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Why drugs fail in clinical trials in pulmonary arterial hypertension, and strategies to succeed in the future

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Abstract

The past three decades have witnessed a welcome expansion of the therapeutic armamentarium for the management of pulmonary arterial hypertension (PAH). But against this backdrop there have been some notable disappointments in drug development. Here we use these as case studies to emphasise the importance of informed drug target selection, the early evaluation of dose-response relationships in human studies and the value of deep-phenotyping of patients in clinical studies to better understand inter-individual variation in patient response. The integration of 'omics' technologies and advanced clinical imaging offer the potential to reduce the risk, and so cost, of drug development in PAH and bring much needed new medicines to those patients most likely to benefit with greater efficiency.

Key Words: Pulmonary Arterial Hypertension, Serotonin, Statins, Vasointestinal Polypeptide, Imatinib, Drug Development

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1. Introduction

Developing new drugs for any disease is both an expensive and risky exercise. On average it takes more than 8 years of clinical development and in excess of \$800 million to bring a drug to market (Kaitin, 2010; Morgan, Grootendorst, Lexchin, Cunningham, & Greyson, 2011). This cost takes into account the high attrition rate along the path from molecule selection to licensing. Success rates have declined over the past 30 years, despite the best efforts of industry. In the early 1990s, 21% of drugs entering clinical development reached the marketplace, but more recently this has fallen to 16% (Hay, Thomas, Craighead, Economides, & Rosenthal, 2014). For the most challenging therapeutic areas, such as cardiovascular and neuropsychiatric agents, it is only 7-8% (Kaitin, 2010).

Historically, the failure to progress through clinical development was often a consequence of unfavourable pharmacokinetics (e.g. poor bioavailability or short half-life) (Kola & Landis, 2004). Today, with greater experience in drug design, this is much less likely. Now, while 25% of drugs in early development fail because of safety concerns, over 50% fail due to lack of efficacy (Arrowsmith, 2011).

Against this background, the past three decades have witnessed a welcome increase in the therapeutic armamentarium for pulmonary arterial hypertension (PAH). These include the prostanoids, endothelin receptor antagonists (ERA), phosphodiesterase type 5 inhibitors and a stimulator of soluble guanylate cyclase (sGC). With the exception of the sGC stimulator, all were initially developed for the treatment of other conditions. With one exception, sitaxsentan, drugs in these classes have been shown to be safe and well tolerated. An acknowledgement of this is that substantial effort has gone into refining selectivity, duration of action or route of administration.

But there have been some notable disappointments in the introduction of drugs directed at other therapeutic targets for PAH. Terguride, statins and vasointestinal polypeptide (VIP) all failed to meet their primary trial endpoint. The tyrosine kinase inhibitor, imatinib, improved haemodynamics, but has not been licensed because of unacceptable adverse effects in a significant number of patients. As the search for new medicines for PAH continues, it is useful to reflect upon this experience.

2. Pathology of PAH

Pulmonary hypertension is defined by a resting mean pulmonary artery pressure (PAP) of 25mmHg. For a diagnosis of PAH, the post-capillary pressure (wedge pressure) has to be less than 15mmHg. The response of the right ventricle to the increase in PAP determines survival (D'Alonzo et al., 1991). Increased pulmonary vascular tone contributes to the elevated pulmonary vascular resistance, but the major pathological change is structural remodeling of pulmonary resistance vessels involving all cellular elements of the vessel wall (Figure 1). The result is a loss of vessels, obstruction of vessel lumen and increased vascular stiffness. Considerable effort is being expended to understand the underlying molecular drivers but endothelial dysfunction, thrombosis and inflammation are all prominent.

The current licensed treatments for PAH were all developed to repair an imbalance of vasoactive factors arising as a result of endothelial dysfunction. These drugs reduce vascular tone but have only a small impact on cardio-pulmonary haemodynamics, reducing mean PAP on average by around 5mmHg. This is associated with improved patient well-being, but mortality remains unacceptably high. With this in mind, attention has turned to developing drugs that address the structural remodeling directly. This is a bigger challenge, not because of a shortage of targets but because of the difficulties of measuring the effects of interventions on the morphology of the pulmonary vasculature in the clinical setting. Lung biopsies are unsafe in the setting of PAH. Moreover, in cancer tissue biopsies permit molecular characterisation of the disease and the prospect of tailoring treatment as well as analyzing drug penetration and interaction with the target tumour. It is clear that other non-invasive strategies are required to evaluate new PAH treatments.

3. Lessons from disappointments

3.1 Terguride

There is a large body of compelling data from animal and human studies supporting a role for serotonin (5-HT) in the pathophysiology of PAH. The association of anorectic agents with PAH sets the scene (Delcroix, Kurz, Walckiers, Demedts, & Naeije, 1998; Simonneau, Fartoukh, & Sitbon, 1998). Both aminorex and fenfluramine are serotonin transporter (SERT) substrates and act as indirect serotonergic agonists (Rothman, Ayestas, Dersch, & Baumann, 1999). 5-HT is a pulmonary artery vasoconstrictor and induces proliferation of both pulmonary arterial fibroblasts and pulmonary arterial smooth muscle cells (Lee, Wang, Lanzillo, & Fanburg, 1994; MacLean, Herve, Eddahibi, & Adnot, 2000; Welsh, Harnett, MacLean, & Peacock, 2004). Inhibition of serotonin receptors, SERT and knockout of the rate-limiting enzyme producing 5-HT (tryptophan hydroxylase-1) have all been shown to inhibit development of PAH in animal models (Guignabert et al., 2005; et al., 2003; Izikki et al., 2007; Keegan, Morecroft, Smillie, Hicks, & MacLean, 2001; Marcos et al., 2003; Morecroft et al., 2007).

Some observations temper the view that inhibition of 5-HT may be a useful therapeutic strategy. Exposure of women to serotonin reuptake inhibitors in pregnancy is associated with an increased incidence of neonatal pulmonary hypertension (Kieler et al., 2012). Variants of the gene that encode SERT have been identified. The LL genotype is associated with increased cellular expression of SERT and therefore increased 5-HT uptake, but this does not convey increased susceptibility to PAH, although PAH patients with the LL genotype may present earlier than those without (Machado, Koehler, & Glissmeyer, 2006; Willers et al., 2006). These reports aside, the prevailing view is that pharmacological manipulation of 5-HT remains a worthwhile therapeutic pursuit.

Seven distinct classes of 5-HT receptors have been defined and 5-HT_{1B}, 5-HT_{2A}, 5-HT_{2B} and 5-HT₇ have been identified in smooth muscle and endothelial cells within the pulmonary vasculature (Ullmer, Schmuck, Kalkman, & Lübbert, 1995). The 5-HT_{1B} receptor mediates serotonin-induced vasoconstriction in human small and large pulmonary arteries (Macintyre et al., 1993; Maclean et al., 1996; Morecroft et al.,

1999) and this receptor is up-regulated in pulmonary arteries from PAH patients as well as animal models of the phenotype(Keegan et al., 2001; Launay et al., 2002; Morecroft et al., 2005; Wang, Dong, Zhang, & Xing, 2001). Combining 5-HT_{1B} inhibition and SERT inhibition has been shown to be effective in preventing and reversing experimental PAH and 5-HT-induced proliferation of pulmonary arterial smooth cells derived from IPAH patients(Morecroft et al., 2010). The 5-HT_{2A} receptor has been explored in animal models(Cogolludo et al., 2006; Hironaka et al., 2003; Morecroft et al., 2005) and pulmonary arterial fibroblasts in culture(Welsh et al., 2004). Although present in human pulmonary arteries, it may only participate in vasoconstriction when exposed to high 5-HT concentrations (Morecroft et al., 1999). However, given that 5-HT levels are significantly increased in PAH, this may be relevant(Dumitrascu et al., 2011). The 5-HT_{2B} receptor is up-regulated in pulmonary arteries removed from PAH patients and the development of hypoxia-induced PAH is inhibited in mice deficient in the 5-HT_{2B} receptor(Dumitrascu et al., 2011; Launay et al., 2002). Pharmacological inhibition of 5-HT_{2B} retards the development of PAH in hypoxic or monocrotaline exposed rodents (Dumitrascu et al., 2011; Porvasnik et al., 2010; Zopf et al., 2011).

The 5-HT_{2B} receptor has also been implicated in determining the fate of bone marrow lineage progenitor cells and their role in pulmonary vascular remodeling; mice with restricted elimination of the receptor in bone marrow cells were resistant to hypoxia or monocrotaline-induced pulmonary hypertension (J-M M Launay et al., 2012). However, unlike the 5-HT_{1B} receptor, there is currently no evidence that the 5-HT_{2B} receptor mediates vasoconstriction of human pulmonary arteries. There is continuing debate concerning the role of 5-HT_{2B} receptor-mediated proliferation of human pulmonary arterial smooth muscle cells or pulmonary arterial fibroblasts(Dumitrascu et al., 2011; Eddahibi et al., 2001; Marcos et al., 2003). Interestingly, there is a case report of a patient with a loss-of-function mutation in 5-HT_{2B} with fenfluramine-associated primary pulmonary hypertension(Blanpain et al., 2003).

