

Repurposing of medications for pulmonary arterial hypertension

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Abstract

This manuscript on drug repurposing incorporates the broad experience of members of the Pulmonary Vascular Research Institute's Innovative Drug Development Initiative as an open debate platform for academia, the pharmaceutical industry and regulatory experts surrounding the future design of clinical trials in pulmonary hypertension. Drug repurposing, use of a drug in a disease for which it was not originally developed, in pulmonary arterial hypertension has been a remarkable success story, as highlighted by positive large phase 3 clinical trials using epoprostenol, bosentan, iloprost, and sildenafil. Despite the availability of multiple therapies for pulmonary arterial hypertension, mortality rates have modestly changed. Moreover, pulmonary arterial hypertension patients are highly symptomatic and frequently end up on parental therapy and lung transplant waiting lists. Therefore, an unmet need for new treatments exists and drug repurposing may be an important avenue to address this problem.

Keywords

pulmonary hypertension, preclinical studies, drug repurposing

Date received: 17 June 2020; accepted: 20 June 2020

Pulmonary Circulation 2020; 10(4) 1–12

DOI: 10.1177/2045894020941494

This position statement outlines the PVRI Drug Repurposing Committee's views on the:

- Innovation needed to create the best environment for drug repurposing.
- Importance of both academic and industry contribution and collaboration.
- Ways to enhance preclinical pipelines of drug discovery.
- Importance of early stage trial design.
- Critical role of current funding models and how they might facilitate change.

Take-home message

Drug repurposing is a potential method to develop novel therapies for pulmonary arterial hypertension (PAH), however multiple barriers exist. Here, we propose numerous

methods to hopefully enhance the success of drug repurposing for PAH moving forward.

Introduction

Drug repurposing is the process of using a drug that failed in its initial indication, while drug repositioning is the use of an already-approved therapy in a different disease. These two terms are frequently used interchangeably in the literature, and for the purpose of this manuscript, we will use drug repurposing as the umbrella term encompassing both

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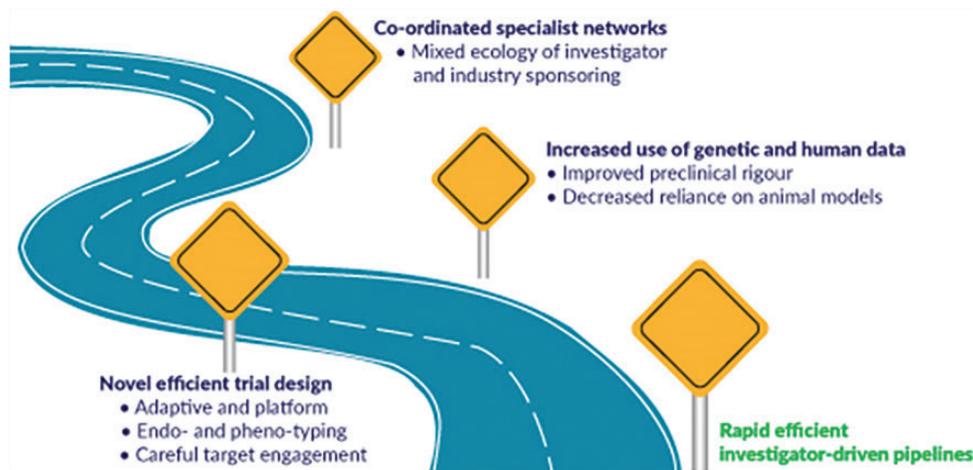


Fig. 1. Roadmap for successful drug repurposing in PAH.

definitions.^{1,2} Drug repurposing is gaining attention in general drug discovery and development for multiple disease states.³ In pulmonary arterial hypertension (PAH), drug repurposing has been a major success story with multiple positive phase 3 trials. For instance, the survival benefit documented with intravenous epoprostenol was a major breakthrough in PAH treatment;⁴ however, continuous intravenous therapy comes with unique challenges and drawbacks including risk of infection, thrombosis/sudden obstruction and the burden on patients due to drug mixing and administration. Moreover, the era of pulmonary vasodilators was ushered in by repurposed oral drugs targeting the phosphodiesterase-5 (sildenafil)⁵ and the endothelin receptor pathways (bosentan).⁶

