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REVIEW

Myocardial fibrosis: biomedical research from bench to bedside

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Myocardial fibrosis refers to a variety of quantitative and qualitative changes in the interstitial myocardial collagen network that occur in response to cardiac ischaemic insults, systemic diseases, drugs, or any other harmful stimulus affecting the circulatory system or the heart itself. Myocardial fibrosis alters the architecture of the myocardium, facilitating the development of cardiac dysfunction, also inducing arrhythmias, influencing the clinical course and outcome of heart failure patients. Focusing on myocardial fibrosis may potentially improve patient care through the targeted diagnosis and treatment of emerging fibrotic pathways. The European Commission funded the FIBROTARGETS consortium as a multinational academic and industrial consortium with the primary aim of performing a systematic and collaborative search of targets of myocardial fibrosis, and then translating these mechanisms into individualized diagnostic tools and specific therapeutic pharmacological options for heart failure. This review focuses on those methodological and technological aspects considered and developed by the consortium to facilitate the transfer of the new mechanistic knowledge on myocardial fibrosis into potential biomedical applications.

Keywords Myocardial fibrosis • Animal models • Biomarkers • Cardiac imaging

Introduction

Cardiomyocytes, fibroblasts, and vascular cells in the heart are connected by a complex matrix principally composed of fibrillar collagen, which is instrumental in preserving structural integrity and plasticity. In the diseased heart, the matrix undergoes structural and subcellular changes that progressively influence heart function.[1] Beyond the cardiomyocyte-centric view of heart injury, it is now accepted that alterations of the cardiac extracellular matrix (ECM) and cardiac remodelling play a major role in the development and evolution of cardiac diseases leading to heart failure (HF).[1] These ECM alterations result in cardiac fibrosis. At the site of myocardial infarction (MI), acute focal fibrotic scarring provides myocardial healing and prevents rupture.[2] In contrast, chronic diffuse or focal reactive myocardial fibrosis is a consequence of either pressure or volume overload due to persisting hypertension, metabolic disorders, valvular heart diseases, ischaemic injury (in areas remote from the infarction), or diffuse myocardial diseases, such as cardiomyopathies. Myocardial fibrosis is characterized by dysregulated collagen turnover (increased synthesis predominates over unchanged or decreased degradation)[3,4] and excessive diffuse collagen accumulation in the interstitial and perivascular spaces.[5] The dysregulation of distinct pro- and antifibrotic factors, including cytokines and chemokines, growth factors, proteases, hormones, and reactive oxygen species, is responsible for the alteration of the collagen matrix[6] (*Figure 1*). This dysregulation of collagen turnover takes place mainly in phenotypically transformed fibroblasts, termed myofibroblasts.[1,7] The phenoconversion of fibroblasts into myofibroblasts involves the expression of alpha-smooth muscle actin, a characteristic of smooth muscle

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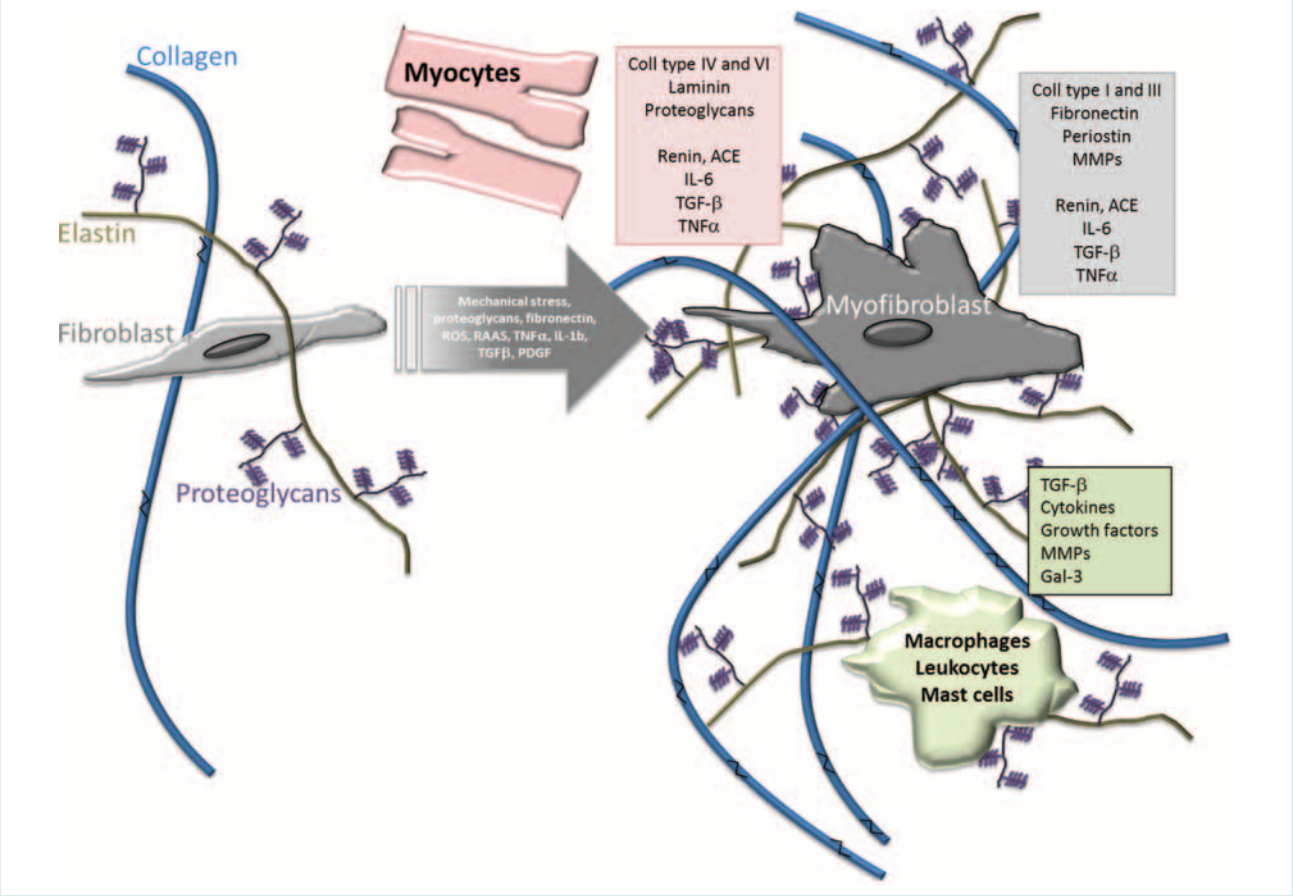

