**What role do pharmaceuticals play in the treatment of Peyronie's disease and is there a need for new emerging drugs?**

Peyronie’s disease is a chronic inflammatory disease affecting the connective tissue sheath (tunica albuginea) surrounding the erectile tissue of the penis. Patients with this fibrotic disorder initially present to their health care provider/urologist with a locally painful area of the penis during erection. After 12-24 months this progresses into a painless, but chronic and visible curvature of the penis. In turn, this can lead to coital failure (due to the curvature), erectile dysfunction and subsequent psychosocial issues. Currently, apart from surgery, the only non-surgical EMA/FDA-approved treatment option remains injection of *Clostridium histolyticum* collagenase into established chronic plaques. A recent systematic review has shown that there is insufficient evidence to recommend mechanotherapy or iontophoresis in the chronic phase of the disease1. Additionally, at this moment, there are no approved curative or preventive treatments in the initial, painful phase 1.

In the past, chronic PD plaques have largely been considered as scars that never manage to heal. Imbalances between extracellular matrix anabolism/catabolism and myofibroblast apoptosis have been put forward as the driving forces. Genes responsible for extracellular matrix (ECM) homeostasis remodelling are suggested to be dysregulated. Matrix metalloproteases (MMPs) (removal of collagen fibers) and their inhibitors, tissue inhibitors of metalloproteases (TIMPs), are possible accountable proteins for this imbalance (inhibition of MMPs and upregulation of TIMPs leads to profibrotic conditions) 2–5. Increased plasminogen activator inhibitor 1 (PAI-1) activity has been shown to be a potent inhibitor of fibrinolysis and down-regulator of MMP activity in PD and renal fibrosis alike 6. Davila et al. 7 found a significantly increased mRNA and protein levels of PAI-1 in human PD plaques and PD-derived cell cultures, leading to myofibroblast and fibrin persistence. However, judging from our current inability to treat PD through oral or injection therapy, there must be a “missing link” in this pathophysiological model. Recent research using state-of-the-art RNA sequencing has painted quite a different picture compared to what is currently known in PD (unpublished data) 8. Even though there is still a high turn-over of extracellular matrix, it seems that this process is not necessarily maintained by myofibroblasts. Instead, a persistent immunological signature has become apparent mainly involving macrophages. The novel hypothesis that was proposed, states that damage-associated molecular patterns (DAMPs), through NF-kB-signalling can activate and maintain the presence of macrophages, which in turn activate the adaptive immune system involving T-cells. DAMPs are released in response to mechanical stress 9, in this case due to erections and/or sexual intercourse 10. This is in line with the original hypothesis by Devine et al. that PD is caused by repetitive micro-trauma during intercourse 10.

Due to the large number of pathways acting during acute or chronic inflammation, and tissue injury as well as the complex intertwining of these pathways, it has been hard to develop efficacious drugs, the reason why very few drugs have been FDA/EMA approved as “antifibrotic” 11. Moreover, even when choosing to target one pathway, there is a likelihood of one or several other pathways acting in a compensatory fashion. Inhibiting one cog in this large machinery usually leads to activation of auxiliary mechanisms. This is likely to be one of the reasons why target-based therapeutic strategies have mostly failed, not only in fibrosis, but also in drug discovery in general, where fewer successful drugs are discovered via this approach. This could explain the up rise of stem cell research, since mesenchymal stem cells can act as a local “drug store”, affecting multiple targets simultaneously 12. While Scannell and colleagues provide an excellent insight into the productivity crisis in drug development 13, the approach for drug discovery needs to be taken into consideration. The landmark paper by Swinney and Anthony revealed that successful new first-in-class were mainly discovered by a phenotypic screening approach as opposed to the classic target-based approach to drug screening 14. There has been a resurgence in the use of phenotypic screening assays in drug discovery and development for small molecule discovery, while target selection and validation appear to be exhausted 15. Others have suggested phenotypic screening to be one of the ways to successfully tackle drug discovery in fibrotic diseases 16 as it can be more biologically relevant and hence more disease relevant by modulating the disease-linked phenotype 17. Recently, high-throughput phenotypic drug testing was applied to PD by Ilg et al 18 using a TGF- β1 based *in vitro* and *in vivo* model, the investigators screened twenty-one compounds suggested for treatment of PD and have demonstrated that phosphodiesterase type 5 inhibitors (PDE5is) and selective estrogen receptor modulators (SERMs) exert a synergistic anti-fibrotic effect. Further clinical studies will determine its impact on PD clinical practice. Tamoxifen, despite not being recommended by international guidelines, could be shown exerting an effect in the acute PD phase 19,20 but not in chronic plaques 21. The use of PDE5is in PD is further corroborated in the randomized controlled trial by Chung et al. 22 where daily administration of tadalafil in patients with isolation septal scars (without penile deformity) led to spontaneous resolution of the septal fibrosis in 69% of the patients (compared to 10% in the control group.

