Molecular imaging approaches

how can it help drug development?

The current challenges facing drug discovery and development in terms of attrition are well documented. This article discusses how molecular imaging approaches from bench to bedside can not only streamline drug development, but also open up new opportunities in the treatment management of targeted therapies.

By Dr Susanta K. Sarkar

High attrition rates, particularly at the late stage of drug development, is a major challenge faced by the entire pharmaceutical community. The average success rate from first in man to registration for all therapeutic areas combined is 11%[1]. For oncology, this is even lower at 5%. Approximately 59% of all oncology compounds that enter in Phase III of development undergo attrition[1]. In fact, the estimated cost of bringing a potential drug to the market has increased significantly and at the current cost growth rate the projected cost for a new drug approval (assuming the R&D was initiated in 2001) is $1.9 billion in 2013[2]. It is therefore critical to develop and implement strategies and technologies that reduce these attrition rates to improve the efficiency of the overall drug development process.

In recent years an increased understanding of the disease biology has led to the development of therapeutics targeted towards specific pathways. For example, a number of targeted agents in oncology, including angiogenesis inhibitors and signal transduction inhibitors, have been under clinical development. The optimal evaluation of these novel molecularly targeted agents would require a shift in the current drug development paradigm. For example, it would be important to show in a patient population whether the particular target is relevant to the growth of that specific tumour and whether the drug is interacting with its intended molecular target. It would also be beneficial to identify a biologically optimal dose of the drug based on its effect on the target in the same patient population. Molecular imaging approaches can provide quantitative repetitive non-invasive measures of the assessment of molecular targets and its interaction with drug molecules *in vivo*[3,4]. Therefore proper development of these approaches will allow better understanding of a particular drug's interaction with its target in patients and thus aid in determining the mechanisms of sensitivity and resistance of the drug and its optimal dosing and scheduling.

Molecular imaging approach

Molecular imaging allows for non-invasive four-dimensional (3-D spatial information over time) visualisation and quantitative characterisation of biological processes in intact living subjects. In this

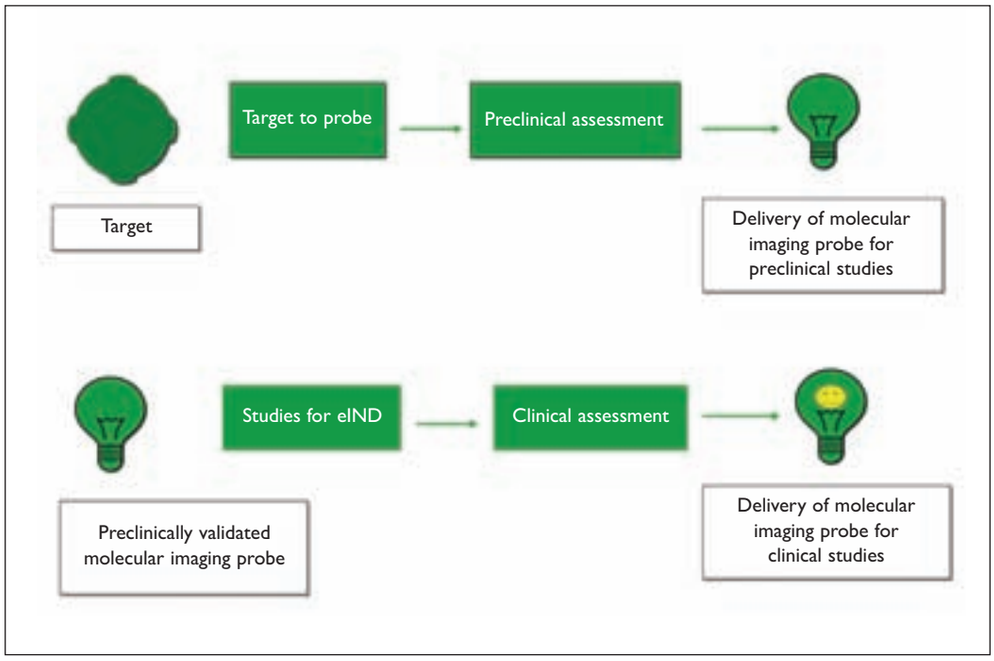

Figure 1
Schematics of the steps for developing a molecular imaging probe specific to a target

approach a vast array of imaging technologies such as Magnetic Resonance Imaging (MRI), Positron Emission Tomography (PET), Single Photon Emission Computed Tomography (SPECT), ultrasound and optical imaging are utilised either in a single or multi-modality fashion. Several excellent descriptions of these technologies are available[5-8]; a brief outline of the principles of these imaging technologies is summarised below.

