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Review

Strategies for biomarker discovery in fibrotic disease ☆

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ABSTRACT

The discovery and development of biomarkers for fibrotic diseases have potential utility in clinical decision-making as well as in pharmaceutical research and development. This review describes strategies for identifying diagnostic, prognostic and theranostic biomarkers. A range of technologies and platforms for biomarker discovery are highlighted, including several with specific relevance for fibrosis. Some challenges specific to fibrotic diseases are outlined including; benchmarking biomarkers against imperfect clinical measures of fibrosis, the complexity resulting from diverse aetiologies and target organs, and the availability of samples (including biopsy) from well-characterised patients with fibrotic disease. To overcome these challenges collaboration amongst clinical specialities as well as between academia and industry is essential. This article is part of a Special Issue entitled: Fibrosis: Translation of basic research to human disease.

1. Introduction

The potential value of biomarkers in revolutionizing both the way we define and monitor diseases, and the way we target and assess the impact of therapies, is well recognised. This potential undoubtedly applies to fibrotic disease where major clinical outcomes may take years, if not decades, to manifest. This need to identify short term readouts of progression and prognosis has driven increasing effort in the field of biomarker discovery for fibrosis.

It is worth recognizing at the outset that despite their potential impact, only a small number of biomarkers are currently used in routine clinical practice, and few have gained broad acceptance, even as tools in experimental settings. This begs a number of questions: do we have the right expectations for what biomarkers offer both clinical practice and drug discovery? Are we identifying biomarkers in the right way? Are we applying existing biomarkers correctly to ensure they are most informative?

In this review we will draw upon some specific examples to explore how we might identify and utilise biomarkers for the study and treatment of fibrotic diseases by setting appropriate expectations and definitions of 'validation', and by the application of newer biotechnologies and statistical methodologies. In the authors' view, the prospects for meaningful biomarker discovery in fibrotic disease are good. But although progress is being made in some areas, there remains much work to be done.

2. Concepts and pitfalls

There are some general principles that can help to define the quality of a biomarker—its accuracy, for example; these apply to any new test or assay under evaluation and are not covered here. However, what represents a valuable, valid biomarker will very much depend on the question it is trying to answer. Similarly, the standards against which we assess new markers, themselves, may be imperfect, which can lead to a vicious loop in the validation process, and a not uncommon scenario in the early stages of biomarker discovery. This should not deter us from moving forward, but a degree of caution will be required as we look to define new gold standards.

2.1. Biomarker types: what question are we trying to address?

Biomarkers can be measurements of physiological and pathological processes, or drug effect. In practice, biomarker discovery has generally aimed to answer one of three key questions: can they distinguish disease from health or other similar diseases (a diagnostic); can they predict mortality, exacerbation, disease progression (a prognostic); or can they predict a response to therapy (a theranostic).

Candidate biomarkers are often derived, in the first instance, from preclinical *in vitro* and *in vivo* models that try to mimic relevant processes e.g. bleomycin-induced pulmonary fibrosis in rodents [4] or

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human lung myofibroblasts *in vitro*. [60] More directed approaches (e.g. measurement of Transforming Growth factor (TGF)beta and its downstream effects [12,33], or extracellular matrix components (ECM)[11,64]) have also been used. Biomarkers identified in this way will therefore come with the same pros and cons inherent in such non-human systems. However if human cells and tissues can be used, and open-platform techniques (e.g. transcriptomics, metabolomics, proteomics, etc.[50]) applied with no *a priori* assumption of a biomarker's value, these more controlled settings can be valuable. Ultimately, of course, it is in the clinical setting that a biomarker has to prove its worth, and some common approaches are outlined below, with their application to drug discovery summarised in Fig. 1:

Diagnostics: Biobanked samples from patient cohorts may be used to compare putative biomarkers in fibrotic disease vs. non-fibrotic disease or healthy controls. Retrospective analysis can be performed on these stored samples which may identify candidate biomarkers. Any candidate biomarker should then be confirmed in patients recruited prospectively. Recruitment bias can be a problem depending on the method for subject identification, e.g. enrollment criteria and this is at least one reason why markers may fail to replicate across studies.

Prognostics: For this purpose, a baseline sample (e.g. blood, urine, bronchoalveolar lavage) can be correlated with clinical outcomes such as disease progression, exacerbations, and mortality. One important issue with this approach is that 'baseline' represents a point in the disease process when the patient presents at the clinic but the underlying disease may have been present for years if not decades before diagnosis. Therefore, there is little consistency between patients as to what 'baseline' represents and this can complicate any relationship with outcomes. It may also be helpful to measure change in a biomarker over a short period of time (1–3 months) in relation to a longer-term outcome at, say, one year, which still offers a considerable time advantage. Deciding whether markers are best assessed statically or dynamically may be difficult to predict, and erring on making assessments at multiple time points is probably ideal, in the first instance.