However, there is a need to develop better the clinical trial design especially when treatment of the early disease is envisioned. Moreover, there is a lack in current trials in terms of placebo controls, randomization and study power. Currently, non-invasive biomarker (measured in serum or urine) are severely missing and further complicate early detection and should be addressed in PD, but also in fibrosis in its entirety 23. Currently, for definitive diagnosis ultrasound (US) remains the gold standard, with a detection rate of around 100%. Both calcified and non-calcified plaques can be distinguished using US. Conversely, X-ray and magnetic resonance should be avoided as either they only are able to detect calcified plaques or their sensitivity is too low and thus unreliable 24.

Additionally, low-cost, high-throughput gene expression profiling technology can connect genes, drugs and disease states by virtue of common gene expression profiles (<http://clue.io>) 25. In short, this technology (L1000 platform: a next generation connectivity map) takes advantage of the large quantity of sequencing data publicly available to record cellular signatures and catalog transcriptional responses of human cells to chemical and genetic perturbation. In this way, novel perturbagens and small molecules that can modulate a cluster of pathways can be inferred with a high reproducibility and comparable to RNA sequencing.

Regardless of a trend for utilizing the target-agnostic approach, there have been excellent reviews dealing with the targets and drugs developed in various fibrotic disease such as liver, pulmonary, and kidney fibrosis 16,26. The highly recommended works of these authors describe the numerous pathways that have been suggested as pharmacological targets in fibrotic disorders. The targets are diverse and range from signaling pathways such as TGF-β, JAK-STAT, CCL2, TNF, PGE2, PDGF, to miR-21, epigenetic targets, NADPH oxidase, cytoskeletal changes, integrin inhibition, PPAR targeting, mTOR inhibition, oxidative stress reduction, and even targeting ageing mechanisms such as cellular senescence, telomere attrition, mitochondrial damage, and loss of proteostasis. Despite this range of innovative targets for treatment, it is not a given that each of the proposed targets will lead to effective treatment in the entire range of different fibrotic diseases. Hence, further research is needed to evaluate the above pathways and the drugs targeting them for their suitability as treatment options in PD.

As it is suggested that there will be differences when using the same compound for different fibrotic diseases and even for different phenotypes of the same disease, care must be taken when considering the ideal model system to investigate the above outlined targets for PD. Targeting these factors and especially their interplay might be facilitated by the development of novel culturing systems such as co-culture systems, scaffolds and three-dimensional culture systems to mirror the human situation more closely. Although none of these assays would be sufficient to represent the complexity in the patient’s situation, they would provide a more relevant model system that hopefully might aid in the search for novel treatments that are more likely to translate from bench to bedside.

It has become increasingly clear that cytokine signaling never acts alone but is entwined in a dense network of distinct cascades. The activity of each separate pathway is monitored and regulated through crosstalk and feedback mechanisms creating a complex regulatory environment. Moreover, the signals offer the cell general clues about the situation in its surroundings, but do not offer precise instructions. The cell itself, more than the involved pathway determines the response. Despite that, it has been shown that the environment of a cell can also significantly affect its response. Previous research has shown that extracellular matrix is more than just a scaffold for cells. It has been suggested that the matrix that fibroblasts reside in can have a distinct influence on their fibrotic response 27 and thereby providing another novel target for pharmacological intervention. This was reviewed recently by Pakshir & Hinz 28, where they describe a complex interplay between ECM, myofibroblasts and macrophages as the main drivers of fibrosis. The ECM activates different precursors into myofibroblasts by transducing mechanical stress and “unwrapping” latent TGF- β1 stores into active TGF- β1 29. This stiffening and remodelling of the ECM enhance the profibrotic cycle by further facilitation of myofibroblast activation. Moreover, myofibroblasts “tug” macrophages into their vicinity by using collagen fibrils, thus providing a continuous stream of new profibrotic factors (e.g. TGF- β1). To achieve this aim of pharmacologically targeting matrix proteins in PD, it would be necessary to further characterize the extracellular matrix composition in different stages of the disease given the ubiquitous nature and clinical importance of these proteins. Further research is needed to uncover the precise mechanisms of how the ECM affects the fibrotic response before it becomes a viable drug target in the future.

We believe that for successful discovery of novel drugs against PD, it will be necessary to design the clinical trial in a standardized and meaningful way with clear cut-off points, sufficient power and comparable on-sets of the disease. Clear pre-screening with precise inclusion and exclusion criteria for selecting patients will help to increase trial efficiency by reducing the number of non-responders. Previous clinical trials have shown different responses to the same drug when administered in different stages 30, therefore the proposed mechanism of action for a drug that is to be tested needs to be taken into careful consideration when selecting the patients for the clinical trial. If the drug is supposed to prevent the formation of a plaque, then it should not be tested on patients with an already formed plaque and stable disease. The same is true for drugs that are tested to dissolve the plaque, as these should not be tested on patients with an unstable plaque. Furthermore, the use of a clinically validated molecular biomarker to monitor the disease progress or a biomarker linked to the decrease of fibrosis will be helpful in improving the outcome of clinical trials. Ideally such a biomarker would be measurable by using non-invasive methods, highlighting the need for more research in this area, despite the best efforts. Overall, we believe that the careful design of clinical trials with clear cut-off points and appropriate patient selection can indeed increase the success rate for drugs that are being tested.

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