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

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**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. 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. 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) and excessive diffuse collagen accumulation in the interstitial and perivascular spaces. 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 (Figure 1). This dysregulation of collagen turnover takes place mainly in phenotypically transformed fibroblasts, termed myofibroblasts. The phenocconversion of fibroblasts into myofibroblasts involves the expression of alpha-smooth muscle actin, a characteristic of smooth muscle.
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 matrixome expansion. Pink, grey, and green boxes list part of the secretome of myocytes, 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.

Myocardial fibrosis disrupts the myocardial architecture, contributes to myocardial disarray, and determines mechanical, electrical, and vasomotor dysfunction, thus promoting the progression of cardiac diseases to HF. Of note, fibrosis persists in the myocardium of HF patients under the current treatment regimens recommended by the official guidelines, 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. In this regard, the detection, prevention, and regression of myocardial fibrosis have emerged as important targets for improving HF therapy.

In order to achieve significant diagnostic and therapeutic advances, it appears to be critical to identify new pro-fibrotic 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.

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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 (\textit{in silico}) and basic (\textit{in vitro}) studies with pre-clinical (\textit{in vivo}) research findings to be distilled into clinically actionable information for the fight against myocardial fibrosis. The FiBRO-TARGETS 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.\textsuperscript{6} Targets and biomarkers under investigation include proteins, proteoglycans, and microRNAs (miRNAs), and have been reviewed previously.\textsuperscript{5} 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.

\textbf{Translational animal models of myocardial fibrosis}

Sophisticated \textit{in vitro} models (\textit{organ-on-a-chip}) for cardiac tissue based on induced pluripotent stem cells have recently been developed and allow, for example, cardiotoxicity screening.\textsuperscript{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.

\textbf{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.\textsuperscript{28} Mutations or depletion of type I collagen,\textsuperscript{29} and \(\alpha\)- or \(\beta\)-cardiac myosin heavy chain\textsuperscript{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.\textsuperscript{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 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.\textsuperscript{34} Repeated brief ischaemia and reperfusion resulted in chemokine induction, inflammation, cardiac dysfunction, and fibrosis in the absence of MI.\textsuperscript{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.\textsuperscript{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).

\textbf{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.\textsuperscript{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.\textsuperscript{38} Ischaemic cardiomyopathies can be simulated in canines through coronary microembolization,\textsuperscript{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
| Model         | Species | Fibrosis generation | Degree of fibrosis | Mechanism                                                                 | Advantages                                                   | Limitations                                                                 | Fibrosis-affected organs | Examples       | Relevance for human therapy                      | Reference |
|--------------|---------|---------------------|--------------------|---------------------------------------------------------------------------|--------------------------------------------------------------|-----------------------------------------------------------------------------|--------------------------|---------------|----------------------------------------------------|-----------|
| 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-ATPase 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) |
| Model         | Species 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 | Surgical procedure, development of fibrosis from hypertrophy requires longer time | Not restricted to myocardium | Cardiac hypertrophy | Caustive 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 preventative 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 | Not restricted to myocardium | SHHF, Zucker rat, ZSF1-Lep⁺/Lepr⁻/ CrI, ZDF-Lepr+/⁻ rats, Lep⁺ and Lepr⁻ 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 | 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) |           |
| Model | Species | Fibrosis generation | Degree of fibrosis | Mechanism | Advantages | Limitations | Fibrosis-affected organs | Examples | Relevance for human therapy | Reference |
|-------|---------|---------------------|--------------------|-----------|------------|-------------|------------------------|----------|-----------------------------|-----------|
| D     | Coronary artery microembolization | 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 amiodar 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 cardiomyopathy | Mild to severe | Altered signalling pathways, according to knockout gene | Commerically available, reproducible, long-term progressive interstitial fibrosis | Highly relevant for humans, through anticancer therapy | Diffuse, biventricular fibrosis with normal heart size | Cardiotoxicity after cytotoxic treatment | 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, endoplasmic/sarcoplasmic reticulum pathways | 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) |
| 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 | Myocardial, biventricular enlargement of the heart, thin wall, hypertrophic or atrophic fibres, infiltrating mononuclear cells | 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.
| 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                  | Low        | Indirect, perfusion defect reflects myocardial scar | Myocardial infarction | Segmental | Indirect proof of collagenous scar | Good         | Easy              |
| SPECT myocardial perfusion scintigraphy | Low | Targeted myofibroblast receptor labelling | Myocardial scar | Segmental | Only experimental | Specialized institution | Complicated |
| SPECT myofibroblast 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       | 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              |
| Delayed enhancement with T1 imaging | High | Contrast-enhanced T1 mapping, use of modified Look-Locker inversion–recovery prototype sequence | Non-ischaemic cardiomyopathy | Diffuse | Quantitative assessment | Good         | Easy              |
chordae have been disrupted with arterially placed grasping forceps in a closed-chest procedure in dogs. 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 LVH42 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 HF54 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) |                                                                                                    | 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.
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. 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.

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). 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.

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. Radiolabelling of myofibroblasts with technetium-99m-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. Non-invasive molecular imaging of fibrosis was demonstrated using collagelin, a peptidomimetic of the platelet collagen receptor glycoprotein VI. The suitability of collagelin as an in vivo probe was tested in a rat model of healed MIs. Injecting Tc-99m-labelled collagelin, scintigraphy imaging showed that uptake of the probe occurred in the cardiac area of rats with infarction, but not in controls.

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. Myocardial fibrosis can be indirectly assessed through calculation of the perfusible 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.

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. 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.
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 (osteonectin) 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
earliest detectable stage within the disease spectrum is an ultimate goal to support timely interventions and enhance HF prevention. 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.

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. These technologies termed high throughput screening (HTS) are now miniaturized to allow automated 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. 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 FIBRO TARGETS 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 FIBRO TARGETS 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.
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.

**Acknowledgements**

The authors want to express their gratitude to all the partners of the FIBROTARGETS consortium: Faiez Zannad and Frédéric Jaisser, Institut National de la Santé et de la Recherche Médicale, France; Catherine Clusel and Marie-Alix Fauvel, Institut National de la Santé et de la Recherche Médicale-Transfert SA, France; Javier Diez and Arantxa González, Fundación para la Investigación Médica Aplicada, Spain; Kenneth Mac Donald, University College Dublin, National University of Ireland, Ireland; Stephane Heymans and Anna Papageorgiou, Universiteit Maastricht, The Netherlands; Thomas Thum, Medizinische Hochschule Hannover, Germany; Mariann Gyöngyösi and Johannes Winkler, Medical University of Vienna, Austria; Quoc-Tuan Do, Greenpharma S.A.S., France; Hueseyin Firat and Kaidre Bendjama, Firalis S.A.S., France; Isbaal Ramos and Clarisa Salado, Innovative Technologies in Biological Systems, Spain; and Natalia López-Andrés, Fundación Pública Miguel Servet, Spain.

**Funding**

This work was supported by the European Commission FP7 Programme [FIBROTARGETS project grant HEALTH-2013-6029047].

**Conflict of interest:** T.T. filed and partly licensed patents regarding the diagnostic and therapeutic use of non-coding RNAs. F.Z. reports personal fees from Janssen, Bayer, Pfizer, Novartis, Boston Scientific, Resmed, Amgen, CVRx, Quantum Genomics, EliLilly, Takeda, and General Electric, all outside the submitted work. The other authors declare no conflict of interest.

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