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Translational research in the pharmaceutical industry: from theory to reality

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Translational research is the collaboration between scientists and clinicians to identify novel targets and develop biomarkers that increase confidence in rationale and therefore help select the mechanisms that are most likely to lead to breakthrough therapies. Here, we describe examples of the utility of linked preclinical and clinical biomarkers to measure pharmacological effects, to estimate clinical dose range, to determine efficacy, and to determine differentiation compared with existing therapies. The use of pharmacogenomics to identify novel drug targets and define enriched patient subpopulations is also discussed. We illustrate how biomarkers and a deep understanding of disease biology are used to discover additional indications for licensed drugs.

Introduction

The theoretical benefit of translational research has been previously described [1]. This article describes examples of how this theory has been put into practice and, in so doing, has benefited the research and development process. For translational research to be successful, a two-way dialogue between scientists and clinicians is necessary, to foster cooperation towards common goals and the setting of complementary strategies, whereby work in one area informs efforts in the other. Translational research enables researchers to capitalize on recent technological breakthroughs and advances in basic sciences, such as the mapping of the human genome, the availability of techniques such as proteomics and metabonomics that enable the detection of small changes in tissue composition, and improvements in imaging platforms that enable a better understanding of the functional changes in normal and disease states. This greater understanding, in turn, facilitates the identification of new targets and the development of linked animal models and clinical biomarkers that can be used to increase confidence in rationale in the preclinical and early development stages (Figure 1). This is particularly relevant with unprecedented drug targets; discarding ineffective mechanisms early on enables more efficient use of resources and better-focussed efforts on targets that are more likely to deliver effective medicines.

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Target biomarkers

Target biomarkers are measures of direct pharmacological effects that result from interaction with the target receptor, enzyme or transport protein. Because they are not linked to a specific disease or condition, they are useful, regardless of the indication being evaluated.

Proof of pharmacology and building knowledge of dose–response

With new chemical entities, it is important to define the pharmacologically active dose range before embarking on large proof-of-concept (POC) studies. This particularly applies to unprecedented drug targets, where it is important that the efficacy hypothesis is adequately tested. By conducting the POC study using doses known to display the appropriate pharmacology, appropriate decisions whether to proceed or not can be made on the drug target rather than just an individual compound. In some instances, the upper end of a pharmacological dose range can be characterized by the emergence of dose-limiting side effects related to that drug class. Doses up to and including the maximum tolerated dose can then be studied in POC studies to determine the therapeutic window. With more selective compounds, however, drug-class side effects might represent a loss of selectivity or extra-pharmacological effects. In this case, the therapeutic margin can be defined by demonstrating the dose ranges showing the selective pharmacological activity relative to the appearance of nonselective side effects.

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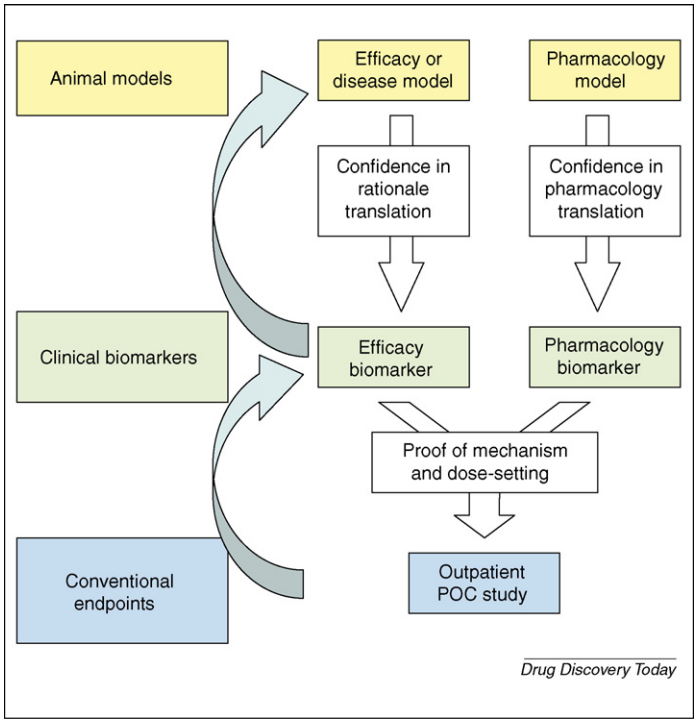

FIGURE 1

Linked animal models and clinical biomarkers can be used to confirm translation of preclinical efficacy and pharmacology to clinical effects. Clinical measures are used to set dose range and optimise the design of outpatient studies.

