New era for drug discovery and development in renal disease

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Abstract | Drug discovery and development is a lengthy and expensive process. Testing new agents in humans at an early stage could reduce the time and costs involved in identifying drugs that are likely to succeed in clinical studies. New guidance has outlined the concept of exploratory clinical trials, which provide important information on a drug's distribution as well as its physiological and pharmacological effects in humans. This strategy reduces the need for preclinical testing by limiting the dose and duration of exposure to a new drug in humans to below those required by the traditional testing of investigational new drugs. Exploratory, first-in-man studies should provide insights into human physiology and pharmacology, identify therapeutic targets relevant to disease and increase our knowledge of a drug's characteristics. Implementation of a new drug also requires the development of useful biomarkers of disease and of the drug's efficacy, as well as sensitive molecular imaging techniques. In this Review, we outline the benefits of exploratory clinical trials, especially in academia, and provide an overview of the experimental tools necessary for rational drug discovery and development.

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Introduction

Research in the pharmaceutical industry is curbed by the time required to develop new compounds, the attrition of the tested molecules and potential adverse effects of newly discovered drugs, all of which entail high costs.[1–3] The time it takes to bring a therapeutic drug to the market is ~10–17 years, with a cost of US$0.8–1.7 billion.[1,3,4] Not only is the number of newly approved drugs decreasing, but some areas of medicine, such as rare diseases, are ignored as they lack economic potential.

Very few drugs are being developed for kidney disease, despite the large number of patients who would benefit from new therapies.[5] The treatment of most chronic kidney diseases therefore relies mainly on drugs that were developed for other conditions, such as antihypertensives, glucose-lowering agents, lipid-lowering agents, or immunosuppressants. As an example of this paucity, a search of the NIH Clinical Trials database in May 2011 produced 2,950 trials involving investigational new drugs (INDs), but among these trials only 218 related to kidney disease, and 13 to diabetic nephropathy. Exploratory clinical trials are a strategy that could accelerate the development of new drugs. In this Review, we outline the benefits of exploratory clinical trials and provide an overview of the experimental tools necessary for rational drug discovery and development.

Drug discovery and development

The traditional process of drug discovery and clinical development is a lengthy and expensive process (Figure 1). Research into the pathophysiology of a disease identifies potential target molecules, which are usually proteins. 'Hit compounds' that interfere with these targets are then searched for by high-throughput screening or *in silico* structure-based drug design (SBDD) using computer-aided docking simulation. After identifying a series of lead compounds, optimization is carried out whereby hundreds or thousands of derivative compounds are synthesized. Once a candidate compound is selected, a large number of experiments are undertaken in animals to test the physical and toxicological properties of this molecule. Several years usually elapse before the compound is ready for clinical studies in humans. Phase I studies focus on the pharmacological characteristics of the drug rather than on whether the compound has any clinical benefit. Phase II studies, in which the proof of concept is tested, are usually conducted several years after the initial identification of the target molecule.

The effect of a drug in the body is summarized by two processes. The first, expressed by pharmacokinetics, includes the drug's absorption, distribution, metabolism and excretion, as well as its concentration–time profiles in the circulation and at the target site. The second, expressed by pharmacodynamics, is initiated when the drug interacts with its target (for example, a receptor or enzyme) and is followed by downstream events such as signaling and transcription. As a drug's pharmacokinetics and pharmacodynamics differ between humans and animals, some compounds that show an effect in preclinical studies fail at a late stage in clinical development. Drug attrition is highest during phase II trials (62%).[6] The causes of attrition have changed over time: in 1991, pharmacokinetic properties were the most important cause of attrition (accounting for around

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The authors declare no competing interests.

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Key points

- New drug approvals are decreasing and very few drugs are developed for the treatment of kidney disease despite the large number of patients who might benefit from these drugs
- Understanding the pathophysiology of disease and assessing a drug's effects in humans at an early stage of drug development is important to reduce the time and costs involved in research
- New guidance has defined the concept of exploratory clinical trials, which involve the administration of a small dose of compound to humans for a limited time
- Exploratory clinical trials should provide insights into human physiology and pharmacology, identify therapeutic targets relevant to disease and expand our knowledge of a drug's distribution in the kidney
- The implementation of a new drug requires the identification of biomarkers of the disease and of the drug's effectiveness, as well as development of sensitive molecular imaging techniques
- Collaboration between regulators and researchers is essential to find more efficient strategies for preclinical and clinical development of a drug

40%), but decreased considerably during the subsequent decade to <10%. The lack of efficacy in humans is now the main reason for attrition.[1,7] Research in the area of renal disease is limited by the lack of experimental animal models equivalent to human disease and by the absence of appropriate surrogate clinical biomarkers able to substitute for hard end points such as renal death or creatinine doubling time. Alternative strategies, such as exploratory clinical trials, could avoid the expensive and time-consuming process of lead optimization and preclinical studies.