Figure 1 Schematic representation of biochemical and cellular mechanisms of cardiac fibrosis. Under physiological conditions (left), fibroblasts secrete extracellular procollagen chains into the interstitium that assemble into fibrils and are cross-linked by lysyl oxidase. Several cell types are implicated in fibrotic remodelling of the heart either directly by producing matrix proteins (fibroblasts), or indirectly by secreting fibrogenic mediators (macrophages, mast cells, lymphocytes, cardiomyocytes, and vascular cells). Under pathological conditions (right), alterations in the matrix environment, induction and release of growth factors and cytokines, and increase of mechanical stress dynamically modulate fibroblast transdifferentiation into myofibroblasts. Higher collagen cross-linking results in increased myocardial tensile strength. Resistance to degradation by matrix metalloproteinases (MMPs) increases cross-linked collagen, which favours matrisome expansion. Pink, grey, and green boxes list part of the secretome of mycocytes, myofibroblasts, and macrophages/leucocytes/mast cells, respectively, that trigger and maintain fibrosis. Gal-3, galectin-3; IL, interleukin; PDGF, platelet-derived growth factor; RAAS, renin–angiotensin–aldosterone system; ROS, reactive oxygen species; TGF, transforming growth factor; TNF, tumour necrosis factor.

cells, as well as the appearance of an extensive, synthetically active endoplasmic reticulum, and is stimulated by a number of bioactive effectors.[1,7–12] Fibroblasts and particularly myofibroblasts secrete extracellular procollagen chains that assemble into collagen type I and III fibrils and become cross-linked to form the final fibres.[3] Collagen cross-linking is an important post-translational modification because it increases myocardial tensile strength and the resistance of collagen fibres to degradation by matrix metalloproteinases.[13,14]

Myocardial fibrosis disrupts the myocardial architecture, contributes to myocardial disarray, and determines mechanical,[15,16] electrical,[17–19] and vasomotor[20] dysfunction, thus promoting the progression of cardiac diseases to HF.[21] Of note, fibrosis persists in the myocardium of HF patients under the current treatment regimen recommended by the official guidelines;[22] thus, the treatment of HF patients improves clinical symptoms, but does not reverse fibrosis. In aortic stenosis patients, aortic valve replacements result in regression of LV hypertrophy (LVH), indicating that hypertrophy and fibrosis are reversible. Furthermore, the severity of histologically proven myocardial fibrosis has been reported to be associated with higher long-term mortality in patients with cardiac diseases, particularly those with HF.[23,24] In this regard, the detection, prevention, and regression of myocardial fibrosis have emerged as important targets for improving HF therapy.[25,26]

In order to achieve significant diagnostic and therapeutic advances, it appears to be critical to identify new profibrotic mechanisms not yet targeted by currently available therapies, and to translate these mechanisms into individualized diagnostic tools and specific therapeutic targets. Besides meaningful functional and therapeutic outcomes, valid molecular targets must not induce serious adverse effects, such as influencing inflammation processes

or wound healing. Therefore, there is a need for a systematic collaboration between clinical investigators and basic scientists, together with industry, to allow integration of data from computer biomedical (*in silico*) and basic (*in vitro*) studies with pre-clinical (*in vivo*) research findings to be distilled into clinically actionable information for the fight against myocardial fibrosis. The FIBROTARGETS consortium is a multinational consortium with industrial and academic partners, funded by the European Commission and primarily aimed at characterizing novel emerging mechanisms of myocardial fibrosis.[6] Targets and biomarkers under investigation include proteins, proteoglycans, and microRNAs (miRNAs), and have been reviewed previously.[6] This review highlights the methodological issues considered by the consortium to transfer these mechanisms into diagnostic biomarkers and therapeutic agents amenable to improve patient care.

Translational animal models of myocardial fibrosis

Sophisticated *in vitro* models ('organ-on-a-chip') for cardiac tissue based on induced pluripotent stem cells have recently been developed and allow, for example, cardiotoxicity screening.[27] Human cells adequately represent human biology, and thus will probably improve pre-clinical drug screening. However, these cell culture models can currently not sufficiently mirror complex physiological and pathological processes and the interplay of distinct cell types in the heart. Thus, animal models are essential in reliably assessing pathological features and for evaluation of potential new drugs. As the development of myocardial fibrosis is characterized by a complex dysregulation of a number of different factors including inflammatory chemokines, angiotensin II, and endothelin signalling, there is a need for accurate and adequate animal models, which ideally closely reflect the pathological mechanisms found in humans. These animal models are instrumental in investigating molecular mechanisms of myocardial fibrosis, for identification and validation of disease targets, and for efficient pre-clinical drug development and testing. Fibrosis is induced by various genetic dispositions, pressure or volume stress, heart injuries, and diseases. There is evidence that depending on the particular trigger, distinct molecular pathways have varying importance for the individual types of fibrosis. Consequently, the type of fibrosis induction in animal models is important for their translational value for distinct diseases. Several aspects of HF and myocardial fibrosis are not fully reproducible in animal models: often, a cluster of risk factors, such as metabolic syndrome, is essential in the development of HF. CAD is characterized by gradual narrowing of arteries due to atherosclerosis, but infarction in animal models is triggered by sudden artery occlusion.

Small animals

Several rodent models are available that reproduce some of the main causes of chronic HF such as hypertension, diabetes, metabolic syndrome, or a combination of several of these factors (*Table 1*). The various animal models differ not only in their availability and ease of use, but also importantly in the mechanism, time course, and severity of cardiac fibrosis. Transgenic and knockout mouse models are used with the aim to model genetic phenotypes and predispositions to cardiac hypertrophy and HF.[28] Mutations or depletion of type I collagen,[29] and α- or β-cardiac myosin heavy chain[30,31] have been introduced in mice to model fibrosis or hypertrophic cardiomyopathy, respectively. The respective animals are used to simulate congenital cardiac hypertrophy, and they develop severe cardiac hypertrophy with substantial development of interstitial fibrosis and collagen deposition.[32] While a more gradual development of pressure overload can be considered to be more clinically relevant, maintenance over a relative long period of time is necessary for development of severe HF.

Infusion of angiotensin II induces severe cardiac interstitial fibrosis in mice. More frequently, surgical interventions in rodents[33] are used as models of fibrosis. Artificial MI by ligation of the coronary artery in mice eventually followed by reperfusion results in scarring, cardiac remodelling, and fibrosis.[34] Repeated brief ischaemia and reperfusion resulted in chemokine induction, inflammation, cardiac dysfunction, and fibrosis in the absence of MI.[35] A partial occlusion of the ascending or descending aorta by a ligature or clip (aortic banding) which is followed by an abrupt increase in pre-occlusion pressure is commonly used to model LVH in rodents.[28,36] Cardiomyocyte hypertrophy and extensive diffuse fibrotic remodelling occur after several days. However, surgical partial occlusion of the aorta requires an open-chest procedure, and causes an immediate compromise of the circulation and a sudden increase in pressure stress, in contrast to the more gradual development of myocardial hypertrophy and fibrosis within pathological settings. Furthermore, the sudden pressure increase can cause myocardial injury. A gradual increase of overload is difficult to achieve in rodents. In addition, there are important deviations between rodent and human hearts on the macroscopic (e.g. size, beating frequency) and molecular levels (e.g. relative predominant expression of major histocompatibility complex isoforms and the importance of distinct signalling pathways).

