Magnetic resonance imaging in drug discovery: lessons from disease areas

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Imaging technologies are presently receiving considerable attention in the pharmaceutical area owing to their potential to accelerate the drug discovery and development process. One of the principal imaging modalities is magnetic resonance imaging (MRI). The multiparametric nature of MRI enables anatomical, functional and even molecular information to be obtained non-invasively from intact organisms at high spatial resolution, thereby enabling a comprehensive characterization of a disease state and the corresponding drug intervention. The non-invasiveness of MRI strengthens the link between pre-clinical and clinical drug studies, making the technique attractive for pharmaceutical research.

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▼ There is significant interest in the use of imaging technologies in pharmacological research because of their potential to considerably accelerate the drug discovery and development process [1–3]. Imaging plays an important role in several phases of the drug development process (Figure 1). The first step of this long pathway consists of identifying and validating potential drug targets. Genetically engineered mice are being employed increasingly in this early phase [4], and non-invasive imaging modalities such as magnetic resonance imaging (MRI) are used for phenotyping. Once potential drug candidates are identified in screening programs, their pharmacokinetic properties, efficacy and safety profiles are characterized in animal models of human diseases.

The average tissue concentration of most compounds is too low to be detected by magnetic resonance (MR) methods so their primary role in pharmacological research is to study the effects of drugs on tissue morphology, physiology and biochemistry. Repetitive measurements are feasible and inter-individual variances are reduced by using each animal as its own control, thereby enhancing the statistical power of experiments. As an established animal and clinical imaging modality, MRI has the potential for enabling the transfer of pre-clinical study paradigms to clinical drug development. It is therefore an important tool for translational research.

The principal assets of MRI are high spatial resolution and the excellent soft-tissue contrasting capabilities. MRI is inherently a multi-dimensional technique providing cross-sectional images as well as full three-dimensional (3D) data of body regions. Depending on the spatial resolution required and the imaging protocol applied, acquisition times for one dataset typically range from 80 ms to 1 h. Because individuals need to be kept immobilized (and, in the case of animals, anesthetized), total investigation times, including preparation, are usually set to a maximum of one hour. Under these circumstances, the spatial resolution limits are of the order of a few hundreds of microns and 50–60 μm for human and rodent imaging, respectively. Image contrast is determined by several parameters, all of which depend on the biophysical properties of the tissue. In the next sections, we illustrate how this wealth of information is explored in the context of pharmaceutical research with examples from several disease areas in which MRI is routinely employed in preclinical and clinical research.

Phenotyping transgenic animals
Transgenic mice are important for target identification and validation, and as refined models of human diseases. They can also be designed to provide early information concerning drug metabolism and toxicity [4]. MR techniques have been used to characterize transgenic mice that model several types of diseases.
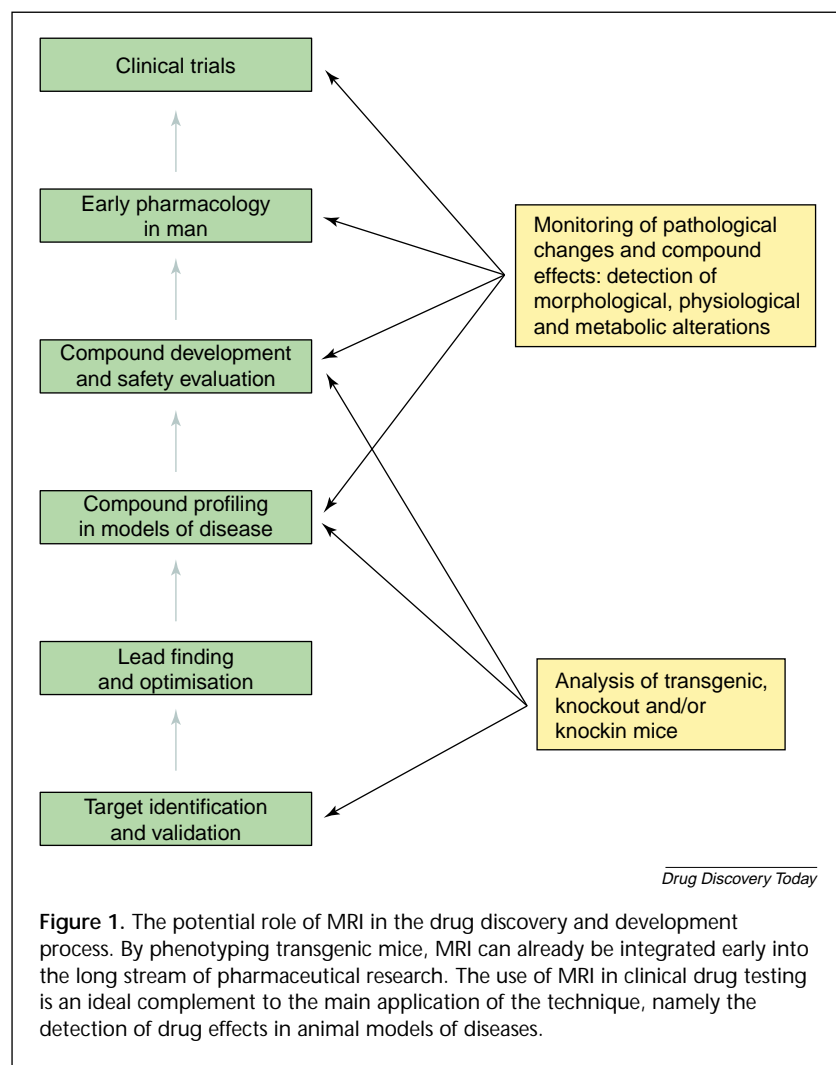
Figure 1. The potential role of MRI in the drug discovery and development process. By phenotyping transgenic mice, MRI can already be integrated early into the long stream of pharmaceutical research. The use of MRI in clinical drug testing is an ideal complement to the main application of the technique, namely the detection of drug effects in animal models of diseases.