Although current PAH therapies mitigate symptom burden, enhance exercise capacity and slow the rate of clinical worsening, PAH patients are still highly symptomatic and life expectancy is poor.⁷ Therefore, new therapies are urgently needed to increase quality of life and enhance survival.⁸ Recently, a novel group of repurposed drugs, selected to modify the underlying disease processes, reverse pulmonary vascular remodelling and right ventricular dysfunction, were tested in small phase 2 clinical trials. These drugs targeted disrupted metabolism,^{9–11} excess inflammation¹² and the bone morphogenic receptor type 2 (BMP2) signalling pathway,^{13,14} and showed early signals of beneficial effects in select patients. However, transition to large phase 3 trials investigating these potentially disease-modifying drugs has been hampered by variable, ambiguous or conflicting results in the small trials, the difficulty to accurately assess the biological response of therapy, lack of funding for often already generic drugs and the insufficient collaboration between academia, governmental research organisations and industry. The only example of a large phase 3 trial studying a repurposed disease-modifying therapy in PAH was the use of the tyrosine kinase inhibitor imatinib, a drug currently approved for treatment of multiple types of cancer.¹⁵ Although imatinib reduced PAH severity, there were higher rates of subdural haematomas in patients on oral

anticoagulation and high drop-out rates potentially due to inadequate dosing in the PAH population.¹⁶ These findings, in addition to imatinib coming to the end of its patented lifecycle, led to Novartis not pursuing further licensing studies.

Drug repurposing to develop novel therapies for PAH remains an attractive option because it can combat the high costs of novel drug discovery and has an added safety benefit as already approved drugs have a known dosing and safety profile. Furthermore, technological advances have allowed for high throughput screening of existing drugs for novel mechanisms of action, which has led to several promising new candidates. Certainly, questions arise over how to best optimise drug repurposing. There is a need to evaluate what early experimental work should be academic-led and sponsored. There are structural questions about how to choose targets/therapies, and what the best pipelines for preclinical and clinical testing of repurposed drugs looks like. In this summary statement, we will briefly discuss the current landscape in repurposing and early phase studies in PAH, discuss the need for collaboration between academia, government and industry, outline ways to improve our preclinical pipeline and discuss the importance of novel clinical trial design when using these therapies in a rare patient population. In summary, we are providing a roadmap (Fig. 1) that will hopefully increase the likelihood of successful drug repurposing in PAH.

Current repurposing landscape and academic and industrial contribution and collaboration

To clarify what repurposing looks like in the modern treatment era, we interrogated clinical trials registered on clinicaltrials.gov in the last decade (January 2010–2020), in addition, reviews as well as literature search sources were used. Only 13.2% of all therapies investigated were not repurposed drugs, and arguably even these therapies were derived directly or indirectly from repurposed predecessors (Table 1). Although all late stage studies leading to

Table 1. Summary of repurposed or novel therapies investigated in pulmonary arterial hypertension.