In this context terguride, a 5-HT_{2A} and 5-HT_{2B} antagonist has been investigated in PAH(Millan et al., 2002). Terguride is approved in Japan for ovulation disorders due to hyperprolactinaemia by acting as a partial dopamine D₂ receptor agonist in the pituitary gland(Ciccarelli & Camanni, 1996). In a proof-of-concept study in 103 patients treated for 12 weeks there was no significant effect on pulmonary haemodynamics or 6 minute walk distance (6MWD) (Al-Hiti, Vonk-Noordegraaf, Behr, & Neurohr, n.d.). The dose used in the study (1.5mg) was based on efficacy in treating hyperprolactinaemia. Studies in the rat suggest that plasma concentrations in excess of 11nM (3.74ng/ml) are necessary to inhibit 5-HT_{2B}(Dumitrascu et al., 2011). Published pharmacokinetic data on terguride suggest that 1.5mg achieves a peak plasma concentration of 2.3ng/ml(Lapka, Marek, Rejholec, & Franc, 1986), falling well short of the levels required to inhibit 5-HT_{2B}. The effect of terguride on D₂ receptors may define the upper limit of the tolerated dose, but it does not follow that a dose effective at the D₂ receptor is also effective at blocking 5-HT receptors.

The lesson: Given the choice of possibilities for pharmacological manipulation of serotonin activity, the scientific rationale for selecting a 5-HT_{2A} / 5-HT_{2B} antagonist for a clinical trial in PAH was not robust and the study most likely pursued the wrong drug target. Furthermore the absence of data on exposure to the drug in PAH patients in the study means there could have been inadequate 5-HT_{2A} / 5-HT_{2B} receptor inhibition. The study does not exclude the strategy of pharmacological manipulation of 5-HT as a potentially useful therapeutic approach in PAH but it is a missed opportunity to clarify the role of 5-HT_{2A} and 5-HT_{2B} receptors in the human disease.

3.2 Statins

An impressive body of animal data suggests a therapeutic role for statins in PAH. At least 30 studies have reported beneficial effects from this drug class in a range of animal models(Chen et al., 2011; Dai & Wu, 2011; DeMarco & Habibi, 2009; Gao, Zhu, Shi, & Jing, 2010; RE Girgis, Li, Zhan, & Garcia, 2003; R. E. Girgis et al., 2007; Guerard et al., 2006; Hsu et al., 2009; Ikeda, Nakamura, Akagi, & Kusano, 2010; Kuang, Wang, Pang, Huang, & Burg, 2010; Laudi et al., 2007; M. Li, Liu, Dutt, Fanburg, & Toksoz, 2007; X.-L. L. Li, Guan, & Li, 2012; X.-L. L. Li, Guan, Xu, & Wu, 2011; Liu et al., 2008; Nishimura, Faul, Berry, & Vaszar, 2002; Nishimura, Vaszar, Faul, Zhao, & Berry, 2003; Pei et al., 2011; Rakotoniaina et al., 2006; M Satoh & Satoh, 2009; K. Satoh et al., 2009; Sun & Ku, 2008; Taraseviciene-Stewart et al., 2006; Wright, Zhou, & Preobrazhenska, 2011; Xie, Lin, Xie, & Xu, 2010; Zhang et al., 2009; Zhang, Zhang, Liu, Yu, & Lu, 2011; L Zhao et al., 2009; Liang Zhao et al., 2007) including the most challenging, with few dissenting voices(McMurtry et al., 2007). Among the most investigated and consistent has been simvastatin, which in doses of 2 to 20mg/kg daily not only prevented but also consistently reversed pulmonary hypertension in animal models. Studies on human cells in culture demonstrated inhibition of vascular smooth muscle and fibroblast proliferation, providing further confidence of a direct anti-vascular remodeling effect but subsequent studies in PAH patients have failed to demonstrate a clear benefit(Kawut, Bagiella, Lederer, & Shimbo, 2011; Wilkins, Ali, Bradlow, & Wharton, 2010; Zeng et al., 2012). Simvastatin 40 to 80mg daily, the higher end of the dose range used for cholesterol reduction, reduced right ventricular mass transiently over 6 months but had no significant effect on 6 minute walk distance(Kawut et al., 2011; Wilkins et al., 2010). Atorvastatin 10mg daily for 6 months had no effect on pulmonary artery pressure or cardiac output(Zeng et al., 2012).

There has been much criticism of animal models of PAH and it is true that none truly recapitulate the human condition(Ryan, Bloch, & Archer, 2011; Stenmark, Meyrick, Galie, Mooi, & McMurtry, 2009). To compensate for this, investigators are often asked to provide evidence of a beneficial effect in more than one animal model but, as is evident with statins, this still has serious limitations as a predictor of response in patients. One contributor to this can be the doses used and tolerated in animal studies.

Statins inhibit HMG-CoA (3-hydroxy-3-methyl-glutaryl-CoA) reductase and have been investigated in PAH on the premise that inhibition of HMG-CoA reductase inhibits

synthesis of farnesyl pyrophosphate and geranyl-geranyl-pyrophosphate. These are isoprenoids responsible for the post-translational prenylation of several proteins (e.g. Ras, Rho, Rac) involved in the regulation of cellular processes including differentiation and apoptosis. These processes are germane to PAH (Connolly & Aaronson, 2011; Oka, Fagan, Jones, & McMurtry, 2008) and to cancer, where statins have been reported to reduce tumour growth and metastasis in patients following cardiac transplantation (Fröhlich et al., 2012). Prenylation of Rho proteins is essential for proper membrane localization, and appears to be required for most, but not all, known Rho functions. RhoA and RhoC are exclusively geranyl-geranylated. RhoB, recently shown to have a fundamental role in hypoxia-induced pulmonary hypertension (Wojciak-Stothard et al., 2012), is both farnesylated and geranyl-geranylated (Ridley, 2006). Allosteric modeling suggests that the doses of simvastatin used in animal studies translate into 22 to 220 mg/day in humans. The maximum licensed dose of simvastatin is 80 mg daily, but this is seldom used due to poor tolerability and increased risk of muscle injury. Doses of 40 to 80 mg reduced circulating cholesterol in the clinical studies in PAH, but may be insufficient to effectively inhibit farnesyltransferase (Turner, Zhuang, Zhang, Boss, & Pilz, 2008).

The lesson: Among their limitations as models for PAH, animals may be able to tolerate doses of drugs that are not acceptable to patients. This may be because of differences in metabolism or because the side effects are less easy to detect in animals or simply not examined for. Examining more than one dose and capturing the level of tissue exposure at each dose is important. Identifying a bridging biomarker that reports the effect of the drug on the drug target of interest in animals and that can also be measured in humans is also invaluable in understanding the dose-response relationship and the translation of an effect from animals to patients.

3.3 Vasoactive Intestinal Peptide (Agiptadil)

Preclinical and open-label studies in humans support a role for vasoactive intestinal peptide (VIP) in the regulation of pulmonary vascular homeostasis (Leuchte et al., 2008; Petkov et al., 2003; Saga & Said, 1984; Said et al., 2007). VIP is a 28 amino acid peptide that has been shown to relax pulmonary vascular smooth muscle in several mammalian species in vitro (Saga & Said, 1984) and inhibits proliferation of pulmonary vascular smooth muscle cells from patients with PAH (Petkov et al., 2003). VIP receptors are expressed in pulmonary arterioles and are more abundant in end-stage disease tissue from IPAH patients (Petkov et al., 2003). Local pulmonary and circulating VIP levels are reduced in patients with PAH. Knockout of VIP in the mouse produces a pulmonary hypertension phenotype that can be reversed by a 4-week infusion of VIP (Said et al., 2007). A polymorphism in the coding region of exon 7 (g.8129T4C) has been reported with a similar frequency distribution in Caucasians with and without PAH (50% vs 46%) (Haberl et al., 2007) but is perhaps more common in Chinese with PAH (40.7%) compared to controls (15.2%) (Y. Zhang et al., 2009). Serum VIP levels were numerically lower and pulmonary haemodynamics worse in the PAH patients with this variant (n=33) than those without (n=48), but the biological differences were only small, and so the clinical significance is uncertain (Y. Zhang et al., 2009).