Exploratory clinical trials

The FDA is keenly aware of the necessity to revise drug development and regulatory processes.[8] Their Critical Path Initiative highlights the importance of translational research and the development of new concepts and tools to increase confidence in the selection of drug candidates early in the clinical development phase.[1] The European Committee for Medicinal Products for Human Use has published its intent to offer a broader guidance for exploratory early clinical studies.[9] As a result, the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH)—a body comprised of European, Japanese and American drug regulators—issued new guidance recommended for adoption by the European Medicines Agency, the Japanese Pharmaceuticals and Medical Devices Agency and the FDA.[10]

The new guidance from the ICH outlines the concept of exploratory clinical trials, in which a small dose of compound is administered to humans for a limited time (Table 1). Compared with the traditional IND application, preclinical testing is markedly shortened and the use of animals is thus minimized. Exploratory clinical trials are intended to be carried out in early phase I trials: they involve limited exposure, have no therapeutic intent and are not supposed to examine clinical tolerability.[10] Although they do not test whether the compound has any clinical benefit, they can be used to investigate a variety of parameters, such as the drug's pharmacokinetics including its distribution in the kidney, and even its pharmacodynamics if useful biomarkers, sensitive drug assays (such as liquid chromatography and mass spectrometry) or molecular imaging techniques (such as PET-CT and single-photon emission CT) are available (Figure 2). Testing very small doses of the drug in humans before preclinical studies should help to identify drug candidates that are likely to fail. A survey of the Pharmaceutical Research and Manufacturers of America member companies concluded that exploratory clinical trials resulted in advancement of the compound to the next development stage in five of seven company responders (71%).[11]

One type of exploratory clinical trial is the microdose trial, which was first described by the European Medicines Agency in 2004.[12] The microdose trial is defined as an early-phase clinical study to obtain information on pharmacokinetic profiles (and tissue distribution if molecular imaging techniques are used).[13] Dose is limited to less than 0.01 of the therapeutic dose or to 100 μg (Table 1). In microdose trials, the single administration of an IND is possible (approach 1), provided that its safety is demonstrated in an extended single-dose toxicity study in one animal species, usually rodents. A maximum of

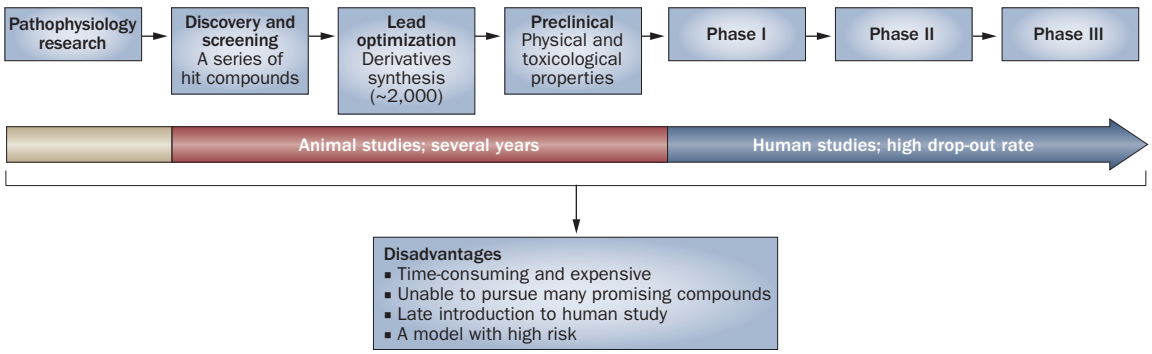

Figure 1 | Traditional process of drug discovery and clinical development. Drug discovery and development involves research into the pathophysiology of a disease, identification of target molecules, *in silico* discovery of new investigational compounds, lead optimization, preclinical studies and early human clinical studies. It usually takes several years for a compound to reach the stage of clinical studies in humans. Proof of concept is tested in phase II trials and it is at this point that most compounds fail. Traditional drug development, therefore, has a number of disadvantages.

Table 1 | Exploratory clinical trial approaches and recommended nonclinical toxicity studies