Repurposed therapies ($n = 72$)	Therapies where pulmonary hypertension arguably first indication ($n = 11$)
7 approved therapies and 65 not approved	8 approved therapies, 2 in development and 1 terminated
Trials in PAH not positive for primary endpoint, terminated early or other results: ¹⁷	Terminated:
Terguride: 5-HT receptor antagonist ¹⁸	Prostacyclin receptor agonist QCC374, NCT02927366, Novartis, due to strategic reasons
Nilotinib: TK inhibitor (AMN107) NCT01179737	In development:
Aspirin and simvastatin: COX and HMG-CoA-reductase inhibitor ¹⁹	Growth factor receptors: Sotatercept: activin receptor type 2A fusion protein acting as a ligand trap (SPECTRA) NCT03738150
Atorvastatin: HMG-CoA reductase inhibitor (APATH)	Sotatercept (phase 2 successfully completed in PAH)
Inhaled aviptadil: VI ²⁰	Oral IP agonist (UT): Ralinepag, phase 2 completed, UT
Imatinib: TK inhibitor (QTI571; IMPRES)	
Sorafenib: TK inhibitor ²¹	
Selonsertib: ASK1 inhibitor (ARROW) ²²	
FK506: calcineurin inhibitor (tacrolimus) ²³	
Pioglitazone: PPAR γ agonist NCT00825266	
Ubenimex: aminopeptidase inhibitor (LIBERTY1/2) NCT02664558/ NCT02736149	
Racecadotril: neprilysin inhibitor	
Anakinra: IL-1 inhibitor	
Ambrisentan plus spironolactone: ERA plus aldosterone antagonist NCT02253394	
Fulvestrant: oestrogen antagonist NCT02911844	
Tocilizumab: anti-IL-6 antibody (TRANSFORM-UK) NCT02676947	
Selective PDHK 1 and 2 inhibitor (Acros) JTT-251 NCT03789643, withdrawn due to priority change	
Current ongoing clinical trials: ¹⁷	
Macitentan and tadalafil and selexipag versus macitentan and tadalafil in combination (TRITON) NCT02558231	
Hormonal modulators:	
Anastrozole: aromatase inhibitor (PHANTOM) NCT03229499	
Tamoxifen: oestrogen receptor inhibitor (T3PAH) NCT03528902	
DHEA (EDIPHY) NCT03648385	
Spironolactone: aldosterone antagonist NCT01712620	
rhACE2: GSK2586881 NCT03177603	
KAR5585: tryptophan hydroxylase 1 inhibitor NCT02746237	
Escitalopram: SSRI NCT00190333	
Fluoxetine: SSRI NCT03638908	
PB1046: VIP analogue NCT03315507	
GPCR pathways: Apelin (EXAP) NCT01590108	
Mitochondrial and metabolic adaptations:	
Ranolazine: sodium channel inhibitor, partial FAO inhibitor NCT01839110	
Ranolazine NCT02829034	
Trimetazidine: FAO inhibitor NCT02102672	
Metformin: biguanide, AMPK activator NCT03617458	
Ferinject or CosmoFer: iron infusion NCT01447628	
Epigenetic alterations and interaction with metabolic pathways:	
Olaparib: PARP inhibitor (OPTION) NCT03782818	
Apabetalone: BRD4 inhibitor (APPRoAch-p) NCT03655704	
Oxidative stress related pathways:	
Bardoxolone methyl: I κ B kinase and NF- κ B inhibitor, Nrf2 activator (LARIAT) NCT02036970	
Bardoxolone methyl (CATALYST) NCT02657356	
Bardoxolone methyl (RANGER) NCT03068130	
CXA-10: nitrated fatty acid compound (PRIMEx) NCT03449524	
Inflammatory mediators:	
Rituximab: anti-CD20 antibody NCT01086540	

(continued)

Table 1. Continued.

Elafin: elastase-specific protease inhibitor NCT03522935

Transcriptional factors:

ABI-009: mTOR inhibitor NCT02587325

Miscellaneous

Carbonic anhydrase (CA) inhibitor:

Acetazolamide, NCT02755259 (results not posted),

Beta-agonist: Albuterol, APD811 NCT03270332, ongoing

Benzbromarone (Medical University Graz) NCT02790450, phase 2 completed, not posted

Carbon monoxide NCT01523548, not posted

Heart failure:

Non-selective beta adrenergic receptor blocker

Carvedilol NCT01586156, safety study only²⁴

Bisoprolol²⁵

Nitro fatty acid CXA-10 NCT04053543

Alpha2 receptor agonist Dexmedetomidine NCT 01072643 use cautioned by²⁶

H2 receptor agonist: Famotidine, NCT03554291, funded by NHLBI, study ongoing

Selective serotonin reuptake inhibitor:

Fluoxetine NCT00942708

Selective thromboxane receptor antagonist Ifetroban NCT02682511, study ongoing

Phosphodiesterase 3 inhibitor Milrinone NCT04391478, study ongoing in PPHN

L-Glutamine NCT01048905, completed, no results posted

Calcium sensitizer **Levosimendan**,²⁷ not pursued

Beta-3 agonist: Mirabegron, NCT02775539 SPHERE-HF, not posted

Pulsed nitric oxide (Bellerophon), NCT02725372, primary endpoint not met (stopped for futility)

Tryptophan hydroxylase inhibitor Rodatristat RVT-1201 (Altavant), NCT03924154 (ELEVATE-I), terminated as not recruitable

Thromboxane synthetase Inhibitor (Boehringer Ingelheim): Terbogrel, NCT02223481, phase 2 terminated due to leg pain,²⁸

Non-selective ETA and ETB receptor antagonist Tezosentan (Idorsia), NCT01077297, terminated due to slow recruitment

PDE-5 inhibitor Udenafil (Dong-A), NCT01553721, phase 2 completed²⁹ Pirfenidone, NCT02951429

Nebivolol^{25,30}

Vasopressin, NCT01370096, paediatric PH, terminated due to slow recruitment

Approved repurposed drugs (n = 7):