Scintillography of PAH patients given intravenous radiolabeled ^{123}I -labelled VIP appears to confirm uptake of the peptide in the lung (Petkov et al., 2003). An exploratory open-label study of VIP 200 micrograms given as 4 single inhalations per day for 3 months in 8 PAH patients showed a 40% reduction in pulmonary vascular resistance, from $1,009 \pm 475$ dyne/s/cm⁵ to 586 ± 165 dyne/s/cm⁵ (Petkov et al., 2003). The 6-minute walk distance increased by 113m, from 296 ± 138 to 409 ± 102 m ($p < 0.01$). In a separate open label study of 20 patients, temporary selective pulmonary vasodilation improved stroke volume and mixed venous oxygen saturations 15 to 30 min after inhalation of 100ug aviptadil, a synthetic VIP (Leuchte et al., 2008). A subsequent randomised placebo-controlled clinical trial of inhaled aviptadil at 3 different doses (12.5 to 200 micrograms four times a day) showed no effect on pulmonary vascular resistance acutely or after 3 months (Said, 2012).

A key question in these studies is the level of exposure to the exogenous peptide when given by the inhaled route. No change in plasma VIP levels was identified at lower and middle doses in the randomized study, and only a small increase was recorded at the higher dose. Local pulmonary vascular exposure may have been higher by the inhaled route than apparent from blood measurements. A concern is that autoantibodies to VIP were detected in some patients (deemed severe in two cases) and may have neutralized the active peptide.

The lesson: The formulation of a drug is a critical determinant of exposure. The convenience of inhaled over intravenous administration is understandable but peptides are a problematic choice as therapeutic agents. Establishing efficacy, even short-term benefit, by intravenous administration in a blinded protocol study would have been a better design, establishing the principle that VIP receptors are a target of interest in PAH before attempting to optimise the formulation and delivery route. Studies with VIP to date do not rule out augmenting VIP activity as a therapeutic strategy, but emphasise again the value of understanding exposure and biochemical engagement with the drug target at the selected dose when interpreting study data.

3.4 Tyrosine Kinase Inhibitors

The development of small molecule tyrosine kinase inhibitors (TKI) has been one of the recent success stories in modern medicine. Understanding the molecular pathophysiology of chronic myeloid leukemia (CML) culminated in the development of the highly efficacious drug, imatinib, which has transformed management of this disease.

Imatinib is a small molecule TKI initially designed to target the BCR-ABL fusion protein in Philadelphia-positive CML (Baselga, 2006). It also has high affinity for a number of other receptors, including platelet derived growth factor receptor (PDGFR), c-kit receptor and Discoidin-Domain receptors (Day et al., 2008; de Kogel & Schellens, 2007). Both PDGFR and c-kit have been implicated in the pathogenesis of PAH (Montani et al., 2011; Perros et al., 2008). Animal models have demonstrated the efficacy of imatinib in both retarding and reversing the development of PAH (Schermuly et al., 2005). These results, complemented by several case reports

describing functional improvements in PAH patients treated with imatinib, warranted further investigation.

A 24-week phase II clinical study in 59 PAH patients (functional class II-IV) tested imatinib as an 'add-on' treatment (Ghofrani et al., 2010). Despite not meeting its primary outcome of change in 6MWD, reductions in pulmonary vascular resistance and improved cardiac output were seen. A non-specified post-hoc analysis demonstrated clinically important improvement in patients with greater haemodynamic impairment ($PVR > 1000 \text{ dyn/s/cm}^5$). A follow-up phase III study (IMPRES) evaluated imatinib in 202 PAH patients with severe haemodynamic impairment ($PVR > 800 \text{ dyn/s/cm}^5$) (Hoeper et al., 2013). This study met its primary endpoint, with significant improvement in 6-minute walk distance. Pulmonary vascular resistance was reduced and cardiac output increased. Unfortunately, time to clinical worsening, an important measure of clinical value, was worse in the imatinib-treated cohort, alongside a higher incidence of drug discontinuations and an unexpectedly high incidence of subdural haematoma. After consideration of these safety concerns, the manufacturer decided to withdraw its application for a licence for imatinib for PAH. There remain well-documented patients in whom licensed treatment options have failed but who appear to benefit from chronic treatment with imatinib. Unfortunately, the clinical and molecular characteristics of these responders have not been defined robustly.

The lesson: Inter-individual variation in drug response is seen across all therapeutic areas (Figure 2). Study design should incorporate the provision to collect data that provide better characterization of responders and non-responders to the study drug. In the case of imatinib, this may have enabled a follow up study that allowed pre- or early identification of patients who might benefit from the drug, increasing the benefit/harm ratio.

4. How can we succeed in the future?

4.1 General comments

Drug development takes place in an environment where most published research findings cannot be replicated adequately (Ioannidis, 2005). This has been emphasized again recently in the context of drug targets, where only 21% of 67 research projects could be satisfactorily replicated (Mullard, 2011; Prinz, Schlange, & Asadullah, 2011). The reasons include the pressure to publish positive findings, data selection bias and inappropriate statistical analysis. In the hunt for new medicines we need to do better, and the drivers for improvement are both financial – the expense of testing a new drug in humans – and ethical – the risks of exposing humans to a new chemical from which likely benefit is small. The first hurdle is target selection.

4.2 Identifying a valid target in the patient with PAH

There are two paths to drug discovery. The first is empirical, with a touch of serendipity. The second is informed, based on an understanding of the molecular mechanisms of the disease.

Documenting the differential expression of a candidate molecule or a dysfunctional molecular pathway in explanted lung or circulating cells can be informative but when provided by patients with advanced disease, any change could be a consequence of vascular damage rather than a cause. The most powerful evidence validating a novel drug target comes from genetic studies. Genes that cause or increase susceptibility to a disease illustrate pathogenic mechanisms and molecular pathways from which 'druggable targets' can be pursued. They also provide a biomarker that allows clinical studies to be enriched with suitable patients and offer a route to personalized medicine. The discovery that mutations in bone morphogenetic protein (BMP) receptor type 2 (*BMPR2*), activin receptor-like kinase-1 (*ACVRL1*, also known as *ALK1*) and endoglin (*ENG*) genes increase susceptibility to PAH is a compelling argument for exploring therapeutic strategies directed at mediators of BMP and transforming growth factor- β (TGF- β) signaling (Deng, Morse, Slager, & Cuervo, 2000; Lane et al., 2000).

The genetic architecture of PAH is immature. The rapid fall in the cost of genome sequencing opens the door to addressing this in well-designed studies. Investigators studying systemic diseases have used Mendelian randomization with some success to provide insight into target validation and safety assessment (The Interleukin-6 Receptor Mendelian Randomisation Analysis (IL6R MR) Consortium., 2012; Smith & Ebrahim, 2004). The large numbers of patients required for this currently limit the usefulness of this approach in PAH. Developments in bioinformatics coupled with deep phenotyping of patients may unlock important insights from gene variants. International collaboration between investigators using common tools for data and sample collection will be vital to ensure that cohorts of patients of sufficient size are assembled. This has brought rewards in other disease areas. The National Cohort Study of Idiopathic and Heritable Pulmonary Arterial Hypertension (ClinicalTrials.gov NCT01907295) is an example of a successful collaboration that is reaching out to other centres of excellence to develop the critical mass needed to make progress in PAH.

4.3 Animal models

The poor fidelity of animal models of pulmonary hypertension for the human disease has been well-rehearsed (Heath, 1992; Stenmark et al., 2009). The best model for human disease is the patient. The use of human cells, particularly induced pluripotent cells from patients, offers attractive possibilities in the near future for "lab on a chip" type experiments (Esch, Bahinski, & Huh, 2015). For the present, animal studies are still valuable but must be used to address specific questions. Animal studies have the advantage that pulmonary vascular tissue can be examined for effects on vascular remodeling and interrogating biochemical and cellular mechanisms of effect. It is important that animal studies include measurements of cardiac output as well as pulmonary artery pressure, to calculate pulmonary vascular resistance and assess effects on myocardial function. It has been argued that haemodynamic data should be coupled with data on clinical endpoints, such as exercise, and conducted with the same attention to rigorous study design that are applied to clinical trials, such as treatment randomization and blinding (Macleod, 2011). Less well discussed is the importance of exploring dose-response

relationships in animal models, beyond defining the maximum tolerated dose. Knowing the minimum exposure needed to produce the desired biological effect, and identifying a bridging biomarker that reports on this effect that can also be used in human studies to ensure equivalent exposure, is invaluable.

4.4 The human experiment

Aside from assessing safety and tolerability, the four main questions when designing an early phase study are:

Does the drug reach the target?

Does the drug modify the target response?

What is the dose-response relationship?

What are the characteristics of the patients that respond?