Large animals

Direct translation of murine or rat models to the clinic is problematic, and large animal models are essential for successful translational aspects. In general, large animals used for translational research share a higher extent of genetic homology with humans as compared with rodents. A number of physiological and pharmacological parameters in large animal models are closer to humans, and they have a longer life span, which facilitates longitudinal studies. Dog, pig, and sheep models of HF and fibrosis have been developed, and these species resemble the human pathophysiology more closely.[37] Dogs have long been studied in cardiology as a model for MI, and ischaemia and reperfusion results in cardiac remodelling and fibrosis. In this dog MI/reperfusion model, the ARB valsartan resulted in decreased infarct size, increased EF, and improved diastolic function.[38] Ischaemic cardiomyopathies can be simulated in canines through coronary microembolization,[39] resulting in reduction of LVEF to <35%. Over the course of a few months, progressive LV dysfunction with neurohumoral activation occurred. For simulating the volume overload HF phenotype, mitral

Table 1 **Animal models for studying myocardial fibrosis**

Model	Species	Fibrosis generation	Degree of fibrosis	Mechanism	Advantages	Limitations	Fibrosis-affected organs	Examples	Relevance for human therapy	Reference
Volume and/or pressure overload-induced fibrosis										
Genetic	M, R	Spontaneous mutation	Varying degree of fibrosis and collagen accumulation, with/without cardiac hypertrophy	Altered signalling pathways, according to gene defect	Commercially available, reproducible, long-term progressive interstitial fibrosis	Expensive, long-term treatment needed to expect prevention/decrease of MIF (at least 3 months)	Not restricted to myocardium	SHR, Dahl salt-sensitive rat	Life-long follow-up for treatment effect possible, symptomatic HF treatment	(28,77)
	M	Transgenesis, homologous recombination, inducible null	Varying degree of fibrosis and collagen accumulation, with/without cardiac hypertrophy	Altered signalling pathways, according to gene defect	Programmed cardiac hypertrophy with accompanying fibrosis	Difficult to obtain; extensive gene manipulation	Mainly myocardium	Muscle lim protein KO; mutation or depletion of type I collagen or alpha- or beta-cardiac myosin heavy chain	Life-long follow-up for treatment effect possible, symptomatic HF treatment	(30,31)
	R	Transgenesis	Diffuse or focal, indirectly associated with cardiac fibrosis	Altered signalling pathways, according to actual gene manipulation	Mostly commercially available, reproducible	Non-specificity and non-pathological levels of expression, indirectly associated with fibrosis generation	Not restricted to myocardium	Ren-2 gene, and TGR(mREN2)27, ACE2, and several others	Life-long follow-up for treatment effect possible, symptomatic HF treatment	(78)
Pharmacological	M, R, GP, D, P, S	NOS inhibitors; activation of RAAS; isoproterenol infusion	Mild, eventually focal fibrosis	Depending on the pharmacological agent, proliferation of non-myocyte cells	Good reproducibility, non-invasive	Questioned applicability	Not restricted to myocardium	Infusion of angiotensin II	Highly relevant for therapeutic target search	(79)
Surgical	M, R	Ascending aortic constriction	Mild, eventually focal fibrosis of the left ventricle	Activated renin–angiotensin system	Resembling human disease, quick onset of hypertension and related fibrosis	High mortality, technically challenging	Left ventricular myocardium	Severe aortic stenosis and cardiac hypertrophy induced cardiac fibrosis	Primarily preventive antihypertensive treatment	(54)
	GP, D, P, S	Descending aortic constriction	Mild, eventually focal fibrosis of the left ventricle	Activated renin–angiotensin system	Reproducible, leads to diffuse severe cardiac hypertrophy	Surgical procedure, development of fibrosis from hypertrophy requires longer time	Left ventricular myocardium	Severe aortic stenosis and cardiac hypertrophy-induced cardiac fibrosis	Primarily preventive antihypertensive treatment	(80)
	M, R,	Pulmonary artery constriction	Right ventricular hypertrophy and dilation, mild, eventually focal fibrosis of the right ventricle	Sarcoplasmic reticulum Ca-ATP-ase and phospholamban down-regulation	Reproducible, leads to diffuse severe hypertrophy of the right ventricle	Surgical procedure, development of fibrosis from hypertrophy requires longer time	Right ventricular myocardium	Severe pulmonary stenosis and right heart insufficiency	Primarily preventive antihypertensive treatment	(81)

Table 1 continued

Model	Species	Fibrosis generation	Degree of fibrosis	Mechanism	Advantages	Limitations	Fibrosis-affected organs	Examples	Relevance for human therapy	Reference
	D	Arteriovenous shunt; disruption of mitral cord; gradual constriction of the renal artery	Mild, eventually focal fibrosis of the left ventricle	Eccentric or concentric cardiac hypertrophy	Diffuse mild to moderate cardiac hypertrophy	Surgical procedure, development of fibrosis from hypertrophy requires longer time	Not restricted to myocardium	Cardiac hypertrophy	Causative treatment, difficult to treat with medicines	(40)
	P	Percutaneous artificial aortic isthmus stenosis	Severe and diffuse	Activated renin–angiotensin system	Very similar cardiac anatomy compared with human	Surgical procedure, development of fibrosis from hypertrophy requires longer time	Left and right ventricular myocardium	Severe aortic stenosis and cardiac hypertrophy induced cardiac fibrosis	Primarily preventive antihypertensive treatment	(56)
Metabolic disease-related cardiac fibrosis										
Genetic	M, R	Spontaneous mutation (colonies)	Varying degree of fibrosis and collagen accumulation, with/without cardiac hypertrophy	Altered signalling pathways, according to gene defect	Commercially available, reproducible, long-term progressive interstitial fibrosis	Expensive, dramatic phenotypes	Not restricted to myocardium	SHHF, Zucker rat, ZSF1-*Lepr*^{fa}*Lepr*^{cp}/Crl, ZDF-Lepr$^{fa/crl}$ rats, Lep^{ob} and Lepr^{db} mice	Life-long follow-up for treatment effect possible, symptomatic HF treatment	(82,83)
	M, R	Transgenesis, homologous recombination, inducible null	Varying degree of fibrosis and collagen accumulation, with/without cardiac hypertrophy	Altered signalling pathways, according to gene defect	Reproduces gene expression alteration seen in disease	Developmental effect of mutant allele	Not restricted to myocardium	Conditional gene targeting in mice	Life-long follow-up for treatment effect possible, symptomatic HF treatment	(90)
Pharmacological	M, R, P	Streptozotocin-induced diabetes	Mild, or no	Non-specific metabolic syndrome	Relative reproducibility, non-invasive	High mortality, dramatic phenotypes; difficult method for large animals	Not restricted to myocardium	Similar to human metabolic syndrome	Treatment of metabolic syndrome	(84)
Dietary-induced	M, R, P	High fat diet, western diet	Mild, or no	Non-specific metabolic syndrome	Resembling human disease	Long and uneven results requiring large number of animals	Not restricted to myocardium	Similar to human metabolic syndrome	Treatment of metabolic syndrome	(85)
Myocardial ischaemia-associated myocardial fibrosis										
Surgical	M, R, D, and S	Coronary ligation (ischaemia w/o reperfusion): AMI-related ventricular remodelling	Localized to the ischaemia site	Ischaemic necrosis and reparative fibrous scar formation, loss of myocytes in the ischaemia-affected region, compensatory hypertrophy in the contralateral areas, later adverse remodelling	Commercially available, reproducible, mid-term progressive interstitial fibrosis distant from the infarct zone (posteroinferior wall)	Moderately expensive; technically challenging; high mortality; mid-term treatment needed to expect prevention/decrease of MIF	Part of the left ventricular myocardium	Pre-clinical chronic coronary artery occlusion model	Anti-ischaemic medication, primary and secondary prevention	(33,34)