In the absence of an appropriate efficacy biomarker, the effective dose range can be characterized using pharmacological measures related to the target that might not be directly linked to a beneficial effect in the indication of interest. The following are examples of the mechanistic biomarkers applied to determine the pharmacologically active dose range.

Peripheral drug targets

Soluble biomarkers can be used to determine a pharmacological response to various drug classes. This approach is particularly relevant for enzyme inhibitor mechanisms, where measurement of the substrate provides direct evidence of pharmacological effect. Neutral endopeptidase is a metallopeptidase enzyme involved in the degradation of several endogenous peptides, including atrial natriuretic peptide (ANP), brain natriuretic peptide, enkephalins, bradykinin, angiotensin II and endothelin 1. Candoxatril is the orally active prodrug of candoxatrilat, a neutral endopeptidase inhibitor (NEPi) assessed as a treatment for hypertension and congestive heart failure. In rodent studies, candoxatril causes a dose-dependent increase in plasma and urinary ANP levels and an associated rise in plasma and urinary cyclic GMP (cGMP), the second messenger mediating the ANP effect. These effects are increased in salt-sensitive animals on a high-salt diet [2]. Clinical studies in healthy volunteers and heart failure patients showed that NEPi treatment causes an acute rise in plasma levels of ANP, and plasma and urinary cGMP [3–5]. These changes are accentuated when candoxatril is administered to subjects with a high sodium intake compared with those on restricted sodium [6]. Hence, ANP and cGMP can be used to quantify NEPi effect.

Serotonin (5-HT) stimulates aldosterone secretion in humans, thought to occur through an effect on 5-HT4 receptors in the adrenal cortex that is independent of hypothalamic–pituitary–adrenal (HPA) axis activation. Therefore, aldosterone secretion can be used to assess the systemic effect of 5-HT4 agonist agents that are developed for the treatment of gastroesophageal reflux disease (GERD). Lefebvre *et al.* demonstrated the acute effect of cisapride on plasma aldosterone levels, although this effect appeared to be transient [7,8]. Gale *et al.* have recently confirmed the robustness of the aldosterone effect, showing that single 15 mg doses of mosapride produced an increase in plasma aldosterone in healthy male volunteers, an effect that was reproducible when administered between one and three weeks apart [9]. This pharmacological measure in healthy volunteers can be used to assess novel 5-HT4 agonists in development, informing the choice of doses for POC studies in GERD.

CNS drug targets

Drug targets in the central nervous system (CNS) present particular challenges because it is important to show that pharmacological activity is due to the CNS drug–target interaction rather than a peripheral effect. For CNS drug targets, it is possible to show pharmacological effects in different ways: (i) peripheral effects, such as the inhibition of prolactin secretion by the anterior pituitary with dopaminergic drugs [10]; (ii) physiological effects due to central drug activity, such as altered sensorimotor processing and motor reaction time seen with dopaminergic drugs [11]; and (iii) brain imaging techniques. Brain imaging technology platforms such as functional magnetic resonance imaging (fMRI), positron emission tomography (PET), and electroencephalography (EEG) are being investigated to assess their usefulness in characterizing central pharmacodynamic effects. For example, fMRI [blood oxygenation level-dependent (BOLD) technique] has been used to characterize the pharmacological effect of dopamine antagonists in preclinical and clinical studies. Sulpiride, an atypical antipsychotic agent used to treat schizophrenia, causes bilateral increases in BOLD signal intensity in the frontal cortex following single dose administration in anaesthetized rats [12]. This effect is also seen in healthy male volunteers following a single 25 mg dose of sultopride, another dopamine antagonist, administered orally [13]. This is consistent with the therapeutic mode of action of dopamine antagonists, which cause an increase in frontal dopaminergic function by antagonism of presynaptically located dopamine D2 receptors in this brain region, demonstrating the usefulness of this translatable measure.