When dealing with agents that affect primarily vascular tone, these questions can be answered in the cardiac catheter laboratory. Patients can be exposed to different drug doses and the effect on pulmonary artery pressure and cardiac output measured, giving confidence in the target and informing dose selection for future studies. This is not applicable to drugs that affect primarily vascular structure, and new approaches with rapid readouts are needed to address these questions.

The pragmatist may say “*The drug will need to reduce pulmonary vascular resistance anyway, so why not design the early study around this?*” This introduces the questions – “*What dose should we explore and for what duration?*” Drugs that are going to fail because of lack of efficacy need to be identified and decisions made early to keep costs down. Waiting six months to give the drug time to effect structural change(s), only to find it is ineffective because either the target or dose is wrong, is unacceptable.

Continuous monitoring of pulmonary artery pressure using an implanted sensor, preferably coupled with a measure of flow, may offer a solution to obtaining haemodynamic data and adjusting dosage in real-time (Abraham et al., 2011; Adamson et al., 2011). This would address an important endpoint in clinical trials, allow early assessment of efficacy and permit patients to trial another agent without having to wait months. The value of building readouts of mechanism of action into studies cannot be underestimated.

In the case of a drug that is directed at halting or reversing structural remodeling, this requires access to a marker that reports on the biology that drives the vascular changes. Lung biopsies are not an option. There is therefore considerable interest in finding a circulating factor, such as a protein, miRNA, metabolite or a cell (as a surrogate of the diseased tissue), that correlates with vascular disease activity and could be used as a biomarker. Advanced imaging is another avenue. Cancer already uses radiotracers to assess the drug efficacy on tumour biology and predict response ahead of changes in tumour size (Stroobants et al., 2003). Radioligands can be designed to interrogate drug-receptor occupancy, answering the question of access of the drug to the tissue of interest and guiding dose selection. Other radioligands

informing on generic processes, such as glucose metabolism (^{18}F -FDG) and proliferation (^{18}F -FLT), already exist and can be engaged to address dose selection and mechanism of action.

4.5 Patient heterogeneity

Traditionally, drugs have been developed with the intention of providing benefit to as broad a patient group as possible. The selection of entry criteria for clinical trials is a delicate balance of, on the one hand, reducing confounding factors to best position the study to detect a signal of effect and, on the other, ensuring adequate and rapid subject recruitment. The usual case mix in clinical trials in PAH includes around a third of patients with connective tissue disease, a group recognized to have a more adverse prognosis than idiopathic PAH, as well as patients clinically assessed to have insignificant septal defects. This inclusivity may help recruitment but introduces noise, so diluting the signal, and works perversely to increase pressure on recruitment as more patients are required to increase the signal to noise ratio. In a competitive field with a limited number of patients, it also reduces the number available for other studies and so obstructs innovation.

There is some evidence of gender and ethnicity differences in the response to ERA antagonists (Gabler et al., 2012) but other factors undoubtedly also contribute. Close inspection of the response to any drug shows a distribution that encompasses the comparator group (Figure 2). Rarely is the therapeutic effect of a drug so dramatic that efficacy is obvious. Some patients respond much better than the mean, but often little is known about the phenotype of that group. If the number is small, this sub-phenotype may be lost in the group result. The dramatic response of a few patients to calcium channel blockers meant that this effect was not missed. Arguably, a lesser but still important response to another novel drug class in a small subset of PAH patients may be missed with current trial designs.

It can be argued that the current clinical classification of patients with pulmonary hypertension, as with clinical classifications for other diseases, is not fit for the purpose of developing targeted drugs. A compelling case has already been made for reforming the taxonomy of human disease by moving away from the traditional descriptive approach based on clinical observations and diagnostic criteria to a more molecular approach based on 'omic' technologies, imaging and pathway and network analysis (Kola & Bell, 2011). This is particularly relevant to PAH. The drugs approved for PAH have been studied in WHO Category 1. This group has undergone refinement over 4 iterations since 1973, based on clinical experience and histological examination of end-stage lung disease. It comprises an eclectic group of diseases that are rarely aggregated together under any other circumstance. Even idiopathic PAH, a diagnosis by exclusion of other associated disease, is heterogeneous at the molecular level. A 'deep phenotyping' strategy that deconstructs general labels into descriptors, both clinical and molecular, and enables a more detailed definition of 'responders' and 'non-responders' would likely rescue drugs that might otherwise be judged unworthy of future development (Robinson, Köhler, Bauer, & Seelow, 2008).

The role of patient registries of well-phenotyped patient cohorts linked to biobanks is important here. Patient registries have proved their worth in identifying risk factors and recording improvements in survival (Benza, Miller, Gomberg-Maitland, & Frantz, 2010; Humbert, Sitbon, Chaouat, & Bertocchi, 2010). Deep mining of these resources to link genomic data, circulating biomarkers and high-fidelity clinical information will identify intermediate phenotypes that can be used to describe more accurately responder groups and select patients for hypothesis testing in clinical trials.

5. Conclusions

The current treatments for PAH focus on restoring the imbalance in vascular tone. The next leap forward in the treatment of PAH depends on pursuing novel drug targets that reverse underlying vascular structural changes.

There is no substitute for the human experiment. Data acquired from cell and animal studies are helpful, but high-quality data from patients are critical for novel target selection. Confidence in a novel drug target is increased if there is a clear genomic link. Genomic markers offer insight into cause and effect, providing a biomarker for patient stratification for treatment. Accessible non-invasive circulating biomarkers and imaging are also useful to examine changes in disease pathways, confirm mechanism of drug action and define dose-response relationships.

Advancing science through trial and error is part of the scientific method. It is an acknowledged cost of science, but one that needs to be managed. In addition to cost, there is an ethical obligation to reduce the number of patients exposed to ineffective drugs (i.e. to terminate early the development of an ineffective drug) and channel resources to new medicines with the most promise of therapeutic gain. That decision has to be based on good information. So early phase studies need to be data rich (Figure 3). These studies need to be designed with measures to capture data to help interpret how an effective drug can be best used or why an ineffective drug has failed. The maxim “All new drug studies should be designed to inform how a drug works or why it doesn’t work” is important ethically and economically to ensure we get the most information out of every patient-drug exposure.

Variability in response to drugs is well recognized. Drugs are tools for dissecting apparently clinically homogenous patient populations. The ‘omic’ technologies provide the means to better understand the molecular basis for this variability and exploit it for personalised medicines. Used in conjunction with well-phenotyped patient cohorts and biobanks, and adaptive trial designs, the future is set for a more intelligent approach to the development of new medicines for PAH.

References

Interleukin-6 Receptor Mendelian Randomisation Analysis (IL6R MR) Consortium; The interleukin-6 receptor as a target for prevention of coronary heart disease: a Mendelian randomisation analysis. (2012). *Lancet (London, England)*, 379(9822), 1214–24. doi:10.1016/S0140-6736(12)60110-X

Abraham, W. T., Adamson, P. B., Bourge, R. C., Aaron, M. F., Costanzo, M. R., Stevenson, L. W et al. (2011). Wireless pulmonary artery haemodynamic monitoring in chronic heart failure: a randomised controlled trial. *Lancet*, 377(9766), 658–66. doi:10.1016/S0140-6736(11)60101-3

Adamson, P. B., Abraham, W. T., Aaron, M., Aranda, J. M., Bourge, R. C., Smith, A. et al. (2011). CHAMPION trial rationale and design: the long-term safety and clinical efficacy of a wireless pulmonary artery pressure monitoring system. *Journal of cardiac failure*, 17(1), 3–10. doi:10.1016/j.cardfail.2010.08.002

Arrowsmith, J. (2011). Trial watch: phase III and submission failures: 2007–2010. *Nature reviews. Drug discovery*, 10(2), 87. doi:10.1038/nrd3375

Baselga, J. (2006). Targeting tyrosine kinases in cancer: the second wave. *Science (New York, N.Y.)*, 312(5777), 1175–8. doi:10.1126/science.1125951

Benza, R. L., Miller, D. P., Gomberg-Maitland, M., Frantz, R. P., Foreman, A. J., Coffey, C. S. et al. (2010). Predicting survival in pulmonary arterial hypertension: insights from the Registry to Evaluate Early and Long-Term Pulmonary Arterial Hypertension Disease Management (REVEAL). *Circulation*, 122(2), 164–72. doi:10.1161/CIRCULATIONAHA.109.89812

Blanpain, C., Poul, E. Le, Parma, J., Knoop, C., Detheux, M., Parmentier, M et al. (2004). Serotonin 5-HT_{2B} receptor loss of function mutation in a patient with fenfluramine-associated primary pulmonary hypertension. *Cardiovascular research*, 60(3), 518–28.