As a representative example, we consider the phenotyping of transgenic mice that model Alzheimer's disease (AD). Neuroanatomical alterations associated with AD comprise the formation of amyloid-β (Aβ)-peptide-containing plaques and the deposition of Aβ-peptide in cerebral vessel walls [5].

The ultimate readout of AD would be the visualization of cerebral plaques *in vivo*. MRI microscopy methods have been developed to image β-amyloid plaques [6], but measurement times are very long (> 10 h), even when enhancing contrast-to-noise by using molecular probes that specifically target β-amyloid plaques [7]. Alternative approaches such as analyzing functional or hemodynamic changes related to the development of AD have been more successful in characterizing transgenic mice *in vivo*. Functional MRI (fMRI) has been applied to assess brain functionality [8] in APP23 transgenic mice that overexpress amyloid precursor protein (APP) [9]. The cerebral hemodynamic response to infusion of the $GABA_A$ antagonist bicuculline was

significantly reduced in aged APP23 mice compared with age-matched wild-type littermates. The decreased response was attributed to a compromised cerebrovascular reactivity associated with perivascular amyloid deposition. High resolution MR angiography demonstrated flow perturbations in principal arteries at the Circle of Willis in old but not in young APP23 transgenic mice [10]. Corrosion casts (a technology that enables the vasculature to be seen in great detail) revealed that, at sites where flow voids were detected *in vivo*, vessel elimination, substitution and/or deformation had taken place.

Compound profiling in animal models of disease

The challenge of integrating MRI into pharmaceutical research resides in exploiting the obvious advantages of the technique, such as non-invasiveness and the multiparametric nature of the MRI signal that provides a unique basis for tissue characterization. Because the approaches that are used depend on the question being asked and/or the organ to be analyzed, this section presents examples from several disease areas that illustrate the benefits of MRI in pre-clinical research. The basic paradigm consists of reproducibly assessing a parameter with which the progression of the pathophysiological condition can be followed (Table 1). Validated markers are then used to evaluate the effects of drug intervention.

Degenerative joint diseases

Treating chronic diseases that affect the joints, such as rheumatoid arthritis (RA) and osteoarthritis (OA), is a major medical challenge. MRI has become the method of choice in the diagnosis of joint diseases in humans [11] and also in animal models of RA and OA because of its ability to differentiate bone, cartilage, tendons, synovium, muscle and adipose tissue. 3D-imaging protocols provide the high spatial resolution that is required and allow for proper image co-registration, thus enabling reproducible monitoring of the complex joint architecture in longitudinal studies.