Disease-oriented biomarkers

Disease-oriented biomarkers are preclinical or clinical measures of efficacy that are specific to the indication of interest. We use this term to include all measures that can be used to predict a desired outcome in a study using registration endpoints such as questionnaire or diary data to record symptom relief in an outpatient study design. The following examples illustrate the translation of preclinical and clinical biomarkers.

Male erectile dysfunction (MED) is defined as the inability to achieve or maintain a penile erection sufficient to permit satisfactory sexual intercourse [14]. Penile plethysmography

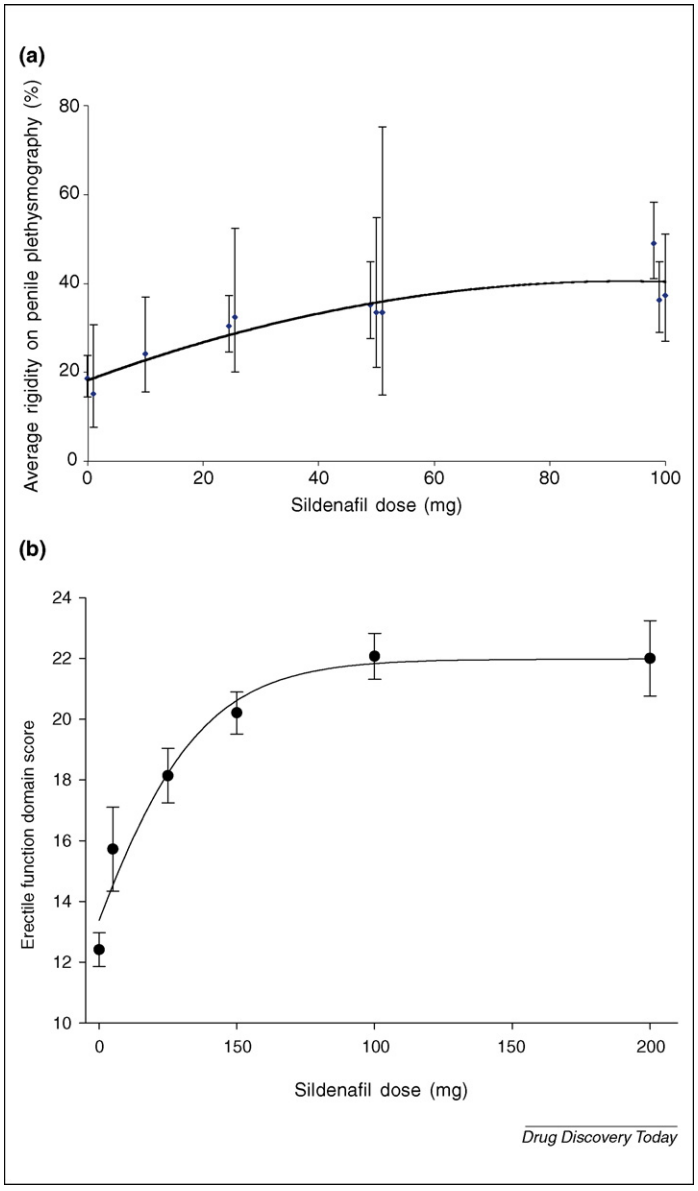

FIGURE 2

Sildenafil dose response in MED patients. (a) Penile plethysmography concentration response. Average penile rigidity during sexual stimulation is shown. **(b)** Outpatient dose response using erectile function domain of International Index of Erectile Function questionnaire [52] is shown. Reproduced with permission from [24]. Copyright Ernst Schering Research Foundation (2007).

(RigiScan® Plus technique) was used by Boolell *et al.* in a laboratory-based study in MED patients to assess the efficacy of sildenafil, a phosphodiesterase type V inhibitor (PDE5i) drug that went on to become the first oral treatment for MED [15]. This technique was used to characterize the efficacious dose range, which was subsequently confirmed in outpatient studies that assessed symptomatic improvement (Figure 2). This clinical methodology has since been used to characterize the efficacy of several other drugs for MED, and has been shown to translate well to outpatient outcome for both centrally acting drugs (e.g. bremelanotide, a nonselective melanocortin agonist compound [16,17]) and peripheral vasodilatory mechanisms (vardenafil, a PDE5i drug [18]). Penile plethysmography can therefore be used to define POC for new compounds or novel approaches in this indication.