Magnetic Resonance Imaging (MRI)
In magnetic resonance, the magnetic properties of the nuclei-like protons of tissue water are exploited. The application of a radiofrequency pulse (RF) to a subject in a uniform magnetic field excites the nuclear spins of the atoms to higher energy levels. Through relaxation mechanisms these excited nuclear spins return back to their initial energy levels and in the process emit RF signals. These signals are then detected and processed to generate MR spectra or an image. In MR spectroscopy, these processed MR signals are plotted against a frequency scale to help identify the chemical entities giving out the specific signals. In MR imaging, the MR signals are acquired in the presence of additional linear magnetic field gradients to encode spatial information thus providing images. In both MR spectroscopy and MRI the signal intensity is directly proportional to the concentration of the specific nuclear spins in the sample and their chemical and magnetic environment.

Positron Emission Tomography (PET)
Here a positron emitting radiotracer like ^{11}C or ^{18}F labelled chemical entity is injected into the body. The emission of positrons results in annihilation photons produced by the positron-electron annihilation process. Each positron decay and annihilation produces two photons. These two photons are of high energy (511keV) and are emitted ~180° apart. In PET, this property of co linearity is exploited to provide the spatial information. Several million of these photon pairs are detected and a corresponding image (distribution of the radiotracer activity) is reconstructed by processing these signals to generate the PET image.

Single Photon Emission Computed Tomography (SPECT)
In SPECT a gamma-emitting radiotracer is injected into the body and the emission of gamma photons are detected by a gamma camera and the distribution of this radiotracer activity within the body is reconstructed as a 3D image.

Computed Tomography (CT)
The principle of CT is based on the fact that x-rays are attenuated to different extent as it passes through the tissues, depending on its composition. These tissue specific absorption coefficients are collected as a set of projection images and their distribution is then reconstructed to generate a CT image.

Optical imaging

Here a fluorescent or bioluminescent probe is administered systemically into the body and the optical signals are then detected and reconstructed to generate the image. Bioluminescence imaging is mostly applicable for near surface imaging of tissues and fluorescent imaging, at near IR frequency, can be applied for deep tissue imaging.

Ultrasound

Here sound waves, generated by the transducer, are propagated into the body and are reflected by different tissues to different extent guided by their acoustic impedance. The corresponding echoes generated are then transformed into an image.

Imaging target and its response to drug

For successful development of targeted therapeutics, it is important to establish that a specific molecular target is associated with the particular disease and that the drug is actually interacting with its intended target. Molecular imaging approaches allow for non-invasive visualisation of the target and quantitative assessment of the functional consequences of its interaction with a drug through the development of molecular imaging probes specific to a particular target.

Molecular imaging probe is a chemical or biological entity with a reporter moiety that can be detected by a particular imaging technique eg, ^{99m}Tc labelled reporter for SPECT imaging, ^{18}F/^{11}C labelled reporter for PET imaging, fluorescence dye labelled reporter for optical imaging and paramagnetic material (eg Gd) labelled reporter for MRI. The development of these probes essentially requires a process similar to drug discovery and the steps involved are outlined in **Figure 1**.