Clinical studies	Nonclinical toxicity studies*
Approach 1	
Total dose ≤100 μg (no interdose interval limitations) Total dose ≤1/100 of the NOAEL and ≤1/100 of the pharmacologically active dose (scaled on mg/kg for intravenous administration and mg/m^2 for oral administration)	Extended single-dose toxicity study in one species, usually rodent‡§
Approach 2	
Total cumulative dose ≤500 μg, maximum of five administrations with a washout between doses (six or more actual or predicted half-lives) Each dose ≤100 μg and each dose ≤1/100 of the NOAEL and ≤1/100 of the pharmacologically active dose	7-day repeated-dose toxicity study in one species, usually rodent Hematology, clinical chemistry, necropsy and histopathology data should be included
Approach 3	
Single-dose trial at subtherapeutic doses or into the anticipated therapeutic range	Extended single-dose toxicity studies in both rodent and nonrodent‡ The top dose should be the MTD, MFD or limit dose Ames assay
Approach 4	
Dosing up to 14 days into the therapeutic range, but not intended to evaluate clinical MTD	2-week repeated-dose toxicity studies in rodent and nonrodent with standard parameters assessed and where dose selection in animals is based on exposure multiples of anticipated clinical AUC at maximum dose Ames assay and an assay capable of detecting chromosomal damage in a mammalian system
Approach 5	
Dosing up to 14 days into the therapeutic range, but not to exceed duration of dosing in nonrodent and not intended to evaluate clinical MTD	2-week repeated-dose toxicity study in rodent The top dose should be the MTD, MFD or limit dose Confirmatory study in nonrodent at the anticipated NOAEL exposure in rodent, with duration of a minimum of 3 days and at least the intended clinical study duration Alternatively, an escalating dose study in nonrodent with duration of a minimum of 3 days and at least the intended clinical study duration at the anticipated NOAEL exposure in the rodent Ames assay and an assay capable of detecting chromosomal damage in a mammalian system; if an *in vivo* assessment is used then this could be part of the rodent toxicity study

*General toxicity studies should be conducted according to good laboratory practice regulations. ‡Extended single-dose toxicity studies should be designed to evaluate hematology, clinical chemistry, necropsy and histopathology data after a single administration, with further evaluations conducted 2 weeks later to assess delayed toxicity and/or recovery. The usual design for rodent studies consists of 10 animals of each sex per group to be assessed on the day following dosing and five animals of each sex at the dose level(s) selected to be assessed on day 14 following dosing. The usual design for nonrodent studies consists of three animals of each sex per group for all groups on day 2 and two animals of each sex for the dose level(s) assessed on day 14 following dosing. §A single-dose level to assess reversibility or delayed toxicity on day 14 can support the microdose approach. The dose level used does not need to be the top dose, but should be a dose that is at least 100 times the clinical dose. Abbreviations: AUC, area under the curve; MFD, maximum feasible dose; MTD, maximum tolerated dose; NOAEL, no observed adverse effect level.

five administrations of 100 μg (a total of 500 μg) is also allowed (approach 2), provided that the drug's safety is documented in a 7-day repeated-dose toxicity study in one animal species, usually rodents. Other types of exploratory clinical trials include single-dose trials at subtherapeutic doses (approach 3) or multiple-dose trials at therapeutic doses up to 14 days (approaches 4 and 5), which aim to evaluate the pharmacological response (Figure 3). Importantly, these studies require more preclinical testing than a microdose trial, but less than the traditional IND approach. The ability to fast-track our understanding of a drug's effects on human physiology and pharmacology, even in small clinical trials in a limited number of patients, should provide a major advantage during drug development, especially in kidney disease.

As of May 2011, a total of 25 microdose trials were registered in the NIH Clinical Trials database. The number of exploratory clinical trials being carried out is still small and their results have not been published as most, if not all, were performed in-house by pharmaceutical companies. The survey of Pharmaceutical Research and Manufacturers of America reported that nine of 16 company responders claimed that they had conducted or were planning to conduct microdose studies.[11] Survey responders indicated that three and 13 microdose studies were conducted in 2006 and 2007, respectively. The projected number of microdose studies planned for 2008 and 2009 were seven and nine, respectively.[11]

The very low doses of the tested compounds has raised the possibility that the pharmacokinetic profiles determined in microdose studies might differ from that calculated in therapeutic dose studies. In Europe, major pharmaceutical companies and academic institutions have formed consortia to verify the usefulness of microdose studies and concluded that this method is useful to investigate pharmacokinetic profiles in humans.[14,15] However, both the number and the diversity of tested drugs remain very limited.

These new approaches that enable the early assessment of drug distribution within the kidney and, through the use of various biomarkers, its physiological and pharmacological effects, will undoubtedly enable the pursuit of many more promising compounds than is currently affordable, with a much greater probability of eventual success. Drug development using this approach might also reduce the reluctance to proceed from animal