Chen, L., Nakano, K., Kimura, S., Matoba, T., Iwata, E., Miyagawa, M. et al. (2011). Nanoparticle-mediated delivery of pitavastatin into lungs ameliorates the development and induces regression of monocrotaline-induced pulmonary artery hypertension. *Hypertension*, 57(2), 343–50. doi:10.1161/HYPERTENSIONAHA.110.157032

Ciccarelli, E., & Camanni, F. (1996). Diagnosis and drug therapy of prolactinoma. *Drugs*, 51(6), 954–65.

Cogolludo, A., Moreno, L., Lodi, F., Frazziano, G., Cobeño, L., Tamargo, J., & Perez-Vizcaino, F. (2006). Serotonin inhibits voltage-gated K⁺ currents in pulmonary artery smooth muscle cells: role of 5-HT_{2A} receptors, caveolin-1, and KV1.5 channel internalization. *Circulation research*, 98(7), 931–8. doi:10.1161/01.RES.0000216858.04599.e1

Connolly, M. J., & Aaronson, P. I. (2011). Key role of the RhoA/Rho kinase system in pulmonary hypertension. *Pulmonary pharmacology & therapeutics*, 24(1), 1–14. doi:10.1016/j.pupt.2010.09.001

D'Alonzo, G. E., Barst, R. J., Ayres, S. M., Bergofsky, E. H., Brundage, B. H., Detre, K. M. et al. (1991). Survival in patients with primary pulmonary hypertension. Results from a national prospective registry. *Annals of internal medicine*, 115(5), 343–9.

Dai, L., & Wu, S. (2011). [Atorvastatin attenuates hypoxic pulmonary hypertension in rats by inhibiting RhoA/Rho kinase pathway]. *Zhong nan da xue xue bao. Yi xue ban = Journal of Central South University. Medical sciences*, 36(1), 58–63. doi:10.3969/j.issn.1672-7347.2011.01.009

Day, E., Waters, B., Spiegel, K., Alnadaf, T., Manley, P. W., Buchdunger, E. et al. (2008). Inhibition of collagen-induced discoidin domain receptor 1 and 2 activation by imatinib, nilotinib and dasatinib. *European journal of pharmacology*, 599(1-3), 44–53. doi:10.1016/j.ejphar.2008.10.014

Delcroix, M., Kurz, X., Walckiers, D., Demedts, M., & Naeije, R. (1998). High incidence of primary pulmonary hypertension associated with appetite suppressants in Belgium. *The European respiratory journal*, 12(2), 271–6.

DeMarco, V. G., Habibi, J., Whaley-Connell, A. T., Schneider, R. I., Sowers, J. R., Andresen, B. T., et al. (2009). Rosuvastatin ameliorates the development of pulmonary arterial hypertension in the transgenic (mRen2)27 rat. *American journal of physiology. Heart and circulatory physiology*, 297(3), H1128–39. doi:10.1152/ajpheart.00048.2009

Deng, Z., Morse, J. H., Slager, S. L., Cuervo, N., Moore, K. J., Venetos, G., et al. (2000). Familial primary pulmonary hypertension (gene PPH1) is caused by mutations in the bone morphogenetic protein receptor-II gene. *American journal of human genetics*, 67(3), 737–44. doi:10.1086/30305

Dumitrascu, R., Kulcke, C., Königshoff, M., Kouri, F., Yang, X., Morrell, N. et al. (2011). Terguride ameliorates monocrotaline-induced pulmonary hypertension in rats. *The European respiratory journal*, 37(5), 1104–18. doi:10.1183/09031936.00126010

Eddahibi, S., Humbert, M., Fadel, E., Raffestin, B., Darmon, M., Capron, F. et al. (2001). Serotonin transporter overexpression is responsible for pulmonary artery smooth muscle hyperplasia in primary pulmonary hypertension. *The Journal of clinical investigation*, 108(8), 1141–50. doi:10.1172/JCI12805

Esch EW, Bahinski A, Huh D (2015). Organs-on-chips at the frontiers of drug discovery. *Nature reviews Drug discovery*. 2015;14(4):248-260. doi:10.1038/nrd4539

Fröhlich, G. M., Rufibach, K., Enseleit, F., Wolfrum, M., Babo, M. von, Frank, M. et al. (2012). Statins and the risk of cancer after heart transplantation. *Circulation*, 126(4), 440–7. doi:10.1161/CIRCULATIONAHA.111.081059

Gabler, N. B., French, B., Strom, B. L., Liu, Z., Palevsky, H. I., Taichman, D. B. et al. (2012). Race and sex differences in response to endothelin receptor antagonists for pulmonary arterial hypertension. *Chest*, 141(1), 20–6. doi:10.1378/chest.11-0404

Gao, Y.-F. F., Zhu, X.-D. D., Shi, D.-M. M., Jing, Z.-C. C., Li, L., Ma, D. et al (2010). The effects of atorvastatin on pulmonary arterial hypertension and expression of p38, p27, and Jab1 in rats. *International journal of molecular medicine*, 26(4), 541–7

Ghofrani, H. A., Morrell, N. W., Hoeper, M. M., Olschewski, H., Peacock, A. J., Barst, R. J. et al (2010). Imatinib in pulmonary arterial hypertension patients with inadequate response to established therapy. *American journal of respiratory and critical care medicine*, 182(9), 1171–7. doi:10.1164/rccm.201001-0123OC

Ghofrani HA A-HH, Al-Hiti H, Vonk- Noordegraaf A, Behr J, Neurohr C, Pavel J. et al. Proof-Of-Concept Study To Investigate The Efficacy, Hemodynamics And Tolerability Of Terguride Vs. Placebo In Subjects With Pulmonary Arterial Hypertension: Results Of A Double Blind, Randomised, Prospective Phase Iia Study. *Am J Respir Crit Care Med*. 2012;185: A2496.

Girgis, RE, Li, D, Zhan, X, & Garcia, J. (2003). Attenuation of chronic hypoxic pulmonary hypertension by simvastatin. *American Journal of Physiology - Heart and Circulatory Physiology* Sep 2003, 285 (3) H938-H945
doi:10.1152/ajpheart.01097.2002

Girgis, R. E., Mozammel, S., Champion, H. C., Li, D., Peng, X., Shimoda, L. et al. (2007). Regression of chronic hypoxic pulmonary hypertension by simvastatin. *American journal of physiology. Lung cellular and molecular physiology*, 292(5), L1105–10. doi:10.1152/ajplung.00411.2006

Guerard, P., Rakotoniaina, Z., Goirand, F., Rochette, L., Dumas, M., Lirussi, F., & Bardou, M. (2006). The HMG-CoA reductase inhibitor, pravastatin, prevents the development of monocrotaline-induced pulmonary hypertension in the rat through reduction of endothelial cell apoptosis and overexpression of eNOS. *Naunyn-Schmiedeberg's archives of pharmacology*, 373(6), 401–14. doi:10.1007/s00210-006-0082-1

Guignabert, C., Raffestin, B., Benferhat, R., Raoul, W., Zadigue, P., Rideau, D., et al. (2005). Serotonin transporter inhibition prevents and reverses monocrotaline-induced pulmonary hypertension in rats. *Circulation*, 111(21), 2812–9. doi:10.1161/CIRCULATIONAHA.104.524926

- Haberl, I., Frej, K., Ramsebner, R., Doberer, D., Petkov, V., Albinni, S., et al. (2007). Vasoactive intestinal peptide gene alterations in patients with idiopathic pulmonary arterial hypertension. *European journal of human genetics*: *EJHG*, 15(1), 18–22. doi:10.1038/sj.ejhg.5201711
- Hay, M., Thomas, D. W., Craighead, J. L., Economides, C., & Rosenthal, J. (2014). Clinical development success rates for investigational drugs. *Nature biotechnology*, 32(1), 40–51. doi:10.1038/nbt.2786
- Heath, D. (1992). The rat is a poor animal model for the study of human pulmonary hypertension. *Cardioscience*, 3(1), 1–6
- Hironaka, E., Hongo, M., Sakai, A., Mawatari, E., Terasawa, F., Okumura, N., ... Kinoshita, O. (2004). Serotonin receptor antagonist inhibits monocrotaline-induced pulmonary hypertension and prolongs survival in rats. *Cardiovascular research*, 60(3), 692–9.
- Hoeper, M. M., Barst, R. J., Bourge, R. C., Feldman, J., Frost, A. E., Galié, N., ... Ghofrani, H.-A. A. (2013). Imatinib mesylate as add-on therapy for pulmonary arterial hypertension: results of the randomized IMPRES study. *Circulation*, 127(10), 1128–38. doi:10.1161/CIRCULATIONAHA.112.000765
- Hsu, H.-H. H., Ko, W.-J. J., Hsu, J.-Y. Y., Chen, J.-S. S., Lee, Y.-C. C., Lai, I.-R. R., & Chen, C.-F. F. (2009). Simvastatin ameliorates established pulmonary hypertension through a heme oxygenase-1 dependent pathway in rats. *Respiratory research*, 10, 32. doi:10.1186/1465-9921-10-32
- Humbert, M., Sitbon, O., Chaouat, A., & Bertocchi, M. (2010). Survival in patients with idiopathic, familial, and anorexigen-associated pulmonary arterial hypertension in the modern management era. *Circulation*. doi:10.1161/CIRCULATIONAHA.109.911818
- Ikeda, T., Nakamura, K., Akagi, S., Kusano, K. F., Matsubara, H., Fujio, H., et al. (2010). Inhibitory effects of simvastatin on platelet-derived growth factor signaling in pulmonary artery smooth muscle cells from patients with idiopathic pulmonary arterial hypertension. *Journal of cardiovascular pharmacology*, 55(1), 39–48. doi:10.1097/FJC.0b013e3181c0419c
- Ioannidis, J. P. (2005). Why most published research findings are false. *PLoS medicine*, 2(8), e124. doi:10.1371/journal.pmed.0020124
- Izikki, M., Hanoun, N., Marcos, E., Savale, L., Barlier-Mur, A. M., Saurini, F. et al. (2007). Tryptophan hydroxylase 1 knockout and tryptophan hydroxylase 2 polymorphism: effects on hypoxic pulmonary hypertension in mice. *American journal of physiology. Lung cellular and molecular physiology*, 293(4), L1045–52.