Theranostics: Development of biomarkers that predict a response to therapy have been somewhat neglected, as effective anti-fibrotic therapies have not been available and, therefore, there is no 'gold-standard' treatment. One obvious starting point is to look at changes in prognostic markers in response to a therapeutic intervention, the assumption being that an effective therapy that induces a change in such biomarkers will be more likely to produce longer-term clinical benefit. One pitfall with this approach is that an effective therapy may act via a biological mechanism that does not directly impact the prognostic marker. In this context, having markers that are as closely associated with core processes (i.e. matrix production) likely to affect disease outcome, can reduce this risk.

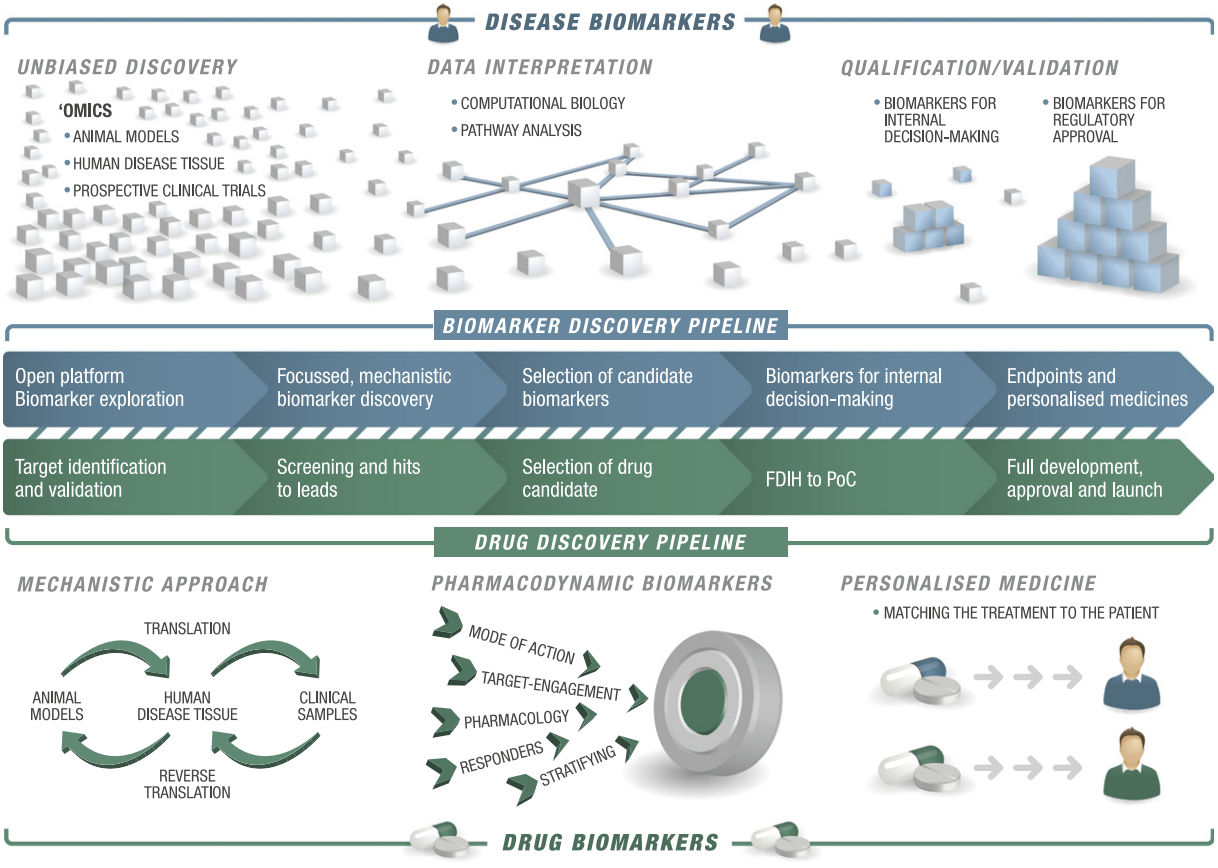

Fig. 1. Discovery and development of drugs and biomarkers. The drug discovery and development pipeline (green chevrons) starts from drug target identification through to approval and launch of the new medicine. Biomarker discovery and development activities (blue chevrons) can be envisioned as occurring in parallel to the drug pipeline. Biomarkers can be divided into two types. The first type of biomarker (Disease Biomarkers) involves the discovery and development of markers that relate to the patient and their disease processes. These biomarkers may be discovered by applying open platform technologies ('omics') to tissues and cells derived from in *vivo* and *in vitro* models as well as from well-characterised patients. The large amounts of complex information derived from these experiments may be analysed and interpreted using computational biology and pathway analytical approaches to reveal those markers with the potential to diagnose disease, predict patient outcomes and to stratify them for therapeutic interventions. Initially biomarkers may be used for internal decision-making and then further developed through to surrogate markers that may be approved for use by regulatory bodies. The second type of biomarker (Drug Biomarkers) involves the discovery and development of markers that relate to the medicine being developed and these can be focussed on the specific downstream events that occur after target engagement (pharmacodynamic effects). These biomarkers may be developed in order to ensure that the medicine is provided to those patients who will derive the most benefit.