Table 1 continued

Model	Species	Fibrosis generation	Degree of fibrosis	Mechanism	Advantages	Limitations	Fibrosis-affected organs	Examples	Relevance for human therapy	Reference
	D	Coronary artery microem-bolization	Localized to the ischaemia site	Ischaemic necrosis and reparative fibrous scar formation, loss of myocytes in the ischaemia-affected region, compensatory hypertrophy in the contralateral areas, later adverse remodelling	Mimics diffuse small-vessel disease of the heart	Focal myocardial ischaemia	Part of the left ventricular myocardium	Pre-clinical chronic coronary artery occlusion model	Anti-ischaemic medication, primary and secondary prevention	(39)
	P	Surgical placement of ameroid constrictor for coronary ligation; closed chest ischaemia/ reperfusion model	Localized to the ischaemia site	Ischaemic necrosis and reparative fibrous scar formation, loss of myocytes in the ischaemia-affected region, compensatory hypertrophy in the contralateral areas, adverse remodelling	Very similar cardiac anatomy compared with human	Focal myocardial ischaemia	Part of the left ventricular myocardium	Pre-clinical chronic coronary artery occlusion, or reperfused infarction model	Anti-ischaemic medication, primary and secondary prevention	(49,50)
Dilated cardiomyopathy-related cardiac fibrosis										
Genetic	M	KO of certain genes inducing dilated car-diomyopathy	Mild to severe	Altered signalling pathways, according to knockout gene	Commercially available, reproducible, long-term progressive interstitial fibrosis	Difficult to obtain; extensive gene manipulation	Mainly myocardium	MybpC	Life-long follow-up for treatment effect possible, symptomatic HF treatment	(86)
Pharmacological	R, Rab	Systemic cytotoxic therapy	Mild to severe, induced by myocyte apoptosis	Mitochondrial, endoplas-mic/sarcoplasmic reticulum pathways	Reproducible, leads to dilated CMP with diffuse fibrosis	Highly relevant for humans, through anticancer therapy	Diffuse, biventricular fibrosis with normal heart size	Cardiotoxicity after cytotoxic treatment	Currently palliative therapy, standard HF therapy	(87)
Immunological	R	Viral or autoimmune myocarditis	Severe, based on acute severe interstitial inflammation	Diffuse inflammation, myocyte loss, and replacement with reactive fibrosis	Reproducible, leads to dilated CMP with diffuse fibrosis	Acute inflammation-induced persistent myocardial fibrosis	Myocardial, biventricular enlargement of the heart, thin wall, hypertrophic or atrophic fibres, infiltrating mononuclear cells	Persistent viral infection, immunization with cardiac myosin fraction	Primarily antiinflammatory agents as therapy, chronic phase is similar to human dilated CMP	(88)
Surgical	D, P, Rab, S	Tachycardia pacing	Mild, diffuse, extracellular matrix remodelling	Myocardial energy depletion, abnormal Ca-channel activity and excitation-contraction coupling	Reproduces congestive HF by low output	Non-specific for structural fibrosis	Left and right ventricular myocardium with enlargement of the heart	Tachycardia pacing	Primarily preventive antitachycardia therapy	(89)

AMI, acute myocardial infarction; CMP, cardiomyopathy; D, dog; GP, guinea pig; HF, heart failure; KO, knockout; Lep, leptin gene; Lepr, leptin receptor gene; M, mouse; MIF, myocardial interstitial fibrosis; MybpC, myosin-binding protein C; NOS, nitric oxide synthase; P, pig; R, rat; RAAS, renin–angiotensin–aldosterone system; Rab, rabbit; Ren, renin; S, swan; SHHF, spontaneously hypertensive heart failure rat; SHR, spontaneously hypertensive rat.

Table 2 Non-invasive techniques for assessment of myocardial fibrosis (adapted from Jellis et *al.*[2])

Technique for fibrosis detection	Specificity	Fibrosis characterization	Fibrotic disease diagnosis	Localization of fibrosis	Description	Availability	Technical challenge
Echocardiography							
backscatter	Low	Increased acoustic brightness, backscatter techniques	Hypertrophy, muscular dystrophy, systemic sclerosis	Transmural trend of fibrosis, diffuse	Quantitative assessment	Good	Easy
Tissue Doppler imaging	Low	Impairment of longitudinal function of the left ventricle, strain and strain rate	Non-ischaemic or ischaemic heart disease	Diffuse	Functional assessment	Good	Easy
Nuclear imaging							
SPECT myocardial perfusion scintigraphy	Low	Indirect, perfusion defect reflects myocardial scar	Myocardial infarction	Segmental	Indirect proof of collagenous scar	Good	Easy
SPECT myofibroblast labelling	High	Targeted myofibroblast receptor labelling	Myocardial scar	Segmental	Only experimental	Specialized institution	Complicated
SPECT collagelin labelling	High	Localization of collagen-producing myofibroblasts	Left ventricular remodelling and prediction of heart failure	Infarct area, peri-infarct zone and remote areas	Only experimental	Specialized institution	Complicated
PET perfusable tissue index	High	Calculated indirect marker, correlates with reduced circumferential shortening in MRI tissue tagging	Ischaemic and non-ischaemic cardiomyopathy	Segmental or diffuse	Quantitative assessment	Moderate	Easy
PET ^{15}O-labelled water	High	Calculation of perfusable tissue index	Ischaemic and non-ischaemic cardiomyopathy	Segmental or diffuse	Only experimental	Specialized institution	Complicated
Cardiac magnetic resonance							
Delayed enhancement with T1 imaging	High	High intensity signal in late enhancement image using inversion recovery gradient-echo sequences, shortening of the inversion time (T1)	Myocardial infarction	Ischaemic area: subendocardial or transmural localization; non-ischaemic fibrosis is rather irregular and intramural, often subepicardial	Quantitative assessment	Good	Easy
T1 mapping	High	Contrast-enhanced T1 mapping, use of modified Look-Locker inversion–recovery prototype sequence	Non-ischaemic cardiomyopathy	Diffuse	Quantitative assessment	Good	Easy

Table 2 continued

Technique for fibrosis detection	Specificity	Fibrosis characterization	Fibrotic disease diagnosis	Localization of fibrosis	Description	Availability	Technical challenge
T2 mapping	High	T2-weighted sequences	Isolated LV non-compaction	Localized collagen fraction	To be validated	Moderate	Complicated
Tissue tagging	Low	Tagging of myocardial tissue with a matrix of radiofrequency saturation	Abnormal cardiac torsion and motion	Diffuse	Functional assessment	Moderate	Easy
Fused PET–MRI							
Delayed enhancement + [^{18}F]FDG PET	High	Combined PET and MRI techniques and data	Myocardial infarction, correlation between viability/non-viability and segmental wall motion and infarct scar location and size	Segmental and diffuse fibrosis	Quantitative assessment	Moderate	Easy to moderate