High resolution 3D MRI was used to follow the arthritic process in the rat paw induced by immunization with heterologous collagen [12] or to investigate soft and hard tissue changes in the rabbit knee during the course of

antigen-induced arthritis [13]. These studies focused on structural readouts such as the joint space for assessment of bone erosion, or on the characterization of synovial fluid effusion and inflamed areas using the contrast agent gadoterate meglumine (Gd-DOTA).

Although contrast agents such as Gd-DOTA accumulate in the extracellular space, superparamagnetic iron oxide particles (SPIO), with a mean diameter of up to 150 nm, are eliminated from the blood by cells of the mononuclear phagocytotic system through absorptive endocytosis [14]. This property has been exploited in rodent models of RA to follow, *in* vivo, the infiltration of macrophages into sites of inflammation [15]. In an antigen-induced model of arthritis in the rat, significant negative correlation was found between the MRI signal intensity in the knee and the histologically-determined iron content in macrophages located in the same region of animals that had received SPIO 24 h before image acquisition [15]. This readout might provide an early marker of disease progression, before more aggressive changes like cartilage and bone erosion take place. Macrophages possess widespread pro-inflammatory, destructive and remodeling capabilities that critically contribute to the acute and chronic phases of RA [16].

In contrast to RA, the aetiopathogenesis of OA, characterized by an initial cartilage damage capable of setting off the destructive process that ultimately affects adjacent bones and muscles, is essentially non-inflammatory in nature. The molecular organization that gives cartilage much of its resiliency also conveys unique MRI properties. Hydration of the cellular matrix is assured both by collagen and proteoglycans (PG). PG depletion and loosening of the collagen network play an important role in the progression of OA. Several MRI approaches have been developed to monitor cartilage degeneration, one of the most promising being gadolinium-enhanced MRI [17], based on the principle that negatively charged gadolinium diethylenetriamine pentaacetic acid [Gd-(DTPA)$^{2-}$] penetrates cartilage to an equilibrium concentration that is inversely proportional to PG concentration.

Animal models that appropriately mimic the slow cartilage degeneration seen in OA, and allow reasonable throughput for drug testing, are not available yet. Using enzymatic treatment as an acute model of OA, a papain dose-dependent effect indicated that partial PG depletion can be detected in

Table 1. List of potential biological markers that can be assessed using MRI

Indication	Biological marker
Rheumatoid arthritis	Bone erosion, osteophyte formation, synovial fluid effusion, macrophage infiltration into synovium
Osteoarthritis	Proteoglycan depletion in cartilage
Stroke	Edema associated with infarction, perfusion, diffusion, oxygenation status and functionality of infarcted tissue, macrophage infiltration
Respiratory diseases	Edema and mucus associated with inflammatory responses, perfusion and ventilation of parenchymal tissue
Cardiac disorders	Myocardial mass and wall motion, end-systolic and -diastolic volumes, stroke volume, ejection fraction
Oncology	Tumor size and extent, angiogenesis (blood flow and volume in the tumor, vessel permeability and size), metastases detection

the rabbit knee cartilage *in vivo* by Gd-(DTPA)$^{2-}$-enhanced MRI [18]. The kinetics of gadolinium enhancement proved to be a potential surrogate for a therapeutic endpoint.

Stroke

The disease models that have been most extensively characterized using MRI are probably of human embolic stroke [2]. This is because of a high medical need for stroke therapy, a large number of animal models that reproduce various aspects of the clinical disease, and the fact that the brain, being relatively motionless, is ideally suited for MRI studies.

A frequently used animal model for stroke is focal ischemia induced by unilateral middle cerebral artery occlusion (MCAO) in rats [19]. The cytoprotective efficacy of anti-ischemic drugs has been assessed with MRI by determining edema volumes 24 or 48 h after MCAO [2,20], a measure that correlates with histologically assessed tissue necrosis [21,22]. This approach does not fully exploit the potential of MRI because the occurrence of vasogenic edema represents irreversible tissue damage. Perfusion- [23] and diffusion-weighted MRI [24], enabling the assessment of perfusion deficits or the consequences of membrane failure, respectively, are examples of alternative techniques that detect compromised tissue at a reversible stage. MRI allows a comprehensive characterization of the pathophysiological cascade of focal cerebral ischemia from a structural and functional point of view [1–3,25].