In recent years, there have been tremendous efforts in developing molecular imaging probes directed against several specific targets[3,4]. Vascular endothelial growth factors (VEGF) and its receptors (VEGFRs) drive angiogenesis and are the focus of major drug development efforts, such as antibodies against VEGF and VEGFRs, and small molecule inhibitors of VEGFR tyrosine kinase, designed to selectively inhibit this pathway[9]. Therefore development of a molecular imaging probe that binds to VEGFR will allow for direct assessment of the target and its response to the drug under development and thus can be very helpful for the successful development of these agents. Recently a single chain VEGF composed of two fused 3-112 amino acid fragments of VEGF121 and an N-terminal 15 amino acid Cys-tag, with unique residues for site specific conjugation of various imaging reporters, have been developed for assessing VEGFR expression[10]. C4-thiol group in Cys-tag of scVEGF was directly labelled with ^{99m}Tc to generate a stable molecular imaging probe, scVEGF/^{99m}Tc for SPECT imaging. This probe rapidly binds to and is internalised by VEGF receptors and thus can be detected using SPECT imaging, providing information on prevalence of VEGFR in tumour vasculature in various tumour models non-invasively[10].

We have used this molecular imaging probe to assess VEGFR expression and its response to the treatment of pazopanib, a small molecule tyrosine kinase inhibitor (targeting VEGFR, PDFR and cKit) currently under clinical development[11]. The results are shown below in **Figure 2**.

Table 1: Surrogate imaging methods

TECHNIQUES	MEASUREMENTS
Dynamic Contrast Enhanced (DCE) MRI	Perfusion/blood flow/permeability
Diffusion MRI (DWI)	Loss of cell membrane integrity/changes in cellularity
Structural and functional MRI	Morphology/blood flow and volume/mechanical characteristics of vessels
^{18}F- labelled 2-deoxy-D-glucose ([^{18}F]FDG) PET	Tumour metabolism
^{18}F- labelled thymidine analogue 3'-deoxy -3'-fluorothymidine ([^{18}F] FLT) PET	Tumour cell proliferation
Ultrasound and Perfusion CT	Perfusion/blood flow

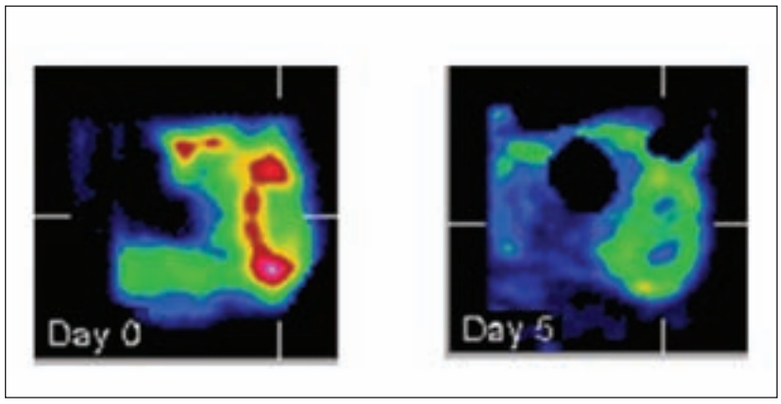

Figure 2
Pazopanib treatment affects scVEGF/^{99m}Tc uptake. Representative SPECT images of the HT29 human colon tumour xenografts from a mouse before (A) and after treatment with pazopanib for five days (B)

