Imaging the Future

Advances in translational imaging are furthering the discovery and optimisation of tomorrow's therapeutics. Careful integration of existing imaging technologies can help to quantify the critical properties and effects of new medicines, meaning patient risk can be mitigated and benefits maximised

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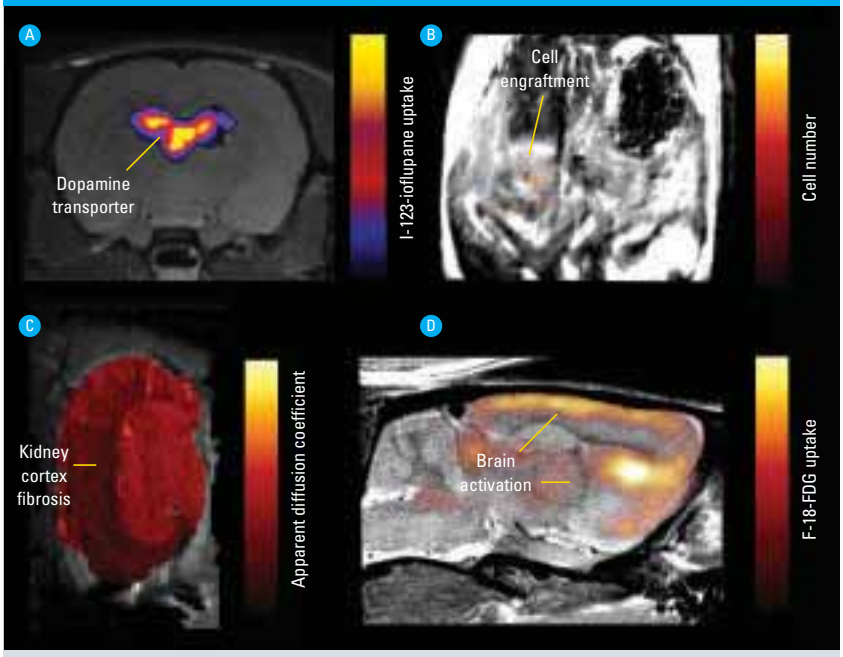
In the 1970s and 1980s, non-invasive medical imaging technologies revolutionised healthcare and medical practice by mapping anatomical structure and disease-based abnormalities throughout the human body, without any need for an intrusive procedure. Today, imaging modalities – including magnetic resonance imaging (MRI), computed tomography (CT), positron emission tomography (PET), single-photon emission computed tomography (SPECT) and ultrasound – are household terms, and the signature two- and three- dimensional (3D) images from these are recognisable to most people. Many of us will directly benefit from medical imaging in our lives.

Very quickly, these technologies have become critical for diagnosis across numerous major diseases afflicting humans. Furthermore, since around the 1990s, the same technologies have enabled a variety of so-called functional or physiological imaging readouts that compliment the imaging of anatomy. These physiological end-points are collectively a broad and powerful toolkit for clinicians and medical researchers that lead to 3D images not just of anatomy, but of important parameters or biomarkers that indicate the severity of a particular condition and, hopefully, the disease response to a prescribed treatment.

In parallel with advances in non-invasive imaging biomarkers, the pharmaceutical industry has found a need for these same imaging technologies. One of the key drivers for drug discovery using non-invasive imaging has been the development of preclinical imaging technologies that can be used in animal models of human disease. These models form the basis for pharmaceutical discovery prior to, and increasingly in conjunction with, clinical trials. This has brought forward the term 'translational imaging', which describes the fact that the same technologies and biomarkers available to clinical imaging can be used in preclinical research to validate or optimise the intended use in clinical practice.

Translational imaging biomarkers have led to substantive advances in clinical care and drug discovery by providing quantitative and highly predictive data that can be used to assess disease progression and therapeutic efficacy.