The measurement of penile rigidity in the penile plethysmography technique correlates well with the preclinical assessment of erectile function achieved by recording penile *corpus cavernosus* pressure in an anaesthetized dog model [19]. This animal model uses electrical stimulation of the cavernosal branch of the pudendal nerve and is suitable for assessing drugs with peripheral sites of action [20,21]. It has been used to characterize the proerectile effect of sildenafil and translates well to a clinical outcome for this mechanism of action.

Benign prostatic hypertrophy (BPH) is an age-related condition that affects a substantial proportion of men. Current medical treatment consists of (i) drugs that relax the smooth muscle component of the prostatic stroma (α-adrenoceptor antagonists), leading to a lowering of urethral resistance and hence improvement in flow, and (ii) those that cause a reduction in prostate size by inhibiting growth (5α-reductase inhibitors). Nonselective α-adrenoceptor antagonists have several dose-limiting side effects, in particular postural hypotension. A methodology to measure drug effect on prostatic tone and blood pressure simultaneously would enable the early assessment of novel, selective $\alpha 1_A$-adrenoceptor (α_{1A}-AR) antagonists, leading to development of those compounds with an improved therapeutic index.

Kenny *et al.* described an anaesthetized dog model in which urethral pressure and cardiovascular changes are recorded simultaneously while infusing phenylephrine, a nonselective α-adrenoceptor agonist that elevates urethral and blood pressures [22]. By comparing the urethral pressor response with blood pressure changes during bolus injections of increasing doses of phenylephrine, this canine model can be used to determine the prostate selectivity of α_{1A}-AR antagonists relative to vascular effects. The ability to record urethral and blood pressure changes simultaneously in a single species that has similar prostatic and vascular responses to humans is a substantial advantage in developing new drugs in this area.

Building on this animal model, Sultana *et al.* developed a urethral pressure challenge methodology that uses a custom-designed urethral catheter containing microtip pressure transducers to record urethral pressure continuously for up to eight hours in healthy male volunteers [23,24]. Similar to the canine model, intermittent escalating infusions of phenylephrine were used to characterize prostatic and blood pressor responses and therefore determine the therapeutic index relative to hypotensive changes. This was used to characterize the efficacy and prostate selectivity of new α_{1A}-AR antagonist compounds, such as UK-338,003 (Figure 3). These findings were subsequently confirmed in outpatient studies (D. McHale. Using genetics to differentiate between alpha 1-adrenoreceptor antagonists in benign prostatic hypertrophy. Pharmacogenetics, Pharmacogenomics and GeneTherapy, London, Novemer 2004).

The examples given above illustrate the usefulness of having linked animal models and clinical biomarkers for progressing novel compounds for these indications. By characterizing the dose responses in preclinical and clinical methodology studies, the relative potency of novel compounds can be determined with respect to current therapy. With the BPH example, in particular,

