

Gu et al (77)	Pulmonary arterial hypertension	16 patients with PAH, prospectively recruited	^{68}Ga-FAPI	PET-CT, echocardiography, RHC	• 12 of the 16 patients had significant RV free wall and insertion point ^{68}Ga-FAPI uptake	• ^{68}Ga-FAPI uptake correlated with TAPSE on echocardiography • No significant correlation between ^{68}Ga-FAPI uptake and pulmonary haemodynamic parameters on RHC
Finke et al (75)	Checkpoint-inhibitor (ICI) associated myocarditis	26 patients who received ICI, 3 with myocarditis, Retrospective analysis	^{68}Ga-FAPI	PET-CT, CMR	• ^{68}Ga-FAPI uptake significantly higher in ICI myocarditis patients	• 2/3 patients with ICI had MRI. 1 had globally elevated T1-mapping and another had basal LGE. T2 mapping was normal
Wang et al (72)	Cardiac AL-amyloidosis	30 patients with AL amyloidosis (27 with cardiac involvement and 3 without) , Prospective	^{68}Ga-FAPI	PET-CT, CMR, Echocardiography	• 80% of AL CA patients had increased intensity of myocardial ^{68}Ga-FAPI uptake	• 4 patients with LGE on CMR also had significant ^{68}Ga-FAPI uptake • FAPI uptake correlated with Mayo stage and NTpro-BNP levels, ECV percentage on CMR and negatively correlated with LVPW thickness and LVEF on CMR

PET-CT: Positron Emission Tomography and Computed Tomography; PET-MR: Positron Emission Tomography and Magnetic Resonance imaging; HFpEF: Heart Failure with preserved Ejection Fraction; HFrEF: Heart Failure with reduced Ejetion Fraction; CMR: Cardiac Magnetic Resonance; ^{68}Ga-FAPI: Gallium-labelled fibroblast activation protein inhibitor; ^{18}F-AlF- FAPI: Aluminium fluoride- labelled fibroblast activation protein inhibitor; FAP: Fibroblast activation protein; LV: Left ventricle; LVEF: Left ventricular ejection fraction; DCM: Dilated Cardiomyopathy; HCM: Hypertrophic cardiomyopathy; SUV_{max}: Maximum Standardised Uptake Value; SUV_{mean}: Mean Standardised Uptake Value SPECT: Single-photon emission computed tomography; LGE: Late gadolinium enhancement; LVEDD: Left Ventricular End Diastolic Diameter; TBR_{max}: Maximum tissue-to-background ratio; NT-proBNP: N-terminal pro b-type natriuretic peptide; ^{13}N- NH_3: ^{13}N- ammonia; LVOT: Left ventricular outflow tract; RV: Right ventricle; hs-cTnI: High-sensitivity cardiac troponin I; RHC: Right heart catheterization; RVEF: Right ventricular ejection fraction; ICI: Checkpoint-inhibitor; CTEPH: Chronic Thromboembolic pulmonary hypertension; PAH: Pulmonary Arterial Hypertension; TAPSE: Tricuspid annular plane systolic excursion; AL- amyloidosis: Light-chain amyloidosis; ECV: Extracellular volume; LVPW: Left ventricle posterior wall

Table 4: Novel anti-fibrotic medications under development

Receptor	Function	Name of drug	Preclinical data		Clinical data	
			Cohort and model	Key points	Cohort and design	Key points
CTGF Also known as CCN2	Mediates ECM production in pathological states	CTGF-mAb	Pressure-overload, mice(117)	• Improved LV systolic function • Reduced cardiomyocyte hypertrophy in murine models	Nil	
			DCM, mice (118)	• Improved LV systolic and diastolic dysfunction	Nil	
			MI, mice (119)	• Improved 7-day survival • Improved LV function	Nil	