- Beraprost – Japan only^{31–33}
- Bosentan (Tracleer[®])³⁴
- Epoprostenol (Flolan[®])³⁵
- Inhaled iloprost (Ventavis[®])³⁶
- I.V. iloprost (Ilomedin[®]) – New Zealand only^{37–40}
- Sildenafil (Revatio[®])⁴¹
- Tadalafil (Adcirca[®])⁴²

Approved non-repurposed drugs (n = 8):

- Ambrisentan (Letairis[®], Volibris[®])⁴³
- Macitentan (Opsumit[®])⁴⁴
- Riociguat (Adempas[®])⁴⁵
- Selexipag (Uptravi[®])⁴⁶
- Inhaled treprostinil (Tyvaso[®])⁴⁷
- Oral treprostinil (Orenitram[®])^{48–51}
- S.C. treprostinil (Remodulin[®])^{52,53}
- I.V. treprostinil (Remodulin[®])⁵⁴

PAH: pulmonary arterial hypertension; TK: tyrosine kinase; IL-1: interleukin-1; PDE-5: phosphodiesterase-5; PH: pulmonary hypertension; S.C.: subcutaneous; I.V.: intravenous.

Note: The information provided in Table 1 was derived from literature search, reviews and clinicaltrials.gov

regulatory approval were industry-sponsored, many early clinical studies were academia driven and either funded by industry in a collaborative manner providing, drug substance or received governmental support. Therefore, the academia/investigator-driven research environment is a vital part of drug development and testing, accounting for most of the hypothesis finding and proof of principle trials in the contemporary era.

However, investigator-led early phase studies are predominantly single-centre or regional in design and rarely cross international borders due to limited resources and funding mechanisms. These limitations may explain why no licensed therapy has come from an exclusively academic funded model, though historically successful therapies have a mixture of industry and academic in their genealogy. Adding to these difficulties, we are in a more challenging era for drug

discovery in PAH as future trials will include patients on extensive background therapies, which will undoubtedly diminish effect sizes. Therefore, it is likely that future trials will require renewed collaborations between academia, government and industry to help overcome these roadblocks.

In addition to the modest change in long-term prognosis, the costs of current PAH treatments are high; as a recent pharmacoeconomic evaluation demonstrated, the discounted-quality-adjusted life year (QALY) costs range approximately £245,566 in oral therapies.⁵⁵ Though the costs of oral therapies are coming down with generic availability, this is not the case for intravenous therapy and previous economic analyses are still valid with the cost-effectiveness ratio at £343,000/QALY.⁵⁶ Healthcare utilisation, especially with intravenous therapy and transplant are not going to change in the foreseeable future. Thus, there remains an urgent need for academia, government and industry to renew their collaborative approaches, learning from each other's best practices, to enhance our current treatment portfolio so we can both improve patient outcomes and mitigate the high economic burden of therapies.

Preclinical pipelines for drug repurposing

Targeted screening approaches

Targeted approaches using drugs with well-documented effects to combat underlying pathophysiology remain the dominant drug discovery model for PAH. Uses of targeted approaches to modulate the BMPR2 signalling are well documented. For instance, chloroquine, based on its known ability to inhibit lysosomal-mediated degradation of BMPR2 in vitro,⁵⁷ has beneficial effects on pulmonary vascular disease severity in monocrotaline (MCT) rats.⁵⁷ Moreover, etanercept, a tumour necrosis factor alpha inhibitor used in inflammatory diseases,⁵⁸ prevents BMPR2 proteolytic cleavage and partially reverses PAH in Sugen-5416 hypoxia rats.⁵⁹ These are just two specific examples showing available pharmaceuticals enhance BMPR2 signalling to mitigate PAH severity in relevant animal models. However, multiple publications showed numerous available drugs can combat multiple pathways implicated in PAH pathophysiology, which have previously been extensively reviewed.^{8,17,60}

High throughput screening

Large drug screens can be used to identify potential compounds for PAH treatment using different physiological readouts in high throughput approaches. One example of a high throughput approach included use of a luciferase reporter-gene approach to probe 3756 Food and Drug Administration (FDA) approved medications that augmented BMPR2 signalling.⁶¹ FK506 (tacrolimus) was identified in the drug screen, and tacrolimus treatment effectively

prevented and reversed pre-clinical PAH.⁶¹ These findings were initially translated in a compassionate use trial of tacrolimus in three end-stage PAH patients. Tacrolimus had important haemodynamic and functional improvements in these three patients,⁶² which eventually led to a single-centre study that evaluated the utility of tacrolimus in PAH patients.¹⁴ Although the trial was ultimately neutral, some patients responded to treatment, which suggests patient selection is crucial when using tacrolimus in PAH.