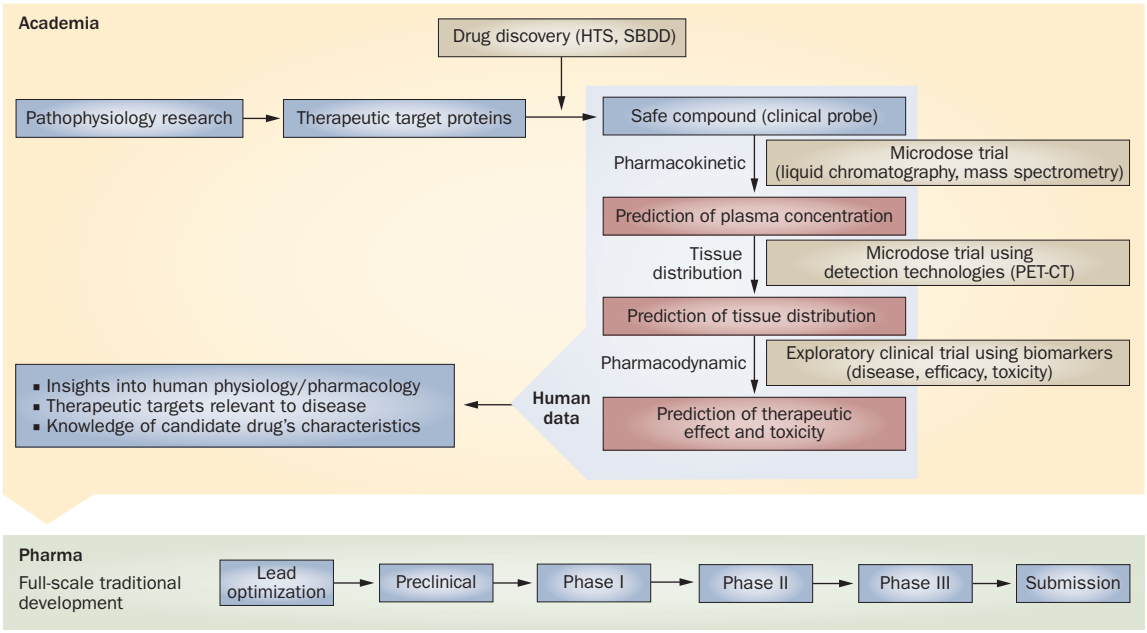

Figure 2 | New framework for drug discovery and development based on exploratory clinical trials. Exploratory clinical trials should improve insights into human physiology and pharmacology, identify therapeutic targets relevant to disease and expand our knowledge of the candidate drug's characteristics, such as pharmacokinetics and pharmacodynamics. Sensitive drug assays (including liquid chromatography and mass spectrometry) or molecular imaging techniques (including PET-CT and single-photon emission CT) help to provide useful information on drug efficacy and response. Abbreviations: HTS, high-throughput screening; SBDD, structure-based drug design.

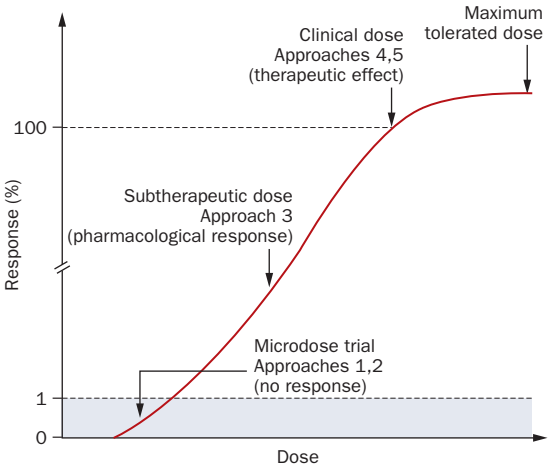

Figure 3 | Relationship between drug dose and response. Approaches 1–5 are included in the definition of exploratory clinical trials as described by new guidance from the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use.

studies to clinical trials, as some human data have already been collected.

Tools for exploratory clinical trials

To take full advantage of exploratory clinical trials, an in-depth understanding of the pathophysiology of human disease is necessary to identify drug targets. Some tools, described briefly in this section, enable this understanding.

Investigational new drugs

A drug candidate should be a probe molecule that enables first-in-human studies to evaluate and, hopefully, confirm the underlying concept. Once a candidate has been identified, high-throughput screening is usually launched by pharmaceutical companies. By contrast, academic researchers have no easy access to large chemical libraries. Fortunately, the availability of information on the tertiary structure of proteins enables the localization of the target and a rational and efficient *in silico* identification of promising candidate compounds by computer-aided approaches, SBDD or fragment-based drug design.[16–18] The integration of detailed protein structural information, computational chemistry, medicinal chemistry and informatics has transformed virtual screening from dream to reality (Figure 4). SBDD is essential not only for drug discovery, design and optimization, but also for understanding a drug's pharmacological mechanisms.[19] This method has contributed to the development of several medical agents currently in use, including neuraminidase inhibitors, HIV-1 protease inhibitors, direct renin inhibitors and tyrosine kinase inhibitors. Some examples of candidate targets from the field of kidney disease, which still require further testing in man, are described below.