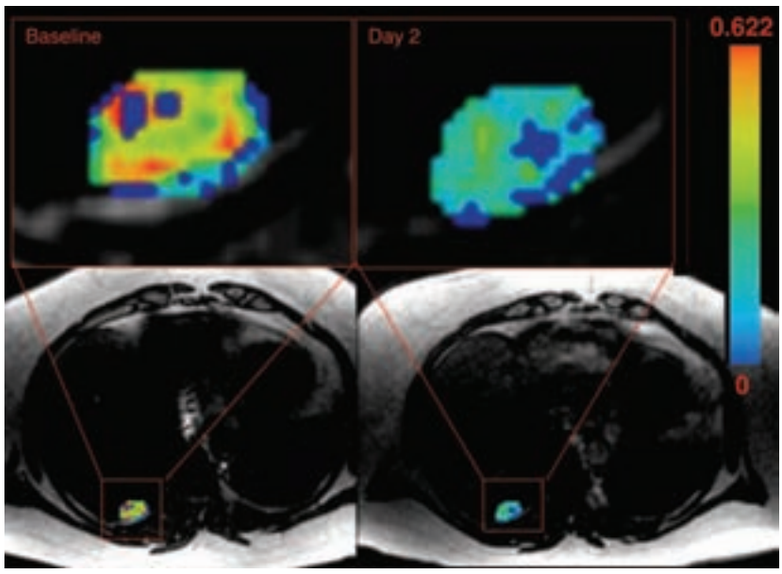

Figure 3
DCE MRI showing a decline in perfusion in a pulmonary metastasis in a patient with renal cell carcinoma (baseline and 2 days after treatment with AG-013736). *[Reproduced, with permission, from J. Clin. Onc. 23, 5464-5473, 2005]*

Our results suggest that in HT29 human colon tumour xenograft model, pazopanib induces a rapid decrease in the scVEGF/^{99m}Tc uptake. Immuno-histochemical analyses of VEGFR-2 and pan-endothelial CD31 markers suggest that the decrease in scVEGF/^{99m}Tc uptake is consistent with a significant depletion of CD31+/VEGFR-2+ endothelial cells in tumour vasculature. This approach allows direct real time evaluation of the complex dynamics of VEGFR expression in the tumour in response to a VEGFR targeted therapy. A clinical extension of this approach will provide new opportunities for treatment management of VEGFR targeted therapies including patient selection and dose optimisation.

Imaging surrogate markers of drug efficacy

When target specific molecular imaging probes are not available, a range of imaging techniques can be applied to monitor drug action by exploiting either intrinsic tissue properties or by administration of imaging agents specific to certain biological processes, eg, metabolic pathways. This is summarised in **Table 1**.

Some examples of surrogate imaging methods and their utility in monitoring the efficacy of drugs are illustrated below.

DCE MRI: tumour response

Angiogenesis, a biological process to form new blood vessels from existing vasculature, is essential for tumour growth and progression[12]. In recent years a significant effort has been devoted to develop effective anti-angiogenesis drugs[9]. As a result there is a need for the development of non-invasive measures to assess the response of these drugs. Dynamic Contrast Enhanced (DCE) MRI provides a convenient means to characterise vascular function, an important parameter to assess tumour response to treatment with anti-angiogenic therapy. In DCE MRI, a contrast agent is administered intravenously that is distributed in tumour tissues and an analysis of the signal intensities from the images provides a measure of the tumour perfusion and microvascular permeability. A number of studies have recently been performed in tumour xenograft models and in patients to monitor changes in vascular parameters as a measure of drug efficacy[13-15]. An example of such a measurement to monitor the effect of AG-013736, in a pulmonary metastasis in patients with renal cell carcinoma is shown in **Figure 3**[15]. A decrease