[^{18}F]FDG, 2-[^{18}F]fluoro-2-deoxy-D-glucose; MRI, magnetic resonance imaging; PET, positron emission tomography; SPECT, single photon emission computed tomography.

chordae have been disrupted with arterially placed grasping forceps in a closed-chest procedure in dogs.[40] Using this model allowed the discovery that beta-adrenergic receptor blockage attenuates sympathomimetic stimulation by the renin–angiotensin system.[41] Gradual constriction of a renal artery in dogs induced LVH[42] as a model for chronic pressure overload. A similar model used banding of the ascending aorta of dogs with gradual increase of aortic constriction at 2-week intervals.[43,44]

Because several confounding factors such as coronary collateral circulation complicate the translation of results gathered in canine models, pigs and sheep have recently been increasingly used as animals of translational models.[37]

In sheep, procedures for causing MI have been elaborated. Selective coronary ligation triggers infarction, followed by progressive cardiac remodelling.[45,46] A couple of issues with sheep, such as zoonotic diseases and anatomical characteristics that complicate detailed imaging, limit the use and translational value of sheep models in cardiology.[37]

Pigs have a very similar cardiac anatomy, circulation physiology, and distribution of blood supply to humans,[47] and are a well-suited species for translational cardiology.[48] In porcine models, cardiac remodelling and HF are most commonly triggered by MI through occlusion of coronary arteries placing ameroid constrictors during open heart surgery.[49,50] For several years, the closed-chest reperfused MI model has been used, by percutaneous occlusion of either the left anterior or left circumflex coronary arteries, followed by balloon deflation resulting in reperfusion; the sudden reopening of the coronary artery closely resembles primary PCI in patients with acute MI.[51] Besides testing of drugs to improve outcome of MI, the swine model is increasingly used for evaluating cardiac regenerative therapies in general, including gene- and cell-based therapies.[52,53] LVH caused by surgical aortic banding is another possibility to provoke cardiac remodelling and HF.[54] Besides the close anatomical resemblance to humans, the adaptation of modern multimodal imaging has enabled detailed evaluation of progressing HF in pig models, and thus this species has emerged as having the best translational value for developing novel treatments for HF and fibrosis.

Few large animal models of pressure overload have been described. An ideal model is based on a gradual increase of LV aortic pressure by a slowly evolving gradient, characterized by an initially preserved EF and cardiomyocyte hypertrophy, and progressive development of fibrosis, diastolic dysfunction, and eventual systolic dysfunction.[55,56] Such models are instrumental in effective and rapid translation from basic research to therapy.

Connecting pre-clinical research with patient care

The field of myocardial fibrosis is continually evolving with regard to the ongoing acquisition of new knowledge on mechanisms and pathways linked to its development. How these novel targets can be utilized as diagnostic tools, for disease monitoring, or for therapeutic targeting is challenging because of two major issues: the cardiac specificity of the target and the complexity and

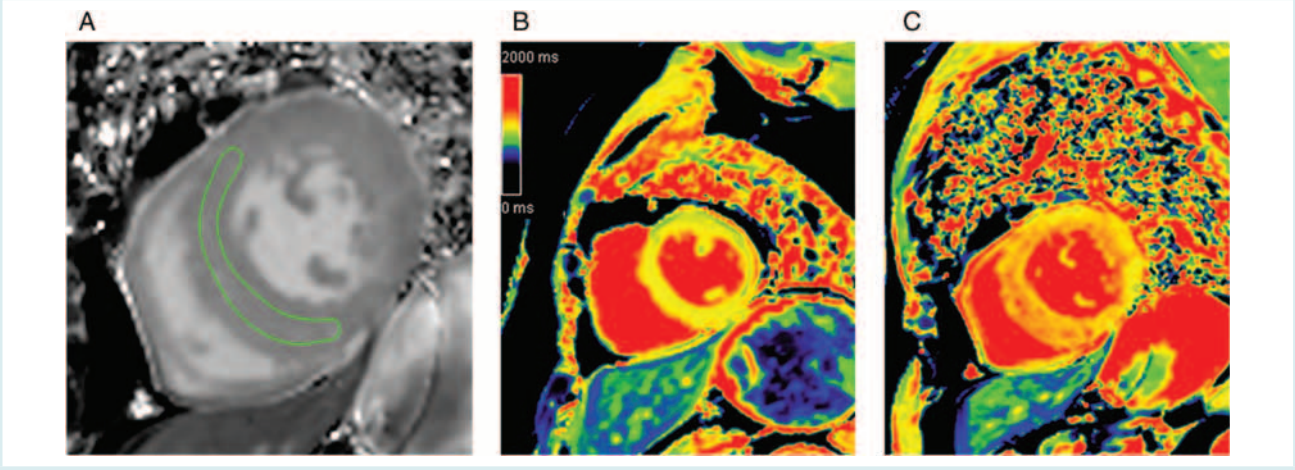

Figure 2 Representative native and T1 cardiac magnetic resonance imaging (cMRI) of diffuse myocardial fibrosis. (*A*) Diffuse myocardial fibrosis on the short-axis view of the cMRI image, with the circumference of the anteroseptal myocardial area (region of interest). (*B*) cMRI T1 map of a patient with moderate aortic stenosis and moderate diffuse myocardial fibrosis. (*C*) cMRI T1 map of another patient with severe aortic stenosis and severe diffuse fibrosis of the left ventricle. Reproduced with permission from the Radiological Society of North America from Lee *et al.*[76]

Table 3 Potential circulating biomarkers for assessment of cardiac fibrosis

Biomarker candidates	Role and correlation to fibrosis	Evidence of association with myocardial fibrosis
ECM formation		
Procollagen type I C-terminal propeptide (PICP)	Cleaved enzymatically from procollagen I (collagen biosynthesis)	Yes
Procollagen type I N-terminal propeptide (PINP)		Unknown
Procollagen type III N-terminal propeptide (PIIINP)	Cleaved enzymatically from procollagen III (collagen biosynthesis)	Yes
Collagen type I C-terminal telopeptide (CITP)	Cleaved by MMP-1 (collagen I degradation), PICP:CITP ratio corresponds to collagen turnover	Inconclusive
Fibrolytic enzymes		
MMP-1 and other MMPs	Degrades collagens I, II, and III	Unknown
TIMP-1 and other TIMPs	Inhibits MMPs	No (TIMP-1), unknown (others)
miRNAs		
miR-21	Correlation with fibrosis in aortic stenosis	Inconclusive
miR-29a	Correlation of plasma levels with hypertrophy and fibrosis in HCM, reduced cardiac expression	Unknown
miRNA panels	Concomitant quantification of several miRNAs increases the diagnostic and prognostic value	Unknown
Others		
TGF-β1	Promotes myofibroblast transactivation and ECM synthesis, deactivates macrophages	Inconclusive
Osteopontin	Matricellular protein involved in macrophage regulation	No association
Galectin-3	Galactosamine binding protein associated with collagen deposition of fibroblasts	Inconclusive
Cardiotrophin-1	Cytokine associated with cardiac fibrosis	No association
Natriuretic peptides	Triggered by myocardial stretch, correlate with HF	Unknown

ECM, extracellular matrix; HF, heart failure; HCM, hypertrophic cardiomyopathy; miRNA, microRNA; MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinases; TGF, transforming growth factor.