Plasminogen activator inhibitor 1

Plasminogen activator inhibitor 1 (PAI) has a key role in renal fibrosis and might therefore be an important therapeutic target in chronic kidney disease.[20] PAI is not expressed in the healthy kidney, but is highly expressed in both the glomeruli and tubulointerstitium

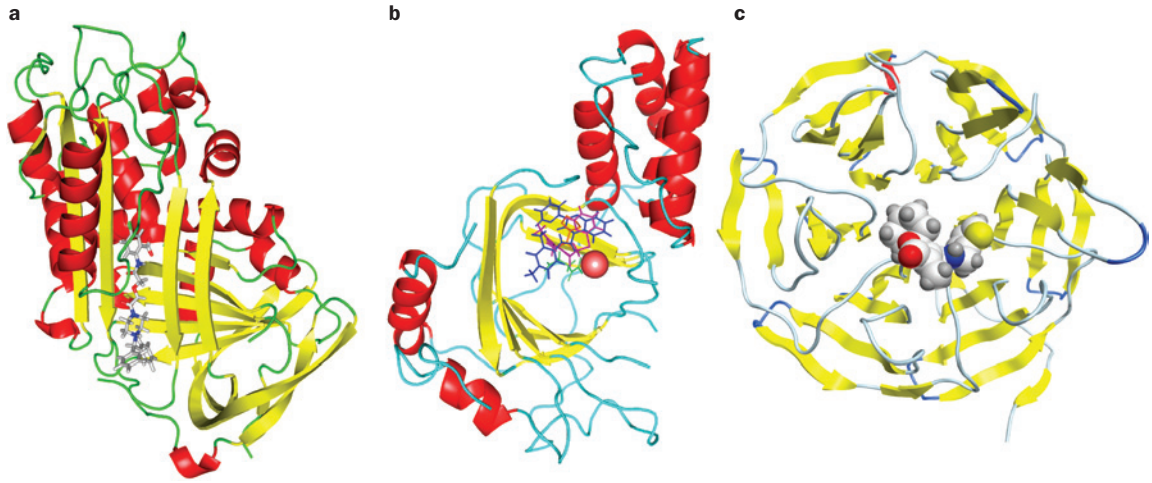

Figure 4 | Predicted binding modes obtained by docking simulations. **a** | The predicted binding mode of the inhibitor TM5275 in plasminogen activator inhibitor 1. **b** | The predicted binding modes of TM6008 (blue) and TM6089 (red) in prolyl hydroxylase 2. **c** | A space-filling model shows an inhibitor of Kelch-like ECH-associated protein 1 bound in the center of the concavity.

of diseased human kidneys.[21–23] Overexpression of PAI in mice exacerbates renal fibrosis in obstructed kidneys,[24] whereas PAI-knockout mice resist renal injury, both in a model of crescentic nephritis[25] and in a streptozotocin-induced diabetic model.[26] Expression of a mutant PAI that does not inhibit the plasminogen activator decreases matrix accumulation in experimental glomerulonephritis.[27] A PAI inhibitor might thus prove not only to be an antithrombotic agent, but also a tool to prevent renal fibrosis.[20]

SBDD was applied to identify a novel inhibitor of PAI *in silico*.[28] Initially, the site essential for PAI inhibition was identified. Structural information on PAI enabled the virtual screening of a chemical library encompassing more than 2 million compounds. Several filters, such as size, charge and drug likeness, reduced the number of candidates. Docking simulation with software subsequently evaluated how the compound fitted within the PAI active pocket.[29] Eventually, 95 candidate molecules were found to bind in this pocket, 28 of which were purchased or synthesized, and their biological activities tested *in vitro*. Finally, two hit compounds were identified.[28] Structural optimization of the hit compounds produced about 500 new derivatives, some of which had ideal pharmacological parameters and were threefold more efficacious than the original compound. In a monkey model of arterial thrombosis, one of these compounds, TM5275, binds to the active site of the PAI moiety (Figure 4a). TM5275 has an antithrombotic effect similar to that of the antiplatelet agent clopidogrel.[30] Interestingly, TM5275 does not prolong bleeding time in rodents and monkeys, which is an advantage over clopidogrel. Exploratory clinical trials are now planned to elucidate the pharmacokinetics and pharmacodynamics of these promising compounds in humans.

Oxygen sensor inhibitor

Oxygen fuels various metabolic processes in mammals, including oxidative phosphorylation during mitochondrial respiration. A decreased oxygen supply (hypoxia) not only induces acute disorders such as ischemic heart disease, but also chronic disorders such as renal fibrosis.[31] In chronic kidney disease, the oxygen supply decreases in the tubulointerstitial space with the attendant hypoxia profoundly altering the functions of tubular cells, eventually leading to tissue fibrosis.[32] Defense against hypoxia relies on the expression of hypoxia-inducible factor (HIF), which activates a broad range of genes that stimulate erythrocytosis, angiogenesis, glucose metabolism, cell proliferation and survival, thus protecting tissues.[33,34] Levels of HIF are determined by its degradation by the intracellular oxygen sensors prolyl hydroxylases (PHDs).[35,36]