There is increasing interest in subchronic and chronic aspects of focal cerebral ischemia. It is known that the size of the infarct can increase within the first 48 h in the rat

MCAO model and sometimes over days in patients [20]. Inflammatory mechanisms might play an important role for the delayed increase of infarct volume. Therefore, non-invasive monitoring of immune-competent cells in the brain might be of predictive value for lesion development. Accumulation of SPIO, transported into the brain by iron-loaded macrophages, has been observed within or near the ischemic brain lesions induced by MCAO in the rat [26]. Anti-inflammatory therapy might be an interesting concept for the post-acute treatment of stroke patients and MRI macrophage imaging should enable the direct monitoring of pharmacological interventions.

The ultimate objective of stroke therapy is improved functional outcome, which is commonly assessed behaviorally or, alternatively, by functional imaging techniques such as fMRI or positron emission tomography (PET). Functional imaging could clarify the question of whether or not an improved performance following cytoprotective therapy is due to functional recovery of the protected brain area or to functional reorganization of the brain [27]. For example, fMRI was used to study the functional recovery of cytoprotected somatosensory cortex in the rat MCAO model following peripheral sensory stimulation of the hind paws. Despite normal morphological appearance of the respective cortical area in drug-treated animals, brain function was still partially impaired, probably because of incomplete restoration of local cerebral perfusion [28]. Therefore, functional information is crucial in evaluating therapeutic responses.

For a recent review on the characterization of disorders of the central nervous system and evaluation of therapy using structural and functional MRI, see ref. [29].

Diseases of the airways

The inflammatory status of the lungs in animal models of respiratory diseases is commonly determined by broncho-alveolar lavage (BAL) fluid analysis or histology. MRI provides a non-invasive alternative for the evaluation of anti-inflammatory compounds in these models [30].

Intense and continuous signals related to edema formation are encountered in the lungs of actively sensitized rats following allergen challenge. These animals develop a response that reflects the key features of asthmatic inflammation. Edematous signals are detected for ~100 h and correlate with inflammatory parameters determined in the BAL fluid [31,32]. By contrast, endotoxin elicits edematous signals in the lungs of a patchy texture and significantly lower intensity. They are detectable eight days after dosing and were shown to be related to secreted mucus [32,33]. Endotoxin induces an inflammation similar to that observed in chronic obstruction pulmonary disease patients.

Edematous signals induced by allergen are markedly suppressed by pre-treating the animals with budesonide, a glucocorticosteroid, which is accompanied by reductions in the inflammatory parameters in the BAL fluid [32]. Post-treatment efficacy is assessed by administering the drugs 24 h after allergen challenge, a time point when extensive edematous signals are found in the lung [31,34]. Treatment with budesonide accelerates the rate of resolution of the MRI signal. Interestingly, BAL fluid analysis does not reveal the rapid resolution of edematous signal. Thus, MRI provides an opportunity for the non-invasive assessment of rapid effects of anti-inflammatory compounds on established inflammation in the lungs, information that is not available from conventional BAL fluid analysis.

Cardiac disorders

Owing to its high spatial resolution and contrast capabilities, as well as the absence of alternative non-invasive approaches to assess structure and function of the heart in small rodents, MRI is an ideal tool for cardiac studies in animal models. The experimental conditions for carrying out such studies in rats and mice have been discussed extensively [35]. There are important issues concerning the high heart rate in small rodents (~300 beats/min for rats and 600 beats/min for mice), requiring synchronization of MRI data acquisition, and the respiratory motion of the thoracic structures. Parameters that are assessed by MRI comprise structural measures such as wall thickness and left and right ventricular volumes, as well as dynamic parameters such as stroke volume and ejection fraction [36]. Furthermore, knowledge of arterial blood pressure, systolic wall thickness and ventricular radius allows estimation of the myocardial wall tension [37,38]. Tagging techniques have also been implemented in small rodents for detailed analysis of the myocardial wall motion [39].

MRI has been applied to characterize pathological conditions of the rodent heart such as myocardial hypertrophy [36,38] or infarction [37]. Pharmacological studies in rats and mice involving MRI addressed the effects of angiotensin-converting enzyme inhibition on functional parameters of the heart [37] and the cardioprotective properties of anti-anginal drugs [40], among others. The technique has also been successfully applied to characterize transgenic models of cardiovascular disorders [41].