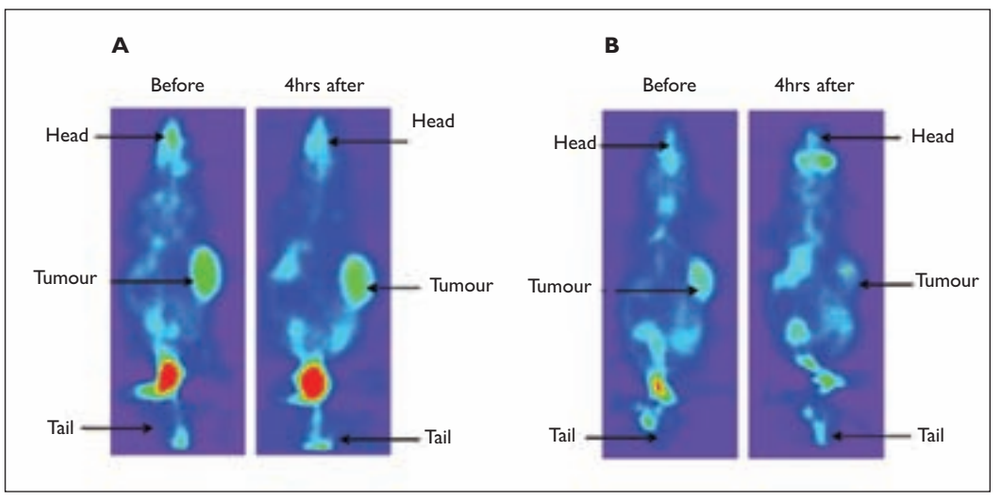

Figure 4
[^{18}F]FDG PET shows inhibition of [^{18}F]FDG uptake by an AKT inhibitor. (A) Representative [^{18}F]FDG PET image of the BT474 human breast tumour xenograft from a control mouse before and four hours after treatment with a vehicle. (B) Representative [^{18}F]FDG PET image of the BT474 human breast tumour xenograft from a mouse before and four hours after treatment with an AKT inhibitor

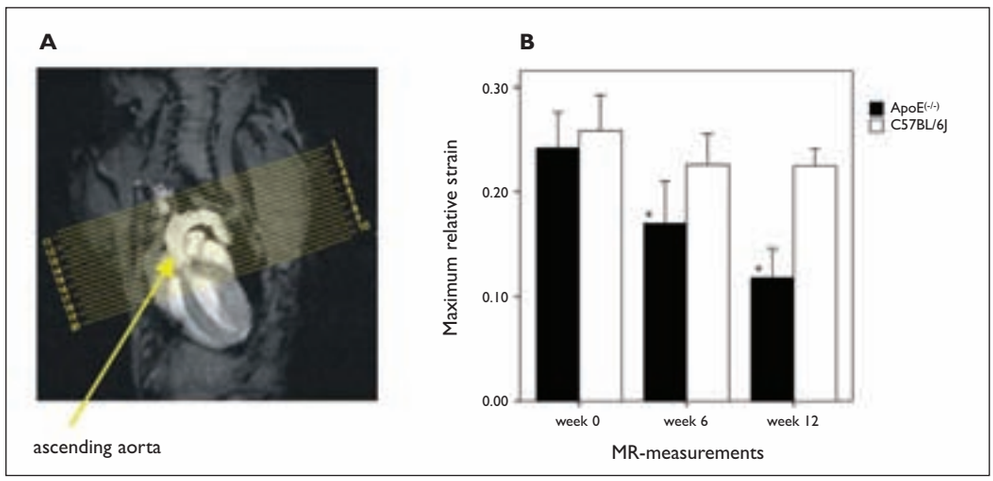

Figure 5: MRI measurement shows decrease in circumferential strain of the ascending aorta in ApoE-/- mice as the atherosclerotic plaque develops. (A) 17.6 T MR image of a mouse heart. (B) Group average of maximal circumferential strain calculated from the ascending aorta of apoE-/- and C57B/6J control mice following 0, 6, and 12 weeks of a high fat diet. *[Reproduced, with permission, from Magn. Reson. Mater. Phy. 22, 159-166, 2009]*

in the perfusion of the tumour after two days of treatment with the drug is clearly visible.

[^{18}F]FDG PET: tumour response

In [^{18}F]FDG PET, ^{18}F-labelled 2-deoxy-D-glucose is administered and it gets phosphorylated to FDG-6-phosphate intracellularly by hexokinase. Unlike glucose it does not undergo further glycolysis and is effectively trapped inside cells and thus can be measured using PET. This property has led to the wide use of [^{18}F]FDG PET to monitor tumour metabolism as a surrogate marker for drug efficacy in a variety of cancers[6,14]. It has been increasingly used to assess tumour response to specific drugs and several studies have shown its potential utility as an early efficacy marker[4,16]. [^{18}F]FDG PET has recently been used to monitor efficacy of drugs in mouse tumour xenograft models as well[17]. **Figure 4** shows the inhibition of [^{18}F] FDG accumulation due to the treatment with an AKT inhibitor in a mouse tumour xenograft model. These changes in [^{18}F]FDG accumulation occurred earlier than tumour volume changes.