doi:10.1152/ajplung.00082.2007

Kaitin, K. I. (2010). Deconstructing the drug development process: the new face of innovation. *Clinical pharmacology and therapeutics*, 87(3), 356–61.

doi:10.1038/clpt.2009.293

Kawut, SM, Bagiella, E, Lederer, DJ, & Shimbo, D. (2011). Randomized Clinical Trial of Aspirin and Simvastatin for Pulmonary Arterial Hypertension ASA-STAT.

Circulation. doi:10.1161/CIRCULATIONAHA.110.015693

Keegan, A., Morecroft, I., Smillie, D., Hicks, M. N., & MacLean, M. R. (2002). Contribution of the 5-HT(1B) receptor to hypoxia-induced pulmonary hypertension: converging evidence using 5-HT(1B)-receptor knockout mice and the 5-HT(1B/1D)-receptor antagonist GR127935. *Circulation research*, 89(12), 1231–9.

Kieler, H., Artama, M., Engeland, A., Ericsson, O., Furu, K., Gissler, M., et al. (2012). Selective serotonin reuptake inhibitors during pregnancy and risk of persistent pulmonary hypertension in the newborn: population based cohort study from the five Nordic countries. *BMJ (Clinical research ed.)*, 344, d8012.

Kogel, C. E. de, & Schellens, J. H. (2007). Imatinib. *The oncologist*, 12(12), 1390–4.

doi:10.1634/theoncologist.12-12-1390

Kola, I., & Bell, J. (2011). A call to reform the taxonomy of human disease. *Nature Reviews Drug Discovery*, 10(9), 641–642. doi:10.1038/nrd3534

Kola, I., & Landis, J. (2004). Can the pharmaceutical industry reduce attrition rates? *Nature reviews. Drug discovery*, 3(8), 711–5. doi:10.1038/nrd1470

Kuang, T., Wang, J., Pang, B., Huang, X., Burg, E. D., Yuan, J. X., & Wang, C. (2010). Combination of sildenafil and simvastatin ameliorates monocrotaline-induced pulmonary hypertension in rats. *Pulmonary pharmacology & therapeutics*, 23(5), 456–64. doi:10.1016/j.pupt.2010.02.00

Lane, K. B., Machado, R. D., Pauciulo, M. W., Thomson, J. R., Phillips, J. A., Loyd, J. E. et al (2000). Heterozygous germline mutations in BMPR2, encoding a TGF-beta receptor, cause familial primary pulmonary hypertension. *Nature genetics*, 26(1), 81–4. doi:10.1038/79226

Lapka, R, Marek, J, Rejholec, V, & Franc, Z. (1986). Pharmacokinetics of oral terguride in patients with a prolactinoma. *European journal of clinical pharmacology* 30:363–365 doi:10.1007/BF0054154

Laudi, S., Trump, S., Schmitz, V., West, J., McMurtry, I. F., Mutlak, H., et al. (2007). Serotonin transporter protein in pulmonary hypertensive rats treated with atorvastatin. *American journal of physiology. Lung cellular and molecular physiology*, 293(3), L630–8. doi:10.1152/ajplung.00110.2006

Launay, J.-M. M., Hervé, P., Callebert, J., Mallat, Z., Collet, C., Doly, S., et al. (2012). Serotonin 5-HT_{2B} receptors are required for bone-marrow contribution to pulmonary arterial hypertension. *Blood*, 119(7), 1772–80. doi:10.1182/blood-2011-06-358374

Launay, J.-M. M., Hervé, P., Peoc'h, K., Tournois, C., Callebert, J., Nebigil, C. G., et al. (2002). Function of the serotonin 5-hydroxytryptamine 2B receptor in pulmonary hypertension. *Nature medicine*, 8(10), 1129–35. doi:10.1038/nm764

Lee, S. L., Wang, W. W., Lanzillo, J. J., & Fanburg, B. L. (1994). Serotonin produces both hyperplasia and hypertrophy of bovine pulmonary artery smooth muscle cells in culture. *The American journal of physiology*, 266(1 Pt 1), L46–52.

Leuchte, H. H., Baezner, C., Baumgartner, R. A., Bevec, D., Bacher, G., Neurohr, C., & Behr, J. (2008). Inhalation of vasoactive intestinal peptide in pulmonary hypertension. *The European respiratory journal*, 32(5), 1289–94. doi:10.1183/09031936.00050008

Li, M., Liu, Y., Dutt, P., Fanburg, B. L., & Toksoz, D. (2007). Inhibition of serotonin-induced mitogenesis, migration, and ERK MAPK nuclear translocation in vascular smooth muscle cells by atorvastatin. *American journal of physiology. Lung cellular and molecular physiology*, 293(2), L463–71. doi:10.1152/ajplung.00133.2007

Li, X.-L. L., Guan, R.-J. J., & Li, J.-J. J. (2012). Attenuation of monocrotaline-induced pulmonary arterial hypertension in rats by rosuvastatin. *Journal of cardiovascular pharmacology*, 60(2), 219–26. doi:10.1097/FJC.0b013e31825c63

Li, X.-L. L., Guan, R.-J. J., Xu, Q.-H. H., & Wu, Z.-Y. Y. (2011). [Effects of rosuvastatin on monocrotaline-induced pulmonary artery hypertension in rats]. *Zhonghua xin xue guan bing za zhi*, 39(3), 247–53.

Liu, B., Wang, X. M., Zhou, T. F., Hua, Y. M., Liu, H. M., Wei, L., et al. (2008). [Expression of connective tissue growth factor and its down-regulation by simvastatin administration in pulmonary hypertensive rats]. *Zhonghua er ke za zhi. Chinese journal of pediatrics*, 46(5), 359–65.

Machado, R. D., Koehler, R., Glissmeyer, E., Veal, C., Suntharalingam, J., Kim, M., et al. (2006). Genetic association of the serotonin transporter in pulmonary arterial hypertension. *American journal of respiratory and critical care medicine*, 173(7), 793–7. doi:10.1164/rccm.200509-1365OC

Macintyre, P. D., Bhargava, B., Hogg, K. J., Gemmill, J. D., & Hillis, W. S. (1993). The effect of i.v. sumatriptan, a selective 5-HT₁-receptor agonist on central haemodynamics and the coronary circulation. *British journal of clinical pharmacology*, 34(6), 541–6.

MacLean, M. R., Clayton, R. A., Templeton, A. G., & Morecroft, I. (1996). Evidence for 5-HT₁-like receptor-mediated vasoconstriction in human pulmonary artery. *British journal of pharmacology*, 119(2), 277–82.