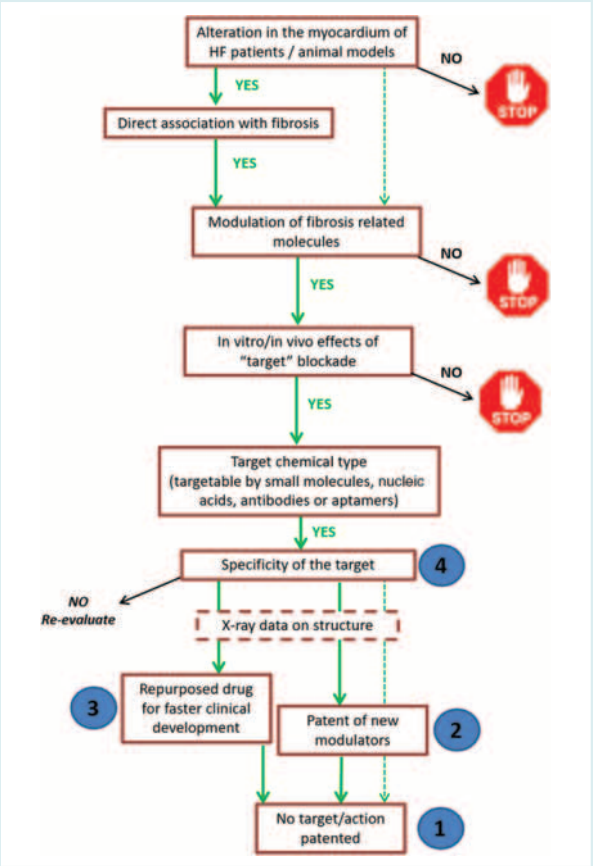

Figure 3 Algorithm for selection of new antifibrotic factors to be further tested as potential therapeutic targets. In order to prioritize the potential antifibrotic targets currently under study in the FIBROTARGETS consortium, and select those to be evaluated in depth from a therapeutic point of view, a number of aspects will be considered in a step-by-step process. Targets need to fulfil the stated criteria, otherwise they will be discarded (stop signs). Numbers in blue circles indicate the prioritization of potential therapeutic agents according to their properties. HF, heart failure.

high cost of the process of drug discovery, respectively. Several developments, however, may help overcome these two challenges for the translation of discovery to routine clinical practice. First, the application of cardiac imaging, and the adoption of a circulating biomarker approach for developing myocardial fibrosis-specific diagnostic assays, and secondly, the use of screening methodologies for early reduction of the number of potential candidates to be further explored as targets with therapeutic potential.

Detection of myocardial fibrosis: imaging and biomarkers

Imaging of myocardial fibrosis

Several single or multimodal imaging technologies have been used to assess the extent and type of myocardial fibrosis (*Table 2*). The distinct imaging modalities reflect specific changes on the molecular, cellular, or functional level, and appropriate selection should be based on the degree and mechanisms of fibrosis. Besides the direct morphological display of the fibrotic tissue, indirect cardiac functional imaging may evidence fibrosis associated with loss of systolic function integrity and increased myocardial stiffness with diastolic dysfunction.[2] Non-invasive morphological multimodal imaging has the advantage that it can be carried out serially, enabling visualization of the turnover of the fibrotic tissue during the pathological processes. The assessment of ECM volume and molecular targeting of essential mechanisms involved in collagen deposition and degradation may eventually also find a role in development of personalized patient treatment.[57]

Cardiac magnetic resonance imaging (MRI) provides detailed tissue characterization, identifying focal myocardial fibrotic scars with late gadolinium enhancement (ventricular LGE) and an estimation of diffuse myocardial fibrosis with post-contrast enhanced T1 and T2 mapping (*Figure 2*).[58] For molecular imaging of fibrosis, a collagen-targeted MRI contrast agent (EP-3533, a cyclic peptide with specific binding to type I collagen) was successfully used to characterize myocardial fibrosis and collagen in a murine model of MI.[59]

Myocardial perfusion scintigraphy is the preferred method to diagnose viable and non-viable cardiac tissue with reduced coronary flow. Extensive scarring identified on perfusion scan is associated with increased mortality.[60] Radiolabelling of myofibroblasts with technetium-99 m-labelled Cy5.5-RGD imaging peptide (CRIP) and positron emission tomography (PET) imaging enabled a non-invasive indirect assessment of collagen deposition in mice with infarct-based cardiac remodelling.[61] Non-invasive molecular imaging of fibrosis was demonstrated using collagelin, a peptidomimetic of the platelet collagen receptor glycoprotein VI.[62] The suitability of collagelin as an *in vivo* probe was tested in a rat model of healed MIs. Injecting Tc-99 m-labelled collagelin, scintigraphy imaging showed that uptake of the probe occurred in the cardiac area of rats with infarction, but not in controls.[62]

Positron emission tomography imaging performed by using ^{15}O-labelled water ($H_2{}^{15}O$) and carbon monoxide ($C^{15}O$) allowed the non-invasive quantification of both myocardial perfusion and fibrosis.[63] Myocardial fibrosis can be indirectly assessed through calculation of the perfusable tissue index (PTI), separating perfusable and non-perfusable tissues. A reduction in PTI serves as an estimate of fibrosis in a chronic MI model and in human dilated cardiomyopathy.[64]

Combining PET and MRI has the potential for sensitive and quantitative imaging of cardiovascular anatomy and function with detection of molecular events at the same time.[65,66] A fused PET–MRI (Biograph mMRI, Siemens AG) image allows the simultaneous detection of myocardial global and regional function, extracellular volume, and tissue perfusion and metabolism.[67]

Circulating biomarkers of myocardial fibrosis

Histopathological analysis of endomyocardial biopsy specimens is the current gold standard for diagnosis and assessment of