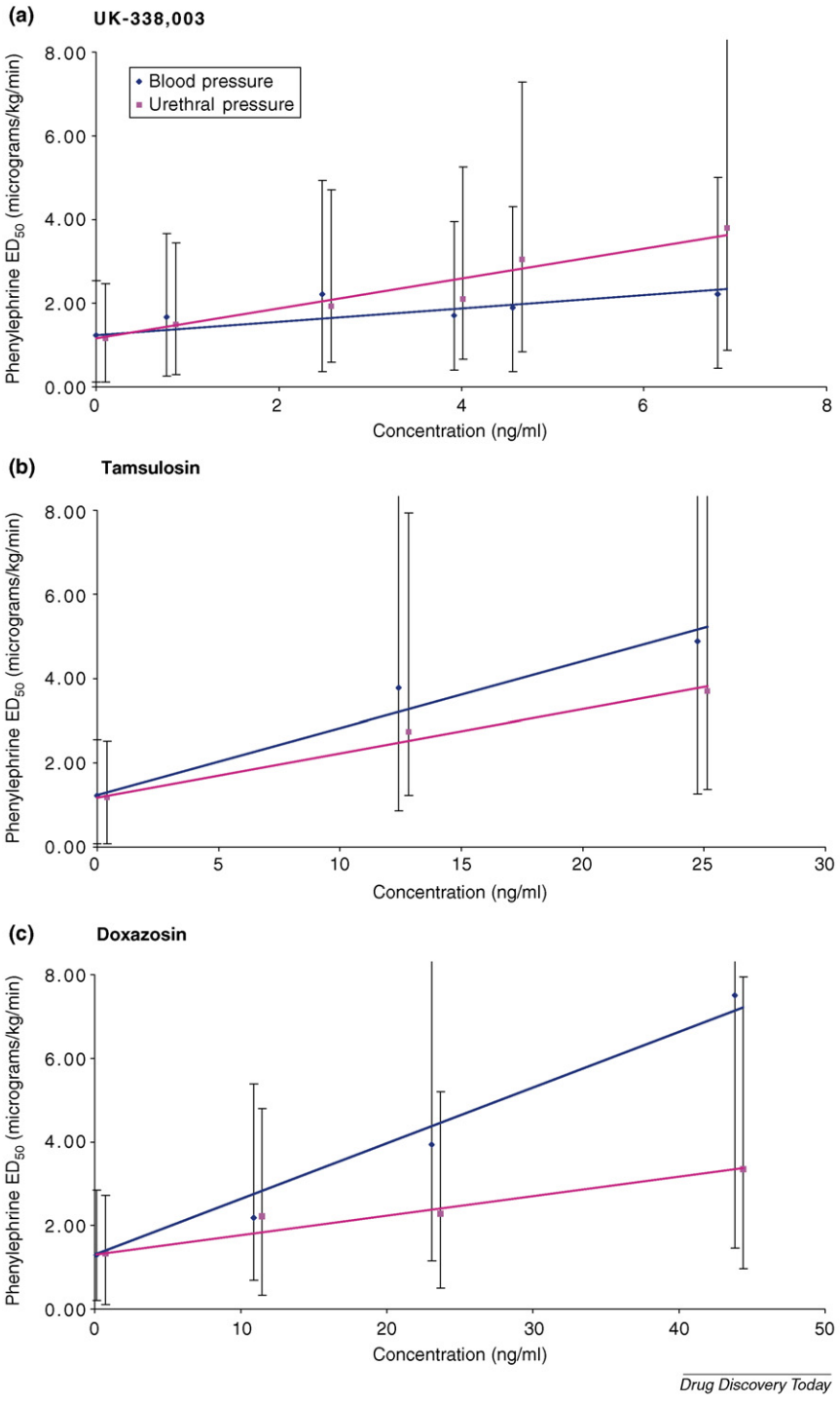

FIGURE 3

Urethral and blood pressure response curves to phenylephrine infusion in healthy male volunteers. The relative effect of **(a)** UK-338003, **(b)** tamsulosin and **(c)** doxazosin on ED_{50} of phenylephrine urethral and blood pressure responses are shown. Urethral versus blood pressure antagonist effect: UK-338,003 > tamsulosin > doxazosin. Pink coordinates represent urethral pressure, blue coordinates represent blood pressure. Reproduced with permission from [24]. Copyright Ernst Schering Research Foundation (2007).

the canine model and volunteer methodology are used to characterize the therapeutic margin relative to one of the common side effects of α-adrenoceptor agonists, which enables the progression of those compounds that are sufficiently differentiated from the current market leaders.

Poly-OMICS

The use of genomics in drug development

One of the key factors in developing improved medicines is a better understanding of the molecular basis of the complex diseases we treat. Genetic association studies have a role in defining

the pathways linked with disease processes and have yielded several novel drug targets, as exemplified below. Pharmacogenomics can also be used to streamline drug development by characterizing the genetic polymorphisms of a given drug target and using these data to explain any variability in drug response or to select an enriched patient population for efficacy studies. The availability of DNA samples from large phase III or IV studies enables whole-genome association studies to be conducted, widening the horizon for novel target identification.