More recently, an unbiased screening approach that evaluated human pulmonary artery smooth muscle cell (PASMC) proliferation in vitro was used to analyse 5562 compounds.⁶³ Emetine, an antiemetic and anti-protozoal drug, was identified as a modulator of PASMC proliferation in this experimental design. In rodent studies, emetine prevented pulmonary vascular remodelling in MCT rats.⁶³ In a reversal approach in Sugen-5416 hypoxia rats, emetine improved haemodynamics and pulmonary vascular disease.⁶³ However, emetine has cardiotoxic effects,⁶⁴ and thus its translatability to PAH patients may be hampered.

Use of human tissue for screening

Another potential avenue to improve drug-screening approaches is to use human tissue via precision-cut lung sections (PCLS) and heart or lung on a chip approach. PCLS is a technique that permits culturing of human lung sections.⁶⁵ In PAH research, PCLS were used to show the tyrosine kinase inhibitors, imatinib and nilotinib, promote vasodilation in precontracted vessels,⁶⁶ and that substance P promotes pulmonary vascular remodelling.⁶⁷ However, use of PCLS for drug screening purposes in PAH has not been implemented yet.

Advances in cell biology and engineering have allowed for the development of organs on a chip, or microdevices that recapitulate the structure, environment and physiology of human organs.⁶⁸ This technology for PAH could be quite useful as both the lung and right ventricle could be modelled and screened for novel drugs. Although not used explicitly in PAH, this technology probed lung physiology and identified angiopoietin-1 as a potential therapy for interleukin-2-mediated pulmonary oedema.⁶⁹ Heart on a chip technology is also available,⁷⁰ but to-date, analyses of right ventricular organ chips from PAH patients have not yet been performed.

Use of artificial intelligence to identify new drugs and enrich PAH populations

Another attractive approach to expand the PAH drug pipeline is the use of artificial intelligence (AI) to understand previously unrecognised drug actions. Recently, a network-based drug-disease analysis of over 900 FDA-approved drugs in 220 million patients provided insights into new purposes for available drugs.⁷¹ The authors identified multiple drug-disease networks and provided specific

examples that carbamazepine is associated with increased risk of coronary artery disease while hydroxychloroquine decreases coronary artery disease risk.⁷¹ Although not explored in detail in the manuscript, there was a drug-pulmonary hypertension network.⁷¹ More recently, a network-based disease module identification and in silico drug repurposing methodology named Genome-wide Positioning Systems network was used to define new therapies for cancer.⁷² The authors showed that integration of patient DNA mutations and gene expression as quantified by RNAseq using approximately 5000 human tumour genomes mapped to the human protein-protein interactome allowed for individualisation of drug therapy. This in silico approach was validated in non-small cell lung cancer as ouabain modulated cell proliferation via hypoxia-inducible factor 1 α and *LEO1* Homolog signaling.⁷² The technology may be used for PAH drug development.

Another example of using AI is deep phenotyping to identify patients who may respond to a certain drug class. Recently, unsupervised machine learning was used to classify patients into four distinct immune categories after they had quantified the levels of 48 cytokines, chemokines and growth factors in a discovery cohort of 281 patients at Stanford University, USA, with a validation cohort of 104 patients at Sheffield University, UK.⁷³ This approach identified low- and high-risk patients independent of other clinical phenotypes.⁷³ This type of cluster analysis could be used to enrich patients for anti-inflammatory therapies with minimised risk of immunosuppressive side-effects. However, it is important to acknowledge that biomarker studies may have confounding variables such as diet, race, ethnicity, age and sex that need to be accounted for when implementing these approaches moving forward.

Summary comments and recommendations for drug and patient screening

1. Both targeted and high throughput drug screens should be used to identify potentially repurposable drugs.
2. Future studies using PCLS and organ-chips for drug screens should be explored as these techniques would use relevant human tissue and probably better predict how the drugs might work in human PAH.
3. Use of AI to expand the drug pipeline and define novel patient clusters that may enrich patients for precision medicine approaches should be investigated further.