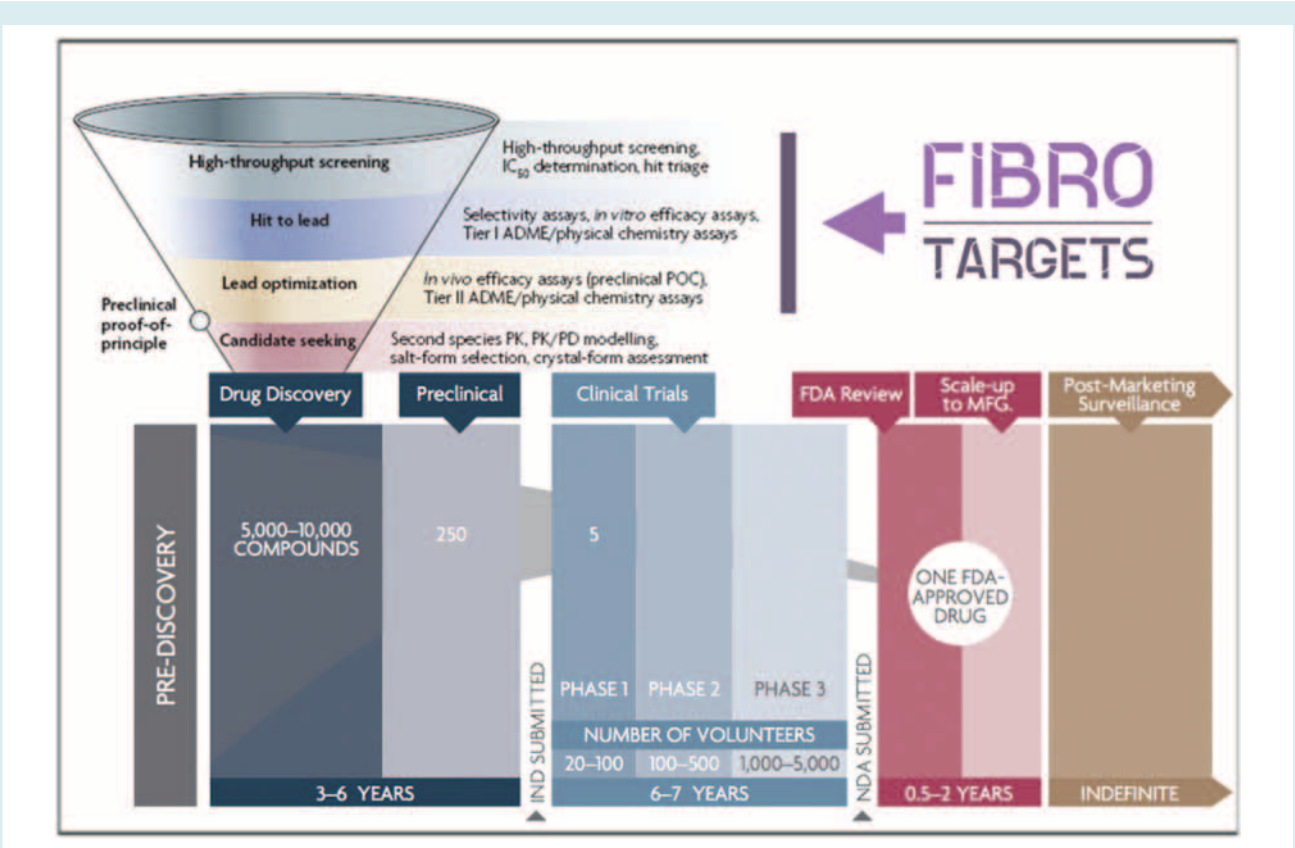

Figure 4 Drug development pipeline highlighting the phases developed by the FIBROTARGETS consortium (modified from Phrma.com). The activities developed by the consortium cover the first steps of the drug discovery strategy; high throughput screening (HTS), hit to lead phase, and lead optimization. By the end of the project, we aim to have identified a set of promising candidates for further evaluation. ADME, absorption, distribution, metabolism, and excretion; FDA, Food and Drug Administration; IC_{50}, half-maximal inhibitory concentration; HCS, high content screening; IND, investigational new drug; MFG, manufacturing; NDA, new drug application; PD, pharmacodynamics; PK, pharmacokinetics; POC, proof of concept.

cardiac fibrosis. A number of circulating biomarkers, including (pro-)collagen cleavage products, processing enzymes, but also miRNAs (*Table 3*), have been proposed and analysed. The most consistent results have been found for the C-terminal propeptide of procollagen type I (PICP) and the N-terminal propeptide of procollagen type III (PIIINP). In many other cases, however, direct correlation to the extent of cardiac fibrosis is lacking or inconclusive.[68] Recent work suggests that urinary peptidomics could provide a further promising alternative to circulating biomarkers of fibrosis.[69,70]

Biomarkers of mechanistic pathways involved in myocardial fibrosis

Beyond detecting ongoing myocardial fibrosis and monitoring overall ECM turnover, one aim of the FIBROTARGETS programme is the identification and clinical validation of circulating biomarkers that inform about the alteration and relative importance of the distinct mechanistic pathways involved in myocardial interstitial fibrosis. Details of these biomarkers and potential targets have been described previously.[6] These include proteins (cardiotrophin-1, galectin-3, NAD phosphate oxidases, neutrophil gelatinase-associated lipocalin, osteonectin, and lysyl oxidase) and proteoglycans (osteoglycin) that impact fibrosis, and miRNAs that act as upstream regulators or downstream effectors of the fibrotic process.[6] For use as biomarkers, it seems reasonable that a combination of several of these increases the predictive and mechanistic power, particularly in the case of miRNAs.[71,72] Stratifying patients according to their myocardial fibrosis bioprofile is an attractive approach for identifying patients with specifically altered expression levels of distinct targets that can be mitigated with respective therapeutic agents.[73] Laying the ground for an antifibrotic precision medicine strategy is the ultimate aim of FIBROTARGETS, all the way from validation of biotargets to identifying mechanistically designed antifibrotic therapeutic agents to biomarker profiling for identification of patients most likely to respond to these agents.

Combination of imaging and circulating biomarkers

A multibiomarker-based strategy should allow for the maximization of the performance of diagnostic tests, and its application at the

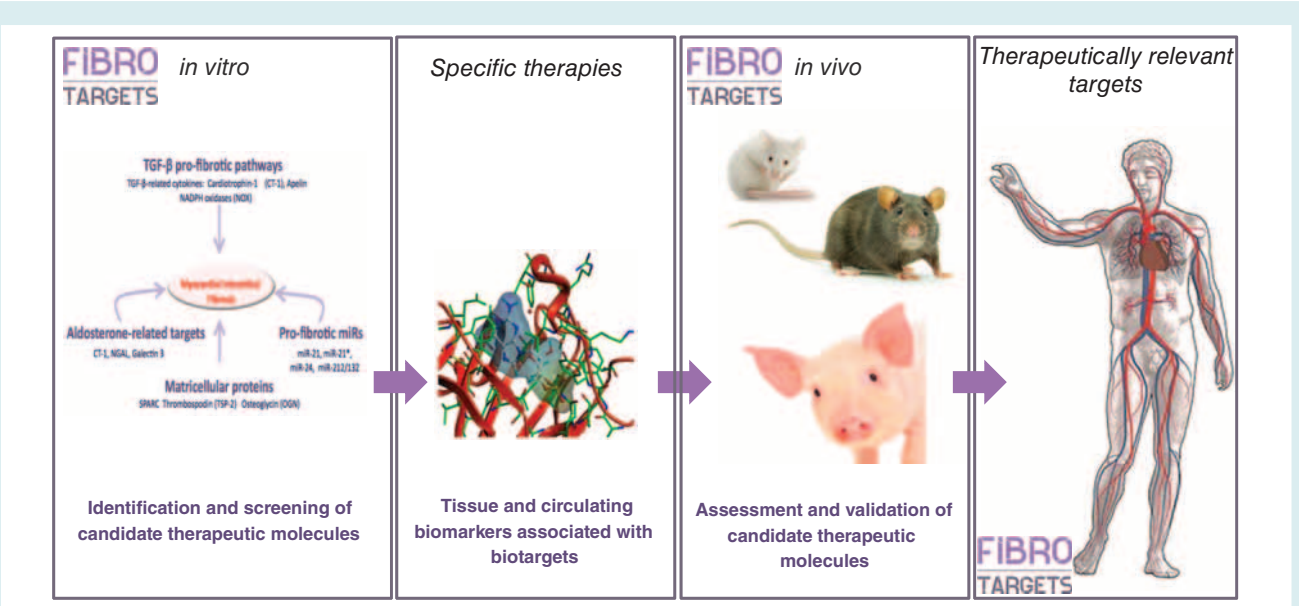

Figure 5 Schematic workflow and aims of FIBROTARGETS.