Structural and functional MRI: atherosclerosis

Atherosclerosis plaque formation involves a cascade of inflammatory processes originating with mononuclear cells adhering to the endothelial surface and evolving to a mass of fibrous capped layers of lipid filled macrophages[18]. Over the course of this evolution, the biochemical changes that occur within the lesion influence the mechanical characteristics of the surrounding vasculature. Since mechanical properties of the vasculature are thought to influence the pathogenesis of cardiovascular disease, there is a need to investigate how functional metrices such as aortic distensibility change as atherosclerotic plaque evolves. MRI can provide functional measurements like vascular elasticity. We have recently performed a high field (17.6 T) MRI study to analyse the influence of atherosclerotic plaque development on the morphological and mechanical characteristics of the aortic vessel

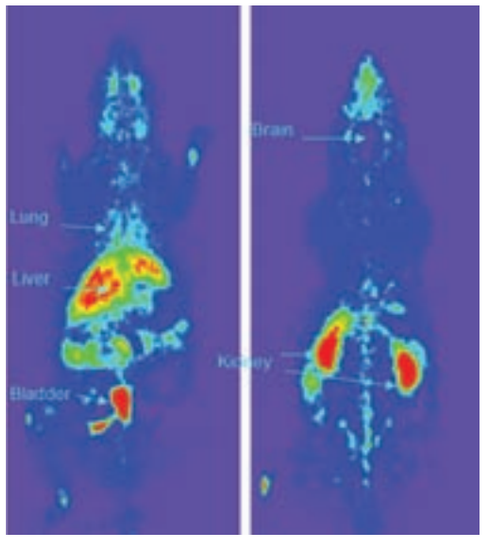

Figure 6: PET image showing the distribution of a drug, labelled with [^{11}C], through different tissues in a live rat. Two separate coronal sections from the same 3-D image are shown to highlight different organs

References

1 Kola, I and Landis, J. Can the pharmaceutical industry reduce attrition rates. Nature Reviews Drug Discovery, 2004, 3, 711-715.

2 DiMasi, JA et al The price of innovation: new estimates of drug development costs. J. Health Econ, 2003, 2, 151-185.

3 Weissleder, R and Pittet, MJ. Imaging in the era of molecular oncology, Nature, 2008, 452, 580-589.

4 Willmann JK et al. Molecular Imaging in drug development, Nature Reviews: Drug Discovery, 2008, 7, 591-607.

5 Mansfield, P and Morris, PG. NMR Imaging in Biomedicine, Advances in Magnetic Resonance, Eds, J.S. Waugh, Academic Press, 1982.

6 Phelps, ME. PET: Molecular imaging and its biological applications, Springer, 2004.

7 Bushberg, JT et al eds. The essential physics of medical imaging, Lippincott, Williams, Wilkins, 2001.

8 Chance, B. Optical Methods, Ann. Rev. Biophys. Biophys. Chem, 1991, 20, 1-28.

9 Hayden, EC. Nature, 2009, 458, 686-687.

10 Backer, MV et al. Molecular Imaging of VEGF receptors in angiogenic vasculature with single chain VEGF-based probes, Nature Medicine, 13, 504-509.

11 Blankenberg, FG et al. Non-invasive assessment of tumor VEGF receptors in response to treatment with pazopanib: A molecular imaging study, Translational Oncology, 2010, 3, 000-000.

12 Bergers, G and Benjamin, LE. Tumorigenesis and the angiogenic switch, Nat. Rev. Cancer, 2003, 2, 795-794.

13 Gillies, RJ et al. Applications of Magnetic Resonance in Model systems: tumor biology and physiology, Neoplasia, 2000, 139-151.

14 O'Connor, JPB et al. Quantitative imaging biomarkers in the clinical development of targeted therapeutics; current and future prospectives, The Lancet, 2008, 766-776.