The same computer-aided strategy used for detecting the PAI inhibitors described above was applied to synthesize two novel inhibitors of PHDs (TM6008 and TM6089).[37] These inhibitors bind to the active site of human PHD2 in which HIF would normally bind (Figure 4b). As anticipated, given orally, these inhibitors stimulated HIF activity in various organs of transgenic rats expressing a hypoxia-responsive reporter vector; given locally, they induced angiogenesis in a mouse sponge assay.[37]

The PHD molecules have three isoforms whose roles have been delineated by the specific disruption of each *PHD* gene.[38] Broad-spectrum conditional knockout of *PHD2* induces expression of vascular endothelial growth factor and an hyperactive angiogenic response, with the formation of mature and perfused blood vessels.[39] In agreement with these observations, TM6008—a compound that binds human PHD2 in docking simulation studies—induces angiogenesis in mice.[37] Adult mice deficient in PHD2 also develop severe erythrocytosis with a dramatic increase in the levels of serum erythropoietin and renal erythropoietin messenger RNA.[40]

The benefits of HIF activation beyond its effects on angiogenesis and erythropoiesis have been demonstrated.[41] Disruption of PHD1 unexpectedly induces hypoxic tolerance in muscle cells, without angiogenesis and erythrocytosis, at least in part through the activation of HIF2α. Basal oxygen metabolism is reprogrammed

and oxidative stress generation is decreased in hypoxic mitochondria. Inhibition of PHD1 is likely to stimulate various protective mechanisms, such as ATP production through increased glycolysis and a restriction of the entry of glycolytic intermediates into the oxidative phosphorylation of glucose through the induction of pyruvate dehydrogenase kinase, with the eventual attenuation of electron entry into the electron transport chain. As a consequence, energy is conserved, oxidative damage is reduced and cells are protected from hypoxic damage.

However, nonspecific inhibition of HIF degradation also augments the expression of other gene products, such as vascular endothelial growth factor and erythropoietin, both of which are proven to have detrimental effects in humans.[42] At present, none of the available PHD inhibitors is specific for a distinct PHD subtype and the human PHD1 structure has not been elucidated. A specific PHD1 inhibitor designed *in silico* by SBDD based on the structure of PHD1 should protect hypoxic tissues through reduced oxidative stress and avoid the adverse effects associated with PHD2 inhibition (such as polycythemia, congestive heart failure and placental defects during pregnancy).[31]

Oxidative stress sensor inhibitor

The involvement of oxidative stress in kidney disease, including diabetic nephropathy, is supported by a large body of evidence from *in vitro* experiments, *in vivo* animal studies and human studies.[43,44] Radical scavenger agents have been used to attenuate neuronal injury after stroke in animals, but a study of the radical scavenger disodium 2,4-disulfophenyl-*N*-tert-butylnitrone (NXY-059) showed that it is ineffective against acute ischemic stroke in humans.[45] However, this conclusion should be taken with caution, as the first individual animal meta-analysis on data obtained from 15 studies (26 conditions, 12 laboratories) involving rats, mice and marmosets found that NXY-059 was neuroprotective in experimental stroke.[46] However, as this effect is not seen in humans, the development of this category of agents has been hampered.

In order to alleviate the effects of oxidative stress we have used an approach different from that of radical scavenging therapy. Our alternative therapeutic approach is based on cellular defense mechanisms against oxidative stress. Nuclear factor erythroid 2-related factor 2 (Nrf2) is a basic leucine zipper redox-sensitive transcriptional factor that regulates the expression of several cellular antioxidant and cytoprotective genes.[47,48] Upon exposure to oxidative stress and/or electrophiles, Nrf2 translocates into the nucleus, heterodimerizes with a small Maf protein, binds to the antioxidant/electrophile responsive element and activates the transcription of antioxidant genes, including heme oxygenase 1, glutathione peroxidase 2, NAD(P)H dehydrogenase [quinone] 1, and glutathione S-transferase. Thus, Nrf2 causes a broad and coordinated set of downstream reactions against oxidative stress.

Induction of renal ischemia and reperfusion has been found to increase Nrf2 levels and expression of downstream target genes in the kidneys of wild-type mice.[49] A deficiency in Nrf2 increased susceptibility to both ischemic and nephrotoxic acute kidney injury: renal function, histology, vascular permeability and survival were considerably worse in Nrf2-knockout mice than in the control mice.[50] Treatment of the Nrf2-knockout mice with the antioxidants *N*-acetyl-cysteine or glutathione improved renal function. Furthermore, Nrf2-knockout mice with streptozotocin-induced diabetes mellitus showed progressively increasing urinary excretion of nitric oxide metabolites, evidence of oxidative stress and renal injury.[51] Nrf2-mediated transcriptional responses were also protective in other experimental diseases, including oxidative lung injury and fibrosis, asthma and brain ischemia reperfusion injury.[52–54]