Challenges of animal and cell modelling

The reproducibility crisis in preclinical science is well documented.⁷⁴ As in many disease areas, the failure of early phase clinical trials in PAH may be due to the over reliance of data from imperfect rodent studies. Initially designed as proof-of-concept, animal studies have been adopted as preclinical

validation of drug targets, which can be problematic. A pre-clinical rigour score was developed⁶⁰ to judge the existing experimental evidence and likelihood that a therapeutic intervention may work in clinical PAH. Furthermore, a recent position paper from *Circulation Research* clearly laid out specific recommendations to improve pre-clinical rigour,⁷⁵ which are briefly outlined below:

1. More robust statistical analysis: Power calculation prior to starting experiments, randomisation of treatment and blinded analysis.
2. More translational analyses used: Closed chest haemodynamic studies, advanced imaging to evaluate the right ventricle and use of human data to corroborate findings.
3. Inclusion of both sexes as PAH is a female predominant disease but most preclinical studies use male rodents.
4. Reversal/regression models rather than prevention models more likely recapitulate the disease state in PAH and thus reversal/regression models should be evaluated rather than prevention alone.

We acknowledge that current standards of study design and reporting in animal studies do not match human studies. Adopting concepts such as more transparent and appropriate power calculations, insistence on randomisation and blinded analyses, registration of preclinical studies to minimise publication/reporting bias will undoubtedly be a step in the right direction. Furthermore, multi-centre evaluations, such as those used in large phase 3 trials, may also help improve reproducibility in preclinical investigations. Such an approach was recently implemented in an international collaborative study that showed RVX-208, a bromodomain and extra-terminal motif inhibitor, partially reverses PAH and has beneficial effects on the right ventricle in pulmonary artery banded animals.⁷⁶ The results have ultimately led to an ongoing phase 2, single-arm, open-label clinical trial evaluating RVX-208 in 10 PAH patients (NCT03655704). While this multi-laboratory, collaborative approach will likely improve translational success, the high demands impose significant costs and challenges. If such cross-Atlantic collaborations became the new standard needed for publication, it would probably shrink the actual research pool for PAH due to cost and feasibility. Moreover, this approach does not address the fundamental over-reliance on small animal data that is an important structural barrier to translation.

Although there are inherent problems with animal models, they are invaluable tools due to the ability to assess multiple physiological parameters relevant to PAH and the disease progression over time. In addition to the molecular data, both haemodynamic assessments and cardiac imaging can be performed in animal models. If possible, experiments in rodent models should also be supplemented by larger animal models (bovine, pig, rabbit). This is crucial to translatability as the strongest predictors of mortality in PAH patients are measures of right ventricular function.⁷

Summary comments and recommendations for preclinical work using animal models

1. Animal models continue to be necessary for drug repurposing analysis. Regression/reversal studies have the most clinical relevance and should be favoured over prevention studies.
2. For drugs that are moving forward to clinical trials, a multi-centre preclinical evaluation with positive results in regression/reversal models and evidence that the molecular pathway is altered in human tissue should take precedence.
3. Assessments of right ventricular systolic and diastolic function (either imaging or haemodynamic evaluation) in preclinical studies should be performed before transitioning to human studies.

Novel patient-based cellular preclinical models

The availability of inducible pluripotent stem cells (iPSCs) has allowed for the development of patient-centric therapies, which may increase the likelihood of translational success, as directly generated patient-derived cells are used. There have been successful demonstrations of modelling approaches using iPSCs to generate vascular cell lineages.^{77,78} This approach was, in part, used to confirm 4-PBA, a chemical chaperone, as a patient-specific therapy for the C118W BMPR2 mutation.⁷⁹ iPSCs have some distinct and important advantages, such as allowing direct modelling of disease-causing mutations rather than relying on knock-in or knock-down systems which frequently do not recapitulate *in vivo* effects. Additionally, iPSCs free researchers from singular reliance on end-stage tissues and cells generated from transplant patients. All of these modelling approaches have their own pros and cons and *ex vivo* culture introduces additional potential biases such as changes to cells through passage or differences in culture conditions, but these techniques expand our toolbox for preclinical studies and are worthy of exploration.

Summary comments and recommendations for patient-based cellular models

1. iPSCs are a valuable tool for PAH and may serve as a way to test patient-specific or mutation-specific drugs.
2. iPSC may facilitate large-scale screenings in both biased and unbiased approaches.