				• No difference in infarct size or 7 week survival		
Galectin-3	Interacts with aldosterone to promote macrophage infiltration and mediates cardiac fibroblast proliferation	Modified Citrus Protein (MCP)	HF, rats (120)	• Improved LV function • Reduced expression of collagen 1 and 3 genes	Nil	
			HF, mice (121)	• Improved LV function • Reduction in cardiac hypertrophy and fibrosis when treated in combination with an aldosterone antagonist		
MicroRNA- 132-3p (mIR-132)	Downregulates expression of Forkhead box O3 (FOXO3) which is anti-hypertrophic and suppresses calcium handling and myocardial contractility	Anti-mIR-132	Post-MI HFrEF, mice (122)	• Improved LVEF and NT pro-BNP levels • Reduction in myocardial interstitial fibrosis on in mice	Prospective randomised, double-blind, placebo-controlled trial of 28 patients with HFrEF (LVEF 30-49%) (123)	• No adverse reactions • Combined endpoint of NT pro-BNP reduction of >10% and LVEF increase of >2% was achieved in 79% in the pharmacodynamically active group vs 46% in the inactive group
Renin	Inhibits angiotensin II and promotes angiotensin II-	Aliskiren	Pressure-overload, mice (124)	• Improved LVEF, LVFS and collagen volume	Nil	

	independent reduction in TGFB-1 and collagen production					
TGF-β	Inhibition of TGF-β reduces collagen deposition in the extracellular matrix	Pirfenidone	Pressure-overload, mice (125)		PIROUTTE trial-randomised, double-blind, placebo-controlled trial in 47 HFpEF patients (126)	• Reduction of ECV and LV mass on CMR • 26% of treated patients has side effects (nausea, insomnia, rash) although similar to placebo group
			MI (127)	• Lower total LV fibrosis • Higher LVEF		
Phosphorylated Smad2 inhibitor	Phosphorylated Smad2, a downstream product of TGF-beta, increases ECM production	FT011	MI, rat (128)	• Improved LVEF • Reduced amount of cardiac fibrosis in non-infarct regions	Nil	
Matrix metalloproteinase (MMP) inhibitors	MMPs promote the degradation of ECM and adverse	PG-116800	Pressure-overload, mice (129)	• Lower LVEDV and intersitial fibrosis in MMP knockout (KO) mice • No difference in LVEF (129)	Randomised placebo-controlled double-blind trial of 253 post-	• No difference in mortality, cardiac admissions or LVEF • Higher incidence of gastrointestinal

	myocardial remodelling		MI, mice (131)	• MMP-29 KO mice had a lower 7-day survival mostly due to cardiac rupture. • LVESD and LVEDD were higher in KO mice than WT, while collagen I and III were lower in KO mice (131)	STEMI HFrEF patients (130)	disturbance and joint stiffness in the treatment group
Relaxin	Stimulates fibroblast differentiation and collagen deposition as well as promoting MMP-induced ECM degradation	Seralaxin	MI, mice (132)	• Lower LVEDP • Reduced collagen volume in infarct and border regions • No significant difference in LVEF	RELAX-AHF trial- a randomised placebo-controlled trial of Seralexin in 1161 acute heart failure patients (133)	• Improved patient-reported dyspnoea but no difference in days alive out of hospital up to 60 days • Reduced number of cardiovascular deaths up to 180 days • No difference in cardiac-related hospitalisations
			Pressure-overload, mice (134)	• No difference in LV mass, LVEF or collagen content		

CTGF: Connective tissue growth factor; ECM: Extracellular matrix; CTGF-mAb: Connective tissue growth factor- monoclonal antibody; LV: Left ventricle; MI: Myocardial infarction; DCM: Dilated cardiomyopathy; MCP: Modified citrus protein; HF: Heart failure; mIR-132: MicroRNA- 132-3p; LVEF: Left ventricular ejection fraction; NT pro-BNP: N-terminal prohormone of brain natriuretic peptide; HFrEF: Heart failure with reduced ejection fraction; PD: Pharmacodynamically; LVFS: Left ventricular fractional shortening; ECV: Extracellular volume; CMR: Cardiac magnetic resonance; LVEDV: Left ventricular end-diastolic volume; LVESV: Left ventricular end-systolic volume; KO: Knock-out; WT: Wild-type; LVEDD: Left ventricular end-diastolic diameter; LVESD: Left ventricular end-systolic diameter; STEMI: ST-Elevation myocardial infarction

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