Figure 1: Examples of *in vivo* imaging-based quantification of function and biodistribution



A. SPECT imaging of the dopamine transporter (DAT) using the DAT binding I-123-ioflupane tracer in a rat's brain. The SPECT signal is shown as a colour overlay on a greyscale anatomical MRI scan. This can be used to image a critical aspect of Parkinson's disease (PD) and treatment response

B. Fluorine-19 (19F) MRI-based stem cell detection after labelling using the 19F Cell Sense reporter and administration of cells in a mouse model of intestinal inflammation. The 19F signal is overlaid on a greyscale 1H anatomical MRI scan. This can be used to determine cell engraftment patterns and kinetics of therapeutic cell transport and localisation

C. Diffusion MRI quantification of kidney fibrosis in the rat unilateral ureteral obstruction model of chronic kidney disease. An apparent diffusion coefficient (ADC) map is shown as a colour overlaid on a greyscale anatomical 1H MRI scan. The water ADC is a biomarker for tissue cellularity and fibrosis, and can be used to image fibrosis progression and treatment response

D. Fluorodeoxyglucose-PET (FDG-PET) imaging of glucose metabolism in the brain after haloperidol treatment, showing cortex and striatum hyper-metabolism/activation. The PET signal is shown as a colour overlay on a greyscale anatomical MRI scan. FDG-PET can be used to image central nervous system (CNS) drug effect and highlight affected brain regions

Imaging Reporter Example: I-123-Ioflupane SPECT Imaging of the Dopamine Transporter

I-123-ioflupane (DATscan) is a radiolabelled cocaine analogue that reversibly binds to the pre-synaptic DAT with high specificity (1). The I-123 isotope emits gamma energies that can be detected through SPECT imaging. This has led to approval and reimbursement for I-123-ioflupane SPECT brain imaging, in supporting PD diagnosis. PD is associated with loss of dopaminergic cells and, therefore, striatal dopamine.

However, as in the case of translational imaging biomarkers originally used for cancer diagnosis, I-123-ioflupane image-based biomarkers may provide a method not just for diagnosis, but also for assessing therapies. New research is, therefore, evaluating whether I-123-ioflupane SPECT imaging and quantification of striatal concentration could be used to quantify efficacy of new treatments that are designed to repair or reverse DAT loss in the striatum, as a treatment for PD. This would allow quantitative methods for considering PD drug or cell-based therapies in animal models, as well as in early clinical trials.

This article will examine several of the recent developments in medical imaging that are helping pave the way for tomorrow's therapeutics.

Imaging Reporters

The development of imaging reporter molecules or probes has been instrumental in the progress made to date. 'Imaging reporter' describes any molecule that can be detected by an imaging instrument, thereby modulating the contrast observed in the image, and providing a means to measure a disease-relevant parameter or biomarker. Imaging reporters comprise a number of strategies, including exogenous molecules that are systemically administered, cell-based reporters (in administered cells or transgenic models), and the quantification of endogenous molecules that can be detected through their inherent properties. These image-detectable reporters provide disease-specific information by:

- Direct imaging of the reporter biodistribution – for example, spatial resolution of image reporter-tagged cells or biologic molecules
- Targeting of the reporter to specific endogenous molecules or receptors – for example, PET or SPECT isotope-tagged, receptor-targeted ligands to enable imaging of receptor occupancy
- Localisation of the reporter in cells, with the localisation dependent on a molecular process – such as 18F-FDG cell glucose metabolism-dependent uptake
- Activation of the reporter from a quiescent state by particular molecules – for example, protease cleavage through caspase or cathepsin-activated reporters
- Physical location specificity of the reporter – like blood pooling agents that cannot escape vessels, enabling vessel mapping

Wider Focus

The power of translational imaging was first realised through detection of deep tissue tumours in cancer patients. Today, the cancer diagnostic applications of MRI, PET, CT and ultrasound are a mainstay of medical practice. The adoption of imaging technologies by the pharma industry was initially focused on oncology therapeutics. More recently, however, the diagnostic and prognostic application of imaging biomarkers has broadened into other major areas: for example, imaging applications in neurodegenerative diseases have become standard, and new applications in Alzheimer's disease, PD and stroke are particularly noteworthy. In addition, learning that a multitude of diseases – such as chronic kidney, pulmonary and liver diseases