MacLean, M. R., Herve, P., Eddahibi, S., & Adnot, S. (2000). 5-hydroxytryptamine and the pulmonary circulation: receptors, transporters and relevance to pulmonary arterial hypertension. *British journal of pharmacology*, 131(2), 161–8. doi:10.1038/sj.bjp.0703570

Macleod, M. (2011). Why animal research needs to improve. *Nature*, 477(7366), 511. doi:10.1038/477511a

Marcos, E., Adnot, S., Pham, M. H., Nosjean, A., Raffestin, B., Hamon, M., & Eddahibi, S. (2003). Serotonin transporter inhibitors protect against hypoxic pulmonary hypertension. *American journal of respiratory and critical care medicine*, 168(4), 487–93. doi:10.1164/rccm.200210-1212OC

McMurtry, M. S., Bonnet, S., Michelakis, E. D., Bonnet, S., Haromy, A., & Archer, S. L. (2007). Statin therapy, alone or with rapamycin, does not reverse monocrotaline pulmonary arterial hypertension: the rapamycin-atorvastatin-simvastatin study. *American journal of physiology. Lung cellular and molecular physiology*, 293(4), L933–40. doi:10.1152/ajplung.00310.2006

Millan, M. J., Maiofiss, L., Cussac, D., Audinot, V., Boutin, J.-A. A., & Newman-Tancredi, A. (2002). Differential actions of antiparkinson agents at multiple classes of monoaminergic receptor. I. A multivariate analysis of the binding profiles of 14 drugs at 21 native and cloned human receptor subtypes. *The Journal of pharmacology and experimental therapeutics*, 303(2), 791–804. doi:10.1124/jpet.102.039867

Montani, D., Perros, F., Gambaryan, N., Girerd, B., Dorfmüller, P., Price, L. C., et al. (2011). C-kit-positive cells accumulate in remodeled vessels of idiopathic pulmonary arterial hypertension. *American journal of respiratory and critical care medicine*, 184(1), 116–23. doi:10.1164/rccm.201006-0905OC

Morecroft, I., Dempsie, Y., Bader, M., Walther, D. J., Kotnik, K., Loughlin, L., et al. (2007). Effect of tryptophan hydroxylase 1 deficiency on the development of hypoxia-induced pulmonary hypertension. *Hypertension*, 49(1), 232–6. doi:10.1161/01.HYP.0000252210.58849.78

Morecroft, I., Heeley, R. P., Prentice, H. M., Kirk, A., & MacLean, M. R. (1999). 5-hydroxytryptamine receptors mediating contraction in human small muscular pulmonary arteries: importance of the 5-HT_{1B} receptor. *British journal of pharmacology*, 128(3), 730–4. doi:10.1038/sj.bjp.0702841

- Morecroft, I., Loughlin, L., Nilsen, M., Colston, J., Dempsie, Y., Sheward, J., et al. (2005). Functional interactions between 5-hydroxytryptamine receptors and the serotonin transporter in pulmonary arteries. *The Journal of pharmacology and experimental therapeutics*, 313(2), 539–48. doi:10.1124/jpet.104.081182
- Morecroft, I., Pang, L., Baranowska, M., Nilsen, M., Loughlin, L., Dempsie, Y., et al. (2010). In vivo effects of a combined 5-HT_{1B} receptor/SERT antagonist in experimental pulmonary hypertension. *Cardiovascular research*, 85(3), 593–603. Retrieved from <https://cardiovascres.oxfordjournals.org/content/85/3/593.full>
- Morgan, S., Grootendorst, P., Lexchin, J., Cunningham, C., & Greyson, D. (2011). The cost of drug development: a systematic review. *Health policy (Amsterdam, Netherlands)*, 100(1), 4–17. doi:10.1016/j.healthpol.2010.12.002
- Mullard, A. (2011). Reliability of “new drug target” claims called into question. *Nature reviews. Drug discovery*, 10(9), 643–4. doi:10.1038/nrd3545
- Nishimura, T, Faul, JL, Berry, GJ, & Vaszar, LT. (2002). Simvastatin attenuates smooth muscle neointimal proliferation and pulmonary hypertension in rats. *American Journal of Respiratory and Critical Care Medicine* 2002 166:10, 1403-1408
- Nishimura, T, Vaszar, LT, Faul, JL, Zhao, G, & Berry, GJ. (2003). Simvastatin rescues rats from fatal pulmonary hypertension by inducing apoptosis of neointimal smooth muscle cells. *Circulation*. doi:10.1161/01.CIR.0000087592.47401.37
- Oka, M., Fagan, K. A., Jones, P. L., & McMurtry, I. F. (2008). Therapeutic potential of RhoA/Rho kinase inhibitors in pulmonary hypertension. *British journal of pharmacology*, 155(4), 444–54. doi:10.1038/bjp.2008.239
- Pei, Y., Ma, P., Wang, X., Zhang, W., Zhang, X., Zheng, P., et al. (2011). Rosuvastatin attenuates monocrotaline-induced pulmonary hypertension via regulation of Akt/eNOS signaling and asymmetric dimethylarginine metabolism. *European journal of pharmacology*, 666(1-3), 165–72. doi:10.1016/j.ejphar.2011.05.035
- Perros, F., Montani, D., Dorfmüller, P., Durand-Gassel, I., Tcherakian, C., Pavet, J. Le, et al. (2008). Platelet-derived growth factor expression and function in idiopathic pulmonary arterial hypertension. *American journal of respiratory and critical care medicine*, 178(1), 81–8. doi:10.1164/rccm.200707-1037OC
- Petkov, V., Mosgoeller, W., Ziesche, R., Raderer, M., Stiebellehner, L., Vonbank, K., et al. (2003). Vasoactive intestinal peptide as a new drug for treatment of primary pulmonary hypertension. *The Journal of clinical investigation*, 111(9), 1339–46. doi:10.1172/JCI17500

Porvasnik, S. L., Germain, S., Embury, J., Gannon, K. S., Jacques, V., Murray, J., et al. (2010). PRX-08066, a novel 5-hydroxytryptamine receptor 2B antagonist, reduces monocrotaline-induced pulmonary arterial hypertension and right ventricular hypertrophy in rats. *The Journal of pharmacology and experimental therapeutics*, 334(2), 364–72. doi:10.1124/jpet.109.165001

Prinz, F., Schlange, T., & Asadullah, K. (2011). Believe it or not: how much can we rely on published data on potential drug targets? *Nature reviews. Drug discovery*, 10(9), 712. doi:10.1038/nrd3439-c1

Rakotoniaina, Z., Guerard, P., Lirussi, F., Goirand, F., Rochette, L., Dumas, M., & Bardou, M. (2007). The protective effect of HMG-CoA reductase inhibitors against monocrotaline-induced pulmonary hypertension in the rat might not be a class effect: comparison of pravastatin and atorvastatin. *Naunyn-Schmiedeberg's archives of pharmacology*, 374(3), 195–206. doi:10.1007/s00210-006-0112-z

Ridley, A. J. (2006). Rho GTPases and actin dynamics in membrane protrusions and vesicle trafficking. *Trends in cell biology*, 16(10), 522–9. doi:10.1016/j.tcb.2006.08.006

Robinson, P. N., Köhler, S., Bauer, S., Seelow, D., Horn, D., & Mundlos, S. (2008). The Human Phenotype Ontology: a tool for annotating and analyzing human hereditary disease. *American journal of human genetics*, 83(5), 610–5. doi:10.1016/j.ajhg.2008.09.01

Rothman, R. B., Ayestas, M. A., Dersch, C. M., & Baumann, M. H. (1999). Aminorex, fenfluramine, and chlorphentermine are serotonin transporter substrates. Implications for primary pulmonary hypertension. *Circulation*, 100(8), 869–75.

Ryan, J., Bloch, K., & Archer, S. L. (2011). Rodent models of pulmonary hypertension: harmonisation with the world health organisation's categorisation of human PH. *International journal of clinical practice. Supplement*, (172), 15–34. doi:10.1111/j.1742-1241.2011.02710.x

Saga, T., & Said, S. I. (1984). Vasoactive intestinal peptide relaxes isolated strips of human bronchus, pulmonary artery, and lung parenchyma. *Transactions of the Association of American Physicians*, 97, 304–10.