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15 Liu, G et al, Dynamic contrast-enhanced magnetic resonance imaging as a pharmacodynamic measure of response after acute dosing of AG-013736, an oral angiogenesis inhibitor, in patients with advanced solid tumors; results from a phase I study, J. Clin. Oncology, 2005, 23, 5464-5473.
16 Van den Abbeele, AD and Badawi, RD. Use of positron emission tomography in oncology and its potential role to assess response of imatinib mesylate therapy in gastrointestinal stromal tumors (GISTs), Eur. J. Cancer, 2002, 38 (suppl 5), 60-65.
17 Tseng, JR et al. Preclinical efficacy of the c-Met inhibitor CE-355621 in a U87 MG mouse xenograft model evaluated by ^{18}F-FDG small-animal PET, J. Nucl. Med., 2008, 49, 129-134.
18 Libby, P. Inflammation in atherosclerosis, Nature, 2002, 420, 868-874.
19 Herold, V et al. In vivo comparison of atherosclerotic plaque progression with vessel wall strain and blood flow velocity in apoE-/- mice with MR microscopy at 17.6 T., Magn,. Reson. Mater. Phy., 2009, 22, 159-166.
20 Lee, JMS et al. Multi-modal magnetic resonance imaging quantifies atherosclerosis and vascular dysfunction in patients with type 2 diabetes mellitus, Diab. Vasc. Dis. Res., 2007, 4, 44-48.
21 Hode, Y et al. A positron emission tomography (PET) study of cerebral dopamine D2 and serotonie-5HT2A receptor occupancy in patients treated with cyamemazine (Tercian). Psychopharmacology (Berl.), 2005, 180, 377-384.
22 Fischman, A et al. Pharmacokinetic imaging: a non-invasive method for determining drug distribution and action, Clin. Pharmacokinet., 2002, 41, 581-602.

wall in a pre-clinical mouse model of atherosclerosis[19]. An image of a live mouse heart along with the calculated circumferential strain, as a measure of distensibility, of the thoracic aorta of ApoE(-/-) knock out and control group of mice as a function of time is shown in **Figure 5**. It is clear from the figure that the circumferential strain is significantly decreased in apoE (-/-) knock out mice compared to that in control animals over 12 weeks, as the plaque is developed.

Similar distensibility measurements using MRI have also been performed in clinic[20] and may be useful for treatment monitoring.

Imaging drug pharmacokinetics and biodistribution

In order for the efficient development of molecularly targeted agents it is critical to know if the drug reaches the intended target and if the optimal biological dose is defined in patients. PET has been increasingly used to measure the level of occupancy of a receptor to determine the effective dosage of a drug for neurological applications[21]. This is usually achieved by analysing the inhibition of the binding of a well characterised radiolabelled ligand (^{11}C or ^{18}F-labelled chemical compound) to a specific receptor by an unlabelled new drug.

Plasma levels of drugs may not necessarily reflect target tissue concentration and therefore it is important to demonstrate that the drug reaches the target tissue and a thorough understanding of target tissue (eg, tumour) drug concentration and its relationship to efficacy will help design the most appropriate dosing and scheduling for clinical trials. Maximum Tolerated Dose (MTD) based dosing may not be appropriate for targeted therapy; long durations of treatment may result in multiple off target adverse events. The physicochemical properties of chemical compounds are not altered by direct labeling them with ^{11}C or ^{18}F and thus wherever possible, this approach can be used to radiolabel the drug for biodistribution studies in patients using PET[22]. The biodistribution and pharmacokinetics of drugs in preclinical species are measured by blood and tissue sampling or autoradiography; however, it is possible to utilise PET for preclinical studies as well. **Figure 6** shows the biodistribution of a drug (radiolabelled with ^{11}C), as measured by micro-PET, through different parts of the body in a live rat.

Conclusion

Molecular imaging approaches provide a unique opportunity to address the current challenges of attrition faced by drug discovery and development. The development of appropriate methodologies for repetitive quantitative assessment of a drug's molecular target, its therapeutic inhibition, and early biological assessment of therapy success or failure will provide a new paradigm for drug development. This will require successful development of molecular imaging probes specific to a molecular target, proper validation of surrogate imaging markers and optimisation of multi-modal imaging methodologies. Since the imaging technologies span from preclinical to clinical domain, a successful integration of molecular imaging approaches from bench to bedside would not only streamline the drug development process but also allow for new opportunities in treatment management of targeted therapies including patient selection, dose optimisation and optimal drug regimen. DDW

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