These data indicate upregulation of Nrf2 as a potential therapeutic target to ameliorate oxidative stress-induced kidney injury. Nrf2 is constitutively transcribed and translated and its level is continuously regulated by its degradation within the proteasome after ubiquitination through the Kelch-like ECH-associated protein 1 (Keap1)–cullin-3 system.[55,56] Keap1 acts as a sensor of oxidative stress and a negative regulator of Nrf2. Under oxidative stress, reactive cysteines within the Keap1 moiety are modified by oxidants and induce conformational changes, leading to the detachment of Nrf2 from Keap1 and inhibition of its ubiquitination. Oxidative stress thus inhibits degradation of Nrf2 and facilitates nuclear translocation of Nrf2. In Keap1-knockdown mice, Nrf2-regulated gene expression substantially increases and ameliorates oxidative injuries in obstructive cholestasis[57] and stroke (S. Takizawa, T. Miyata and M. Yamamoto, unpublished work). Inhibition of Keap1 could thus result in tissue protection through increased nuclear translocation of Nrf2 and subsequent activation of antioxidant genes.

Bardoxolone methyl, a potent inducer of Nrf2, is being tested in an ongoing phase II study of diabetic nephropathy.[58] No effective Keap1 inhibitor is currently available, but the X-ray crystal structure of Keap1 and the molecular mechanism of its interaction with Nrf2 have been elucidated.[59] We therefore used the three-dimensional structure of Keap1 and SBDD to identify a compound binding to the active site of Keap1 and inhibiting its interaction with Nrf2 *in vitro* (M. Yamamoto, N. Hirayama and T. Miyata, unpublished work) (Figure 4c). If its benefits are confirmed in experimental disease models, a specific Keap1 inhibitor may offer an alternative approach to mitigate oxidative stress injury.

Biomarkers and molecular imaging techniques

Useful information for drug discovery and clinical development might be obtained from molecular imaging techniques.[60] Direct measurement of a drug's effects in the body might reduce the time and costs involved in drug development. Critical to this approach are molecular imaging probes that target specific molecular pathways *in vivo*. Such probes visualize the phenotypic expression of key molecular targets associated with the disease process. Molecular imaging might display biochemical

and physiological abnormalities that occur early in the disease process, in contrast to the structural changes that develop late and are identified by standard anatomical imaging techniques. Such techniques will have many potential uses in all phases of the drug development process, from target discovery and validation to use in clinical trials.

Direct assessment of the sequential events involved in the pathophysiology of the kidney, such as renal tissue hypoxia, is difficult to obtain from analyses of human specimens such as blood or urine. By contrast, molecular imaging technologies enable the direct evaluation of renal oxygen levels. For instance, blood oxygen level-dependent (BOLD)-MRI in a healthy individual given 1 l water load after an 8 h water restriction demonstrates a substantial increase in the oxygen level in the outer medulla of the kidney (T. Mori and T. Miyata, unpublished work). Furosemide blocks sodium reabsorption in the outer medulla and thus reduces oxygen consumption. Renal BOLD-MRI shows that intravenous furosemide increases medullary oxygen levels within 15 min after its administration to a healthy individual (Figure 5). These findings document the dramatic alterations of renal oxygen levels that can be visualized and monitored using molecular imaging techniques.

Other more sophisticated molecular imaging probes are available to identify hypoxia. Hypoxic tissues selectively accumulate 2-nitroimidazole analogues. ^{18}F-fluoromisonidazole,[61] first used in humans for the direct visualization of tumor tissue hypoxia, may be followed by administration of an ^{18}F-FRP170 (1-[2-fluoro-1-{hydroxymethyl}ethoxy]methyl-2-nitroimidazole) probe,[62] providing high-contrast images on PET-CT with low background signal. This probe enables the visualization of the ischemic myocardium in patients with ischemic heart disease,[63] but it has not yet been applied in patients with kidney disease. However, a similar probe —pimonidazole—has been utilized to detect renal hypoxia in several experimental models.[64,65] In combination with inhibitors for oxygen sensors, these labeled probes could provide a platform for exploratory clinical trials, improving insights into renal hypoxia, verifying whether some interventions prevent hypoxia and expanding our knowledge of a drug's characteristics (Figure 6). Additional labeled probes might directly elucidate other mechanisms critical to kidney injury, such as oxidative stress, fibrosis, inflammation and carbonyl stress, and should accelerate drug discovery and clinical applications in the field of unmet medical needs.

These emerging biomarkers and improved technologies should also have much to offer for the monitoring of kidney safety during drug development and evaluating drug-induced nephrotoxicity.[66,67] They enable us to identify and monitor specific types of injuries in the kidney with high sensitivity and specificity.