Oncology

Because of its capacity to accurately measure tumor growth in longitudinal studies, to develop and validate new animal models of cancer and monitor the effects of therapy in these models, MRI is considered as a key technique in oncology research [42]. Issues addressed include the early

detection of pre-cancerous tissue, the detailed characterization of cancerous tissue, the role of angiogenesis in tumor development and the mechanisms of tumor invasion and dissemination.

Angiogenic activity facilitates tumor perfusion and plays a crucial role in tumor growth and metastasis. Newly formed vessels are characterized by increased leakiness. Moreover, angiogenesis leads to a local increase of blood volume. Fractional tumor blood volume and vascular permeability, as assessed by dynamic contrast-enhanced MRI, have evolved into markers for angiogenesis. Such studies provide valuable insight into vascular development in a wide variety of tumor models and have been extensively applied to evaluate the effects of drugs on angiogenesis [43–45].

The response to chemotherapy and radiation can be significantly affected by blood flow and tumor oxygenation. Optimization of treatment regimens would profit from knowledge of these physiological parameters. An important technique used in this context is blood oxygen level dependent MRI, which has been successfully applied to assess tumor blood flow and oxygenation under a variety of conditions relevant to tumor therapy [46].

The use and development of contrast agents to help visualize tumors has been fostered by the challenge of detecting cancerous tissue at the earliest stage possible and to enhance the sensitivity and specificity of diagnosis. In many cases, these agents were developed to target tumors in specific organs. For example, iron oxide nanoparticles have been used to visualize lymph node metastases [47], to characterize microvessels in experimental breast tumors [48], to differentiate non-metastatic from metastatic rodent prostate tumors [49], to detect activated microglia in experimental gliomas [50] and, when conjugated to streptavidin, to target specific receptors in breast cancer cells [51]. Moreover, macromolecular contrast agents are being developed to improve the differentiation between highly permeable tumor neovasculature and normal vessels [52].

Clinical trials

Cutting edge drug research has a pressing need for early pharmacodynamic studies in humans. These proof-of-concept studies aim at validating the pharmacological and therapeutic principle in patients and at guiding the dose selection for extensive and expensive clinical efficacy trials. Evidently, having the same technology available for drug testing at the pre-clinical and clinical levels increases the information content regarding compounds. Here, we address how the information derived from MRI studies in animals has been translated into the clinic.

Degenerative joint diseases
Measures such as clinical status and serum markers of inflammation are currently used to assess severity of the disease and response to therapy in RA patients. The main drawbacks of these measures are their subjective character, difficult standardization and the fact that they do not provide a direct measure of inflammation within the synovial tissue. Synovial biopsy could be an alternative, however, it is too invasive for use in longitudinal studies. A non-invasive imaging modality for monitoring synovitis is therefore of great interest.

In early RA, synovitis is the primary abnormality, whereas bone damage occurs in proportion to the level of synovitis [53]. Dynamic gadolinium-enhanced MRI, which measures the leakiness of vessels, has been used to assess synovitis in clinical trials [54]. Alternatively, MRI-based measurement of bone marrow edema and synovial membrane volumes has also served as markers for disease activity [55].

Although validated approaches exist for the characterization of RA, the situation is less straightforward in OA. MRI has contributed little to drug development in this area to date, partly because pain rather than functional disability is the main clinical outcome that regulatory agencies insist on for treating OA. Proper surrogate markers for OA still need to be found. Cartilage loss, for example, provides a small dynamic range – changes in cartilage volume as measured by MRI do not exceed 5% per year [56]. One alternative could be the determination of PG loss using Gd-(DTPA)$^{2-}$-enhanced MRI. In support of this approach, measurements carried out on normal volunteers and late stage OA patients successfully showed a good correlation between cartilage T_1 relaxation in the presence of Gd-(DTPA)$^{2-}$ and *ex vivo* histological data [57].

Stroke
All MRI techniques used in pre-clinical studies involving stroke models are also available for clinical studies and have been extensively used for early diagnosis and to characterize cytoprotective drugs [58]. Despite this, during the last decade, MRI has not contributed to the development of cytoprotective therapy following acute stroke. The only accepted therapy is vessel recanalization using recombinant tissue plasminogen activator, which is amenable only to a small subpopulation of patients.