Candidate gene linkage studies

Clinical observations could lead to candidate gene studies that can identify polymorphisms associated with particular disease phenotypes. This, in turn, can lead to new drug targets being identified. This is illustrated by the discovery of CCR5 antagonists for the treatment of HIV infection. It was observed that certain human chemokines can prevent HIV from entering T lymphocytes [25] and that some individuals repeatedly exposed to HIV-1 remain uninfected. Moreover, CD4+ lymphocytes and macrophages from these individuals were shown to be relatively resistant to HIV-1 infection *in vitro* [26,27]. Genetic analysis showed that this was associated with a homozygous defect (32 base pair deletion) in their CCR5 gene, leading to a lack of expression of the CCR5 (C-C chemokine receptor-5) receptor on the cell surface of the CD4+ T lymphocytes [28]. Subjects who are heterozygous for this deletion have partial resistance to infection [29,30], and individuals who are HIV-1 seropositive have a slower decrease in their CD4 T-cell count and a longer AIDS-free survival relative to those with the wild-type CCR5 gene [31]. These findings led to further work to characterize the role of the human CCR5 receptor, and resulted in a new class of anti-HIV therapy. The search for a selective CCR5 antagonist resulted in the development of maraviroc, a new compound that is safe and efficacious [32,33] and is now in late-stage development for HIV with encouraging results to date. This drug discovery program reflects the use of applied genetics to find novel targets, in this case a host target that is expected to confer a degree of immunity against HIV-1 infection.

The candidate gene approach can be used to great effect in large clinical studies. Mank-Seymour *et al*. reported that variation in the endothelial lipase gene (LIPG) is associated with high density lipoprotein (HDL) cholesterol levels [34]. By optimizing a phase 4 atorvastatin study, they were able to collect a large (n = 3916 subjects) sample set and group subjects into high (n = 355), intermediate (n = 1458) and low (n = 239) levels of HDL cholesterol. Their study also suggested an association between LIPG genotype and myocardial infarction, but further work is needed to characterize this. These data confirm previous preclinical [35] and clinical [36,37] findings demonstrating a link between LIPG and HDL cholesterol, and provide strong evidence in support of LIPG as a potential drug target.

Whole-genome association studies

The examples described above represent candidate gene analyses with a biological hypothesis underpinning gene selection. Whole-genome analysis has been used in family-based linkage studies (e.g. stroke [38], schizophrenia [39], and Crohn's Disease [40]) or in pharmacogenomic studies of large phase 3 and 4 programs. Genetic data from Iceland showed that a single-nucleotide polymorphism of the 5-lipoxygenase activating protein (FLAP) gene is associated with a doubling of risk of myocardial infarction [41]. The same group also showed an association between the leukotriene A4 hydroxylase (LTA4H) gene and a modest increase in risk of myocardial infarction [42]. LTA4H is in the same biochemical pathway as FLAP, providing further support for the role of this pathway in the disease. A FLAP inhibitor is currently in late-phase development for the treatment of coronary artery disease, based on these data. Time will tell if any of these newly identified genes will give rise to new drug targets, but these and other examples provide evidence of the value of genomic analysis to drug discovery.

Optimizing drug response

Besides linkage studies that might yield novel targets, pharmacogenomics can also be used to develop personalized medicines – where the therapy is targeted at individuals most likely to respond. This is particularly true for several recent oncology approaches. For example, tumour analysis from women with breast cancer identified that 30% of patients had tumours that overexpressed the HER-2/neu oncogene [43]. These women were found to have a poorer prognosis, with HER-2/neu overexpression associated with greater degree of lymph node metastasis, reduced disease-free survival and shorter life expectancy. This gene encodes a receptor related to the epidermal growth factor receptor (EGFR) family. Studies in a xenograft mouse model with implanted human tumour overexpressing HER-2/neu showed that a monoclonal antibody against this receptor slowed tumour growth [44]. These findings led to the development of the HER-2 monoclonal antibody trastuzumab (Herceptin®), and clinical studies confirmed its efficacy in women with breast cancers overexpressing this receptor [45]. This antibody is now licensed as a treatment for patients overexpressing HER-2, which constitute ~30% of breast cancer patients.