Said, S. I. (2012). Vasoactive intestinal peptide in pulmonary arterial hypertension. *American journal of respiratory and critical care medicine*, 185(7), 786; author reply 786. doi:10.1164/ajrccm.185.7.786

Said, S. I., Hamidi, S. A., Dickman, K. G., Szema, A. M., Lyubsky, S., Lin, R. Z., et al. (2007). Moderate pulmonary arterial hypertension in male mice lacking the vasoactive intestinal peptide gene. *Circulation*, 115(10), 1260–8. doi:10.1161/CIRCULATIONAHA.106.681718

- Satoh M & Satoh A. (2009) 3-Hydroxy-3-methylglutaryl (HMG)-CoA reductase inhibitors and phosphodiesterase type V inhibitors attenuate right ventricular pressure and remodeling in a rat model of pulmonary hypertension. *Journal of Pharmacy & Pharmaceutical Sciences* 11:118s–130s
- Satoh, K., Fukumoto, Y., Nakano, M., Sugimura, K., Nawata, J., Demachi, J., et al. (2009). Statin ameliorates hypoxia-induced pulmonary hypertension associated with down-regulated stromal cell-derived factor-1. *Cardiovascular research*, 81(1), 226–34. doi:10.1093/cvr/cvn244
- Schermuly, R. T., Dony, E., Ghofrani, H. A., Pullamsetti, S., Savai, R., Roth, M., et al. (2005). Reversal of experimental pulmonary hypertension by PDGF inhibition. *The Journal of clinical investigation*, 115(10), 2811–21. doi:10.1172/JCI24838
- Simonneau, G., Fartoukh, M., Sitbon, O., Humbert, M., Jagot, J. L., & Hervé, P. (1998). Primary pulmonary hypertension associated with the use of fenfluramine derivatives. *Chest*, 114(3 Suppl), 195S–199S
- Smith, G. D., & Ebrahim, S. (2004). Mendelian randomization: prospects, potentials, and limitations. *International journal of epidemiology*, 33(1), 30–42. doi:10.1093/ije/dyh132
- Stenmark, K. R., Meyrick, B., Galie, N., Mooi, W. J., & McMurtry, I. F. (2009). Animal models of pulmonary arterial hypertension: the hope for etiological discovery and pharmacological cure. *American journal of physiology. Lung cellular and molecular physiology*, 297(6), L1013–32. doi:10.1152/ajplung.00217.2009
- Stroobants, S., Goeminne, J., Seegers, M., Dimitrijevic, S., Dupont, P., Nuyts, J., et al. (2003). 18FDG-Positron emission tomography for the early prediction of response in advanced soft tissue sarcoma treated with imatinib mesylate (Glivec). *European journal of cancer (Oxford, England)*: 1990, 39(14), 2012–20.
- Sun, X, & Ku, DD. (2008). Rosuvastatin provides pleiotropic protection against pulmonary hypertension, right ventricular hypertrophy, and coronary endothelial dysfunction in rats. *American Journal of Physiology - Heart and Circulatory Physiology* 294 (2) H801-H809; DOI: 10.1152/ajpheart.01112.2007
- Taraseviciene-Stewart, L., Scerbavicius, R., Choe, K.-H. H., Cool, C., Wood, K., Tuder, R. M., et al. (2006). Simvastatin causes endothelial cell apoptosis and attenuates severe pulmonary hypertension. *American journal of physiology. Lung cellular and molecular physiology*, 291(4), L668–76. doi:10.1152/ajplung.00491.2005
- Turner, S. J., Zhuang, S., Zhang, T., Boss, G. R., & Pilz, R. B. (2008). Effects of lovastatin on Rho isoform expression, activity, and association with guanine nucleotide dissociation inhibitors. *Biochemical pharmacology*, 75(2), 405–13. doi:10.1016/j.bcp.2007.08.031

Ullmer, C, Schmuck, K, Kalkman, HO, & Lübbert, H. (1995). Expression of serotonin receptor mRNAs in blood vessels. *FEBS letters* 370. doi: 10.1016/0014-5793(95)00828-W

Wang, H. L., Dong, X., Zhang, X. H., & Xing, J. (2001). 5-HT_{1B} receptor augmented 5-HT vasoconstrictor response of pulmonary artery in monocrotaline-induced pulmonary hypertensive rats. *Acta pharmacologica Sinica*, 22(3), 269–73.

Welsh, D. J., Harnett, M., MacLean, M., & Peacock, A. J. (2004). Proliferation and signaling in fibroblasts: role of 5-hydroxytryptamine_{2A} receptor and transporter. *American journal of respiratory and critical care medicine*, 170(3), 252–9. doi:10.1164/rccm.200302-264OC

Wilkins, M. R., Ali, O., Bradlow, W., Wharton, J., Taegtmeyer, A., Rhodes, C. J. et al. (2010). Simvastatin as a treatment for pulmonary hypertension trial. *American journal of respiratory and critical care medicine*, 181(10), 1106–13. doi:10.1164/rccm.200911-1699O

Willers, E. D., Newman, J. H., Loyd, J. E., Robbins, I. M., Wheeler, L. A., Prince, M. A., et al. (2006). Serotonin transporter polymorphisms in familial and idiopathic pulmonary arterial hypertension. *American journal of respiratory and critical care medicine*, 173(7), 798–802. doi:10.1164/rccm.200509-1361OC

Wojciak-Stothard, B., Zhao, L., Oliver, E., Dubois, O., Wu, Y., Kardassis, D., et al. (2012). Role of RhoB in the regulation of pulmonary endothelial and smooth muscle cell responses to hypoxia. *Circulation research*, 110(11), 1423–34. doi:10.1161/CIRCRESAHA.112.264473

Wright, JL, Zhou, S, & Preobrazhenska, O. (2011). Statin reverses smoke-induced pulmonary hypertension and prevents emphysema but not airway remodeling. *American Journal of Respiratory and Critical Care Medicine*, Vol. 183, No. 1 (2011), pp. 50-58. doi:10.1164/rccm.201003-0399OC

Xie, L., Lin, P., Xie, H., & Xu, C. (2010). Effects of atorvastatin and losartan on monocrotaline-induced pulmonary artery remodeling in rats. *Clinical and experimental hypertension (New York, N.Y. 1993)*, 32(8), 547–54. doi:10.3109/10641963.2010.503295

Zeng, W.-J. J., Xiong, C.-M. M., Zhao, L., Shan, G.-L. L., Liu, Z.-H. H., Xue, F. et al. (2012). Atorvastatin in pulmonary arterial hypertension (APATH) study. *The European respiratory journal*, 40(1), 67–74. doi:10.1183/09031936.00149011

Zhang, W, Zhang, Y, Liu, C, Yu, B, & Lu, W. (2011). Simvastatin protects against the development of monocrotaline-induced pulmonary hypertension in rats via a heme oxygenase-1-dependent pathway. *Experimental lung research*. doi:10.3109/01902148.2011.591892

Zhang, W.-H. H., Lu, W.-X. X., Zhang, Y.-J. J., Ji, Y.-Q. Q., Liu, C.-P. P., & Li, G. (2009). [Simvastatin prevents the development of pulmonary hypertension in the rats through reduction of inflammation]. *Zhonghua yi xue za zhi*, 89(12), 855–9.

Zhang, Y., Zhang, J.-Q. Q., Liu, Z.-H. H., Xiong, C.-M. M., Ni, X.-H. H., Hui, R.-T. T., ... Pu, J.-L. L. (2009). VIP gene variants related to idiopathic pulmonary arterial hypertension in Chinese population. *Clinical genetics*, 75(6), 544–9. doi:10.1111/j.1399-0004.2009.01196.x

Zhao, L., Sebkhi, A., Ali, O., Wojciak-Stothard, B., Mamanova, L., Yang, Q. et al. (2009). Simvastatin and sildenafil combine to attenuate pulmonary hypertension. *The European respiratory journal*, 34(4), 948–57. doi:10.1183/09031936.00143508

Zhao, L., Zhou, T. F., Liu, H. M., Li, M., Dou, H., & Tang, Z. H. (2007). [Effect of simvastatin on rat pulmonary hypertension induced by high pulmonary blood flow and injected monocrotaline]. *Sichuan da xue xue bao. Yi xue ban = Journal of Sichuan University. Medical science edition*, 38(3), 463–5, 479.

Zopf, D. A., Neves, L. A. A. das, Nikula, K. J., Huang, J., Senese, P. B., & Gralinski, M. R. (2011). C-122, a novel antagonist of serotonin receptor 5-HT_{2B}, prevents monocrotaline-induced pulmonary arterial hypertension in rats. *European journal of pharmacology*, 670(1), 195–203. doi:10.1016/j.ejphar.2011.08.015

Fig. 1

Fig. 2

Fig. 3

Figure legends

Figure 1: Vascular remodelling in pulmonary arterial hypertension: Haematoxylin and eosin staining showing (A) neointimal proliferation (double arrow) in an elastic pulmonary artery; (B) medial hypertrophy and neointimal proliferation leading to occlusion of the vessel lumen (arrows) in muscular pulmonary arteries; and (C) a plexiform lesion, comprising a plexus of capillary-like channels, in a patient with plexogenic arteriopathy.

Figure 2: Variation in patient response: Distribution of change in 6 minute walk distance (6MWD) at 3 months in patients randomised to placebo or study drug.

Figure 3: Biomarker-driven study design: (A) Classical randomised study design; deep-phenotyping of patients entering studies can provide data that can be mined for informative biomarkers. (B) Patients are selected on the basis of a chosen biomarker and those positive in the test are randomised to receive the drug or placebo; (C) A biomarker, e.g. change in glucose uptake, can be used to identify patients who show early evidence of response and treatment is continued in these patients to assess effect on objective trial end point; those that do not show an early response are spared further exposure to a drug from which they are unlikely to benefit and can be recruited to a different study.