Stroke patients display considerable heterogeneity in location and severity of cerebral infarction. Clinical drug trials would benefit from stratification of patients by applying structural and functional imaging. Despite the discouraging results in the development of an effective treatment for cerebral infarction, imaging methods, in

particular MRI, will play a central role in the management of patients suffering from stroke and in the evaluation of novel therapeutic concepts.

Diseases of the airways

Spirometry is a standard, cheap and simple technique to obtain information about the global functional status of the lung. Alternatively, hyperpolarized gas [59] and oxygen-enhanced [60] MRI methods allow the quantitative assessment of lung ventilation at high spatial resolution. It remains to be determined to what extent such advanced imaging methods provide additional information relevant for pharmacological drug evaluation as compared with spirometry. Considering the cost of hyperpolarized gases, and the still questionable added benefit of MRI in this area, there will be hesitance to incorporate this approach into large clinical drug studies. Nevertheless, MRI ventilation techniques could play an important role in proof-of-concept studies.

Cardiovascular diseases

In cardiology, MRI has to compete with ultrasound, a well-established and cheap imaging technology that has been thoroughly validated in studies involving exercise and pharmacological stress paradigms. Significant improvements have been made recently on real-time 3D-echocardiography for quantitative assessment of cardiac volumes, ventricular mass and myocardium with contraction and/or perfusion abnormalities [61]. Thus, it is not surprising that the number of drug trials involving MRI has been quite limited up to now.

Cine MRI has been used to characterize the effects of anti-hypertension drugs in patients with chronic heart failure [62,63]. In addition, the effects of compounds on myocardial contractility have been assessed by tagging MRI [63]. Through its ability to determine plaque morphology, MRI might provide noninvasive surrogates of atherosclerosis [64]. Whereas most of the standard imaging techniques identify luminal diameter stenosis, wall thickness and plaque volume, MRI could provide additional information on plaque composition (lipid, fibrous, calcium, thrombus), indicative of high-risk unstable plaques. Such readouts might help to select high-risk individuals to participate in drug trials. Finally, MRI techniques have been recently developed to identify early changes related to new vessel growth [65]. They include measurement of blood delivery to the myocardium, development of intramyocardial vasculature and incremental changes in regional myocardial contractile function. With these methods, it is expected that key steps of angiogenesis and the efficacy of angiogenic therapies can be tracked *in vivo*.

Oncology

Histochemical analysis of tissue biopsies is a way of characterizing the tumor type and stage in patients. Several MRI techniques are being explored as non-invasive alternatives to histology in clinical studies. Dynamic contrast-enhanced MRI has been used in trials of drugs aimed at slowing down tumor progression [66]. The method has also been applied to assess tumor oxygenation [67], which might become an important biomarker. In addition, detection of metastases and primary tumors through, for example, the administration of iron oxide nanoparticles, is an important contribution that MRI could make to clinical trials [68]. The diagnostic accuracy and specificity of a therapeutic approach could be further enhanced by developing probes and drug delivery devices that selectively bind to tumor cells. For example, the effects of motexafin gadolinium, an MRI-detectable redox mediator that selectively targets tumor cells and enhances the effect of radiation therapy, on brain metastases has been recently evaluated [69].

Final remarks

A major advantage of employing non-invasive technologies in animal research is the possibility of directly linking pre-clinical to clinical findings through relevant imaging biomarkers. Nonetheless, most clinical drug studies involving MRI still need extensive validation to be accepted by regulatory agencies. Thus, pre-clinical studies involving MR-derived biomarkers are going to play a fundamental role in the validation and optimization of clinical protocols. Building a bridge between the two intimately connected, although in practice often too distant, areas of pre-clinical and clinical research should ultimately be one of the main contributions of MRI to pharmaceutical research.

A comprehensive appraisal of the real impact of the technique in this area is still lacking. In pre-clinical research, the major interest resides in applications to chronic models of diseases and in deriving functional information. In clinical applications, non-invasively assessing biomarkers or surrogates could significantly reduce the duration of a trial and/or the number of individuals necessary to determine drug efficacy. Therefore, although being inherently expensive, MRI might eventually lead to cost savings. In the years to come, we are going to witness an increasing use of MRI and other imaging techniques in the drug research and development process.

Acknowledgements

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