Regulatory science

Increased understanding of the latest regulations in pharmaceutical practice and of the newer, more efficient strategies for preclinical and clinical development is of utmost importance. As most physicians are not familiar with these regulations, a close collaboration with the regulator or with experts of regulatory matters is essential. For example, a new division called 'Regulatory science' has been established at our institute that includes three faculty members who previously worked for the Japanese Drug Regulatory Agency. The aim of this division is to provide education on the latest regulations in pharmaceutical practice and of novel strategies for preclinical and clinical drug development.

The new ICH guidance raises several ethical issues that relate to the risks and benefits offered to individuals

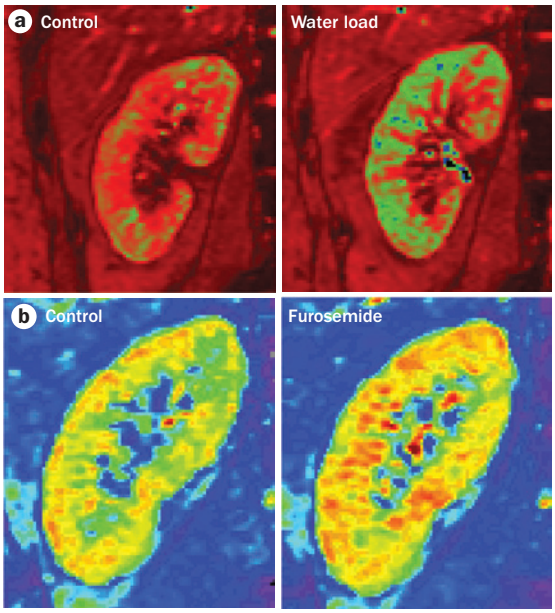

Figure 5 | Utility of BOLD-MRI. **a** | BOLD-MRI of a healthy individual given 1 l water orally after an 8 h water restriction shows a considerable increase in the oxygen level (green) in the outer medulla of the kidney. The control showed no increase in oxygen level. **b** | Furosemide given intravenously in a healthy individual increases medullary oxygen level (red) within 15 min after its administration. The control showed no increase in oxygen level. Abbreviation: BOLD, blood oxygen level-dependent.

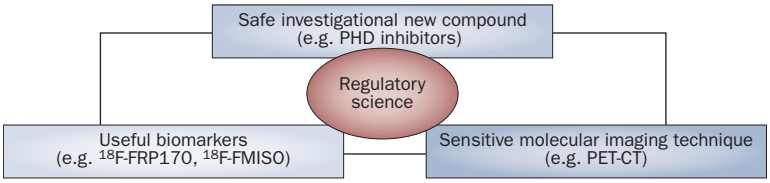

Figure 6 | Tools for exploratory clinical trials. Safe investigational new compounds, useful biomarkers and sensitive molecular imaging techniques are necessary to carry out exploratory clinical trials. Information on the tertiary structure of a protein enables the identification of promising candidate compounds *in silico* by computer-aided approaches, such as structure-based drug design. Molecular imaging probes enable visualization of the phenotypic expression of key molecular targets associated with the disease process. Biomarkers that track physiological events developing *in vivo* will potentially benefit all phases of the drug development process, from target discovery and validation to use in exploratory clinical trials and clinical studies. Abbreviations: ^{18}F-FMISO, ^{18}F-fluoromisonidazole; ^{18}F-FRP170, 1-(2-fluoro-1-[hydroxymethyl]ethoxy)methyl-2-nitroimidazole; PHD, prolyl hydroxylase.

enrolled in exploratory clinical trials.[68] These issues warrant further discussion and require that the results of exploratory clinical trials be published or made available in publicly accessible electronic databases.

Conclusions

Researchers in academia often undertake the entire process of research and development, from research into the disease pathophysiology to the identification of target molecules, *in silico* discovery of new compounds, lead optimization, preclinical studies and, eventually, early phases of human clinical studies. However, academia should not necessarily be involved in clinical trials conducted by pharmaceutical companies to obtain marketing approval. Rather, researchers in academia should concentrate on the selection of useful compounds that are active in humans so as to provide useful information to companies, which should remain responsible for the subsequent full-scale, traditional clinical development of new drugs. Academia should take notice that the treatment of rare diseases[69] and kidney diseases is little addressed by the pharmaceutical industry. Research in these areas that reach the first-in-man stages necessary to attract attention from the pharmaceutical industry is of utmost importance.

Review criteria

We searched the PubMed database for English-language articles published up to October 2010 using the terms "exploratory clinical trial", "microdose trial", "structure-based drug design", "*in silico* screening", "virtual screening", "diabetic nephropathy", "oxygen sensor", "hypoxia" and "oxidative stress". We also searched ClinicalTrials.gov for relevant studies.

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Author contributions
T. Miyata researched data for the article. All the authors contributed to discussion of the content. T. Miyata and C. van Ypersele de Strihou wrote the article and reviewed and edited the manuscript before submission.