Use of Genome-wide association study data and Mendelian randomisation

Genome-wide association study (GWAS) data and Mendelian randomisation are in the process of being

established as an alternative or adjunctive preclinical resource when assessing drug targets. The proposal that GWAS associations with disease might improve preclinical pipelines was published in 2012 and subsequently validated.^{80,81} Though GWAS have caused great excitement, the effect sizes vary and therefore it is not clear how much weight will be put on genetic data by pharmaceutical companies when making stop/go decisions on drugs. Moreover, the utility of this approach is unproven in rare diseases where the numbers of patients with genetic data is very unlikely to reach more than 10.⁵ This can be viewed as both a disadvantage from the perspective of a high false negative rate related to underpowering, but it can also be viewed as a positive factor as power will only be retained for large effects. Therefore, positive Mendelian randomisation data in the 'large effect' zone is likely to represent powerful evidence of effect.

In addition to common variation, the large effects of rare variants will probably not be tractable to Mendelian randomisation but are very useful for proof-of-concept studies. Consideration will need to be given as to whether treatments targeting pathways with rare variants should be generalised to heterogeneous patient groups or evaluated first in the context of the mutation carriers relevant to the therapy. This is directly relevant to PAH where we have a growing risk of very rare mutations causing disease, but no clear idea of the importance of the pathways related to these mutations to other PAH ontologies/classes.

Challenges in early stage trial design

The success rates of therapies in general drug discovery are modest when measured by the metric of successful licensing.⁸² Even for repurposed therapies, entering the drug pipeline at phase 2, only around a third will make it through to phase 3 and few of these will end up licensed in the clinic.⁸² A major hurdle in the post-vasodilator era is the extent to which trial design adopts a personalised medicine approach. It is already challenging to perform clinical trials in PAH, technically classified as rare diseases. The heterogeneous nature of the underlying aetiologies in PAH means that there are clear putative strategies that are more applicable to stratified approaches (BMPR2 modulation in heritable PAH, immunomodulation in CTD-PAH). The use of individual patient biological analysis may be a useful way to combat the heterogeneity in PAH, as it allows each patient to be their own control. For example, analysis of transpulmonary and transventricular gradients of both microRNAs and metabolites are related to important catheterisation-based measures of pulmonary vascular disease, right ventricular function and clinical outcome.^{83,84} Thus, the change in gradient of metabolite or miRNA in each patient may be useful for identifying responders to therapy.

Endotyping and phenotyping

To a modest extent, existing trials have already engaged with endotyping patients, even if this was never explicit. The first wave of trials included heterogeneous groups containing both PAH and chronic thromboembolic pulmonary hypertension (CTEPH) patients, but gradually transitioned to more restricted and refined populations. In CTEPH for example, successful trials were eventually contingent on more stringent screening of distal disease by expert panels.⁸⁵ Vasodilation has been generally successful in the World Health Organization (WHO) Groups 1 and 4 pulmonary hypertension (PH), but they have often failed in adults with WHO Groups 2⁸⁶ and 3⁸⁷ PH. Of interest, Group 3 PH is currently being reinvigorated by two positive randomised controlled phase 2 trial using either inhaled prostacyclin (NCT02630316) or inhaled nitric oxide,⁸⁸ again arguably based on better stratification of populations.

Target selection and demonstration of engagement

Vasodilatation in PAH has benefitted from a target engagement readout that is also an important physiological endpoint: pulmonary vascular resistance (PVR) and PVR/systemic vascular resistance ratio. As novel treatment strategies are proposed, we have not changed this model. Therefore, we have a gap opening up between target engagement and efficacy demonstration. We now need a suite of validated endpoints for demonstrating biological changes. More specifically, we need novel lung imaging that will allow us to develop *in vivo* readouts of common pathobiological targets such as proliferation, inflammation, and specific pathway targeting such as BMPR2. If direct imaging is not feasible, has unacceptable signal to noise ratio or cannot be adequately validated, then robust work will need to be undertaken to demonstrate that surrogate measures using biological specimens that assay pathways of interest can adequately replace these assessments. Focus should be on making sure new endpoints are assessed and developed to regulatory standards.

Recommendations

- Development of novel endpoints that specifically quantify important biological aspects of the disease relevant to specific drug mechanisms such as proliferation and inflammation are needed.
- Continuation of research on the utility of biological specimens to link the drug mechanism of action and patient outcome.