Genetic variation in the drug target can affect the response of an individual to therapy. Pharmacogenomic information that predicts the likelihood of response would therefore help to tailor treatment to those individuals most likely to benefit. UK-338,003 is a highly selective α_{1A}-AR antagonist, with initial studies demonstrating good safety and efficacy in the treatment of BPH. Pharmacogenetic analysis was performed on 1375 DNA samples, collected in three large phase 2b studies assessing the safety and efficacy of UK-338,003 relative to placebo and tamsulosin (D. McHale. Using genetics to differentiate between alpha 1-adrenoreceptor antagonists in benign prostatic hypertrophy. Pharmacogenetics, Pharmacogenomics and GeneTherapy, London, Novemer 2004). A pharmacogenetic effect was observed with a genetic variant (C1475T) that alters an amino acid in the drug target. Subjects homozygous for the C allele showed a trend towards a slightly better response to tamsulosin than UK-338,003, whereas heterozygous subjects, or those homozygous for the T allele, responded better to UK-338,003 with the latter subjects demonstrating the larger response If confirmed, the results of this exploratory pharmacogenetic study suggest that selection of the most appropriate α_{1A}-AR antagonist could be informed by the C1475T genotype of a patient.

Other technologies

Other 'omic' technologies, such as proteomics and metabonomics, offer great promise; however, we have yet to reap any major

benefits from applying these techniques, and it will take time to define the true value of these new technologies. Initial promise needs to be tested in a wider range of indications and patient subpopulations to determine how broadly applicable such given technology would be. Even after a several decades of pharmacogenomic research, we are still in the process of establishing the full potential of this technology across several complex diseases. One would hope that we can learn from experience, and that new technologies will develop faster and establish their proper place in drug development.

Indication discovery

Translational research has an important role in identifying new indications for established therapies – a process we have termed 'indications discovery'. New drug target hypotheses can be generated through preclinical experiments on novel target organs or can result from clinical observations, such as side effects or additional pharmacological effects beyond those expected from efficacy in the main indication.

Sildenafil, a selective PDE5i, is a good example of a drug assessed for one indication but was subsequently licensed for a different one [46]. This drug was originally intended for the treatment of angina as an alternative to nitrate therapy. However, its relatively short half-life and haemodynamic interaction with nitrates led to discontinuation of development in this indication. Following chance observations of enhanced penile erections in healthy volunteers, its potential as a treatment for MED was assessed in an animal model of erectile function and in clinical biomarker studies. This led to the drug being developed and subsequently licensed as the first effective oral treatment for this condition [47].

Subsequent to its launch, sildenafil has been actively assessed in several new indications. Gillies *et al.* provide a comprehensive review of the cardiovascular effects of sildenafil, including its beneficial effects on endothelial dysfunction and arterial compliance – areas that merit further investigation [48]. Basic scientific knowledge linking the abundance of PDE5 in human and rodent lungs and the therapeutic benefits of inhaled NO in pulmonary hypertension led to preclinical studies with sildenafil in animal models [49] and clinical investigation of its effect on pulmonary arterial pressure and pulmonary vascular resistance in patients with primary pulmonary hypertension [50]. Various subsequent studies have confirmed the efficacy of sildenafil in pulmonary arterial hypertension [51], with improvement in clinical outcomes such as symptoms and exercise status. Sildenafil is now approved for use in patients with pulmonary artery hypertension.

Conclusion

Translational research can benefit many aspects of drug discovery and development. The examples given illustrate some of the ways in which it can help in identifying novel targets and increase confidence in rationale for unprecedented mechanisms. Pharmacogenomics can lead to the discovery of new mechanisms as well as help to define patient subpopulations with exaggerated drug response, thus enabling enriched clinical trial designs and the potential for personalized medicines. Measures of pharmacology can be used to determine the active dose range and hence aid dose selection for efficacy testing and enable appropriate decision-making in POC studies.

Translational biomarkers are particularly useful in linking the efficacy seen in preclinical studies with potential therapeutic benefit. The examples given show their use to assess efficacy in early development and establish whether compounds offer improvements compared with existing therapies. These early decision-making studies help to minimise the cost of attrition and success. Translational biomarkers are also useful for identifying novel indications for late-stage compounds or established drugs, opening up new markets for molecules that might already have an established safety profile in the main indication. The development of sildenafil, from a drug intended to treat angina to a successful medicine in male erectile dysfunction and pulmonary artery hypertension, is used as an example of how translational research can give serendipity a helping hand.

Translational research is a rapidly developing area that offers great promise, and this article describes how its successful application can reduce the cost of research and development and assist in the delivery of important new medicines of the future.

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