earliest detectable stage within the disease spectrum is an ultimate goal to support timely interventions and enhance HF prevention.[74] Recently, a combination of some specific circulating and imaging biomarkers of myocardial fibrosis was proposed as a useful tool to assess this lesion non-invasively in HF patients.[68]

Developments in drug screening

According to Pharmaceutical Research and Manufacturers of America, developing a single new drug takes 10–15 years, thousands of researchers, and costs approximately US$1 billion, with a success rate of only ~20% (*Figures 3* and *4*). The failure factor is mainly caused by poor *in vivo* efficacy and serious adverse events. Improvement in pre-clinical research strategies with careful selection of drug candidates for clinical evaluation would increase success rates and lower the financial burden. Therefore, it is important to rationalize drug discovery by using meaningful *in vitro* models to discard irrelevant molecules in terms of efficacy, and pharmacokinetic and toxicological profiles at an early stage. Drug screening technologies are widely used for identifying new potential drug candidates. They comprise protein binding assays and sophisticated cell models in which disease-relevant biomarkers are measured.[75] These technologies termed high throughput screening (HTS) are now miniaturized to allow automatized testing of several thousand compounds per day and measurements of multiple biological parameters simultaneously (high content screening; HCS). With the increasing calculation power of computers, cheminformatics is gaining importance. It is possible to predict biological activities, ADME (absorption, distribution, metabolism, and excretion), and toxicological profiles of molecules based on their chemical structure. For example, this allows the estimation of the affinity of a molecule for a target protein, reducing experimental evaluation to only compounds predicted as most promising.

FIBROTARGETS aims to find promising hits for further development into drugs targeting cardiac fibrosis. The starting points are several potential targets for two major pathways and biological entities involved in myocardial interstitial fibrosis: the mineralocorticoid and transforming growth factor-β (TGF-β) pathways, and non-structural matrix proteins and miRNAs.[6] One target of each group is selected and validated according to the criteria illustrated in *Figure 3*. Screening of commercially available (drug) compound libraries supplemented by *in silico* modelling will provide lead structures that are consequently further screened with high content methodologies in relevant cardiac *in vitro* assays. Toxicity, ADME, and the mechanisms of the molecules in the fibroblast physiology are determined in order to ascertain the therapeutic potential in myocardium interstitial fibrosis treatment. For facilitating further pre-clinical and clinical drug development, preference will be given to novel molecular targets and/or drug repurposing, i.e. the evaluation of therapeutics that have already been tested and approved for other indications (*Figure 3*).

Conclusions and perspectives

The FIBROTARGETS consortium has identified novel factors potentially involved in the effector mechanisms of diffuse myocardial interstitial fibrosis. An excess or deficiency of these individual molecules is hypothesized to contribute significantly to fibrillary collagen turnover. The FIBROTARGETS consortium is now validating and qualifying these factors as imaging and/or circulating biomarkers of myocardial fibrosis in HF, as well as developing effective and safe antifibrotic therapies for HF prevention or treatment of HF patients with the aim of fibrosis regression (*Figure 5*). Target selection and prioritization is based on pathophysiological properties, but also takes drug development aspects into account,

Table 4 Score to rank the relevance and interest of new potential targets of myocardial fibrosis

Aspect to evaluate for each candidate	Score
1. Pathophysiological aspects:	
1.1. Alteration in the myocardium	(max 5)
Heart failure patients	2.5
Animal models	1.5
In more than one model	1
1.2. Myocardial expression/activation associated with end-point fibrosis	(max.5)
Association with fibrosis	3.5
Pleiotropic effects on other cell types	1.5
1.3. Direct modulation of fibrosis-related molecules	4
1.4. Effect of the blockade on myocardial fibrosis	(max. 8)
In vitro data	2
In vivo data	3
Effects on other features of cardiac remodelling	1.5
No detrimental side effects (other pathways, tumour growth, etc.)	1.5
2. Availability of a non-invasive circulating biomarker	2
3. Chemical properties of the target	(max. 2)
Enzyme	2
Receptor	1.5
Transporter	1
Protein-protein interaction surface	0.5
microRNAs	2
4. Drugability	(max. 5)
High specificity of the target	2.5
Repurposing of drugs that enable a faster clinical development	2
X-ray data on the target structure	0.5
5. Intellectual property	(max. 4)
Non-patented target/action	2
Non-patented modulators	2

including the accessibility for chemical compounds (drugability) and intellectual property opportunities (*Table 4*).

The development of fibrosis biomarkers and antifibrotic therapies comprises major challenges, such as lack of organ specificity of biomarkers and the occurrence of related side effects. Is it possible to identify a multibiomarker panel that correlates with the extent of cardiac fibrosis, without being interfered with by fibrosis in other organs such as liver fibrosis, immune processes, or scarring? One of the major challenges in targeting myocardial fibrosis is to avoid side effects such as tendinitis, abnormal wound healing, or adverse inflammation. Hence, a lack of organ specificity might also be an advantage in view of HF being a systemic disease with co-morbidities of different organs. For example, metabolic risk-induced HF with preserved EF is accompanied by renal failure, liver fibrosis, and systemic inflammation in patients with diabetes and hypertension, and finding the key hub for fibrosis in all these organs may lead to promising novel therapies and biomarkers. In particular, miRNAs tend to modulate common pathways in different organs, and are often disease and/or organ specific. The multibiomarker approach represents a way to circumvent this problem: combining different biomarkers may help to increase the specificity and positive predictive value in detecting myocardial fibrosis in HF patients.

Finally, FIBROTARGETS closely works together with industry to develop these novel biomarkers and therapeutic tools to be tested and validated in different phases. This interchange provides a unique opportunity to gain access to dual knowledge and to develop these small molecules and miRNA-based therapies that later on could be applied in humans. Still, the road to the development of individualized therapies or multibiomarkers for human application is a long, expensive and bumpy one. In depth *in silico* screening, a viable strategy for protein, ligand, or miRNAs targeting, and further toxicological and (pre-)clinical testing in translational animal models are mandatory before letting ourselves even dream of human application.

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