Funding trials

The paucity of transnational funding means that the academic community is poorly equipped to adopt some of the critical lessons from industry. Though investigator-initiated trials are more likely to be cutting edge and evaluate novel targets, they are rarely multicentre/multi-national, have limited monitoring capacities and are slow to recruit. To capitalise on the promise of personalised medicine, clinical trials will need to consider stratification of PAH populations. However, stratified trials will need to expand the number of sites to complete enrolment, which will require international collaboration and funding. Thus, there is a need for diverse funding sources and many of the current national funding agencies, like the US-based National Institutes of Health, do not fund studies in this manner. There are recent and notable recognitions of transnational funding opportunities, such as joint British, German and Dutch Heart Foundations collaborative grant, but these remain exceptions, fund only very few groups and to date have rarely been targeted at clinical trials. Collaboration will be vital to success, but currently funding structures and assessment metrics often act as a barrier, rather than an incentive, to setting up longer-term collaborative studies especially in experimental medicine. New funding sources will be required to complete modern clinical trials with more complex designs.

The complexity and expense of PAH clinical trials requires cooperation between the academic and

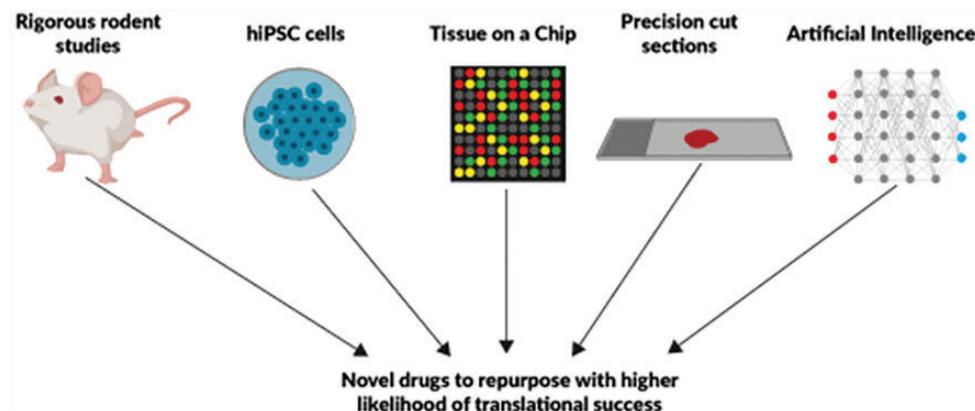


Fig. 2. Strategies to expand the pipelines for successful drug repurposing in PAH.

non-academic medical community, pharmaceutical and other governmental sponsors. Each of them brings special expertise and important operational elements necessary for completion of studies. However, this complex interaction may collide with corporate sponsor perspectives concerning proprietary information that may require a degree of restricted access to data. Therefore, it is important to provide a platform for partnership between the academic, regulatory and industry communities. The PVRI is committed to facilitating multi-national cooperations in this area.

Industry sponsors can gain considerably from interaction with the medical and scientific community in terms of basic disease knowledge and ultimate early study design for clinical trials. On the other hand, the medical, scientific and PAH patient communities can gain from the research, operational and clinical trial knowledge as well as resources (including pharmacologic, toxicologic and others) that industry sponsors possess.

Recommendation

- We need to engage funding agencies at national and international levels to change funding structures so that transnational experimental medicine projects can be launched.

Conclusions and recommendations

There is a need for innovation in preclinical and clinical studies if drug repurposing is going to be successfully adapted to the modern PAH research environment. Our roadmap (Fig. 1) demonstrates the main summary points.

- The importance of a mixed ecology of investigator and industry-led and sponsored clinical trials
- A need to revisit preclinical pipelines of drug discovery (Fig. 2).
- An updated approach including more rigorous study design and physiological assessments in pre-clinical research using imperfect animal models.
- Implementation of human genetic data and novel cell systems will facilitate drug discovery.
- The adoption of precision medicine approaches to clinical trial design including stratification, new methods for demonstrating target engagement and the use of endo- and phenotyping.
- The critical role of current and new funding models and how they might facilitate change in the design and conduct of future PAH research.

Acknowledgments

We thank Paul Corris for the general supervision of the Innovative Drug Development Initiative and their dedicated support of the

Drug Repurposing workstream, as well as Georgie Sutton, PVRI Admin Manager, for her support in proofreading this manuscript.

Conflict of interest

The author(s) declare that there is no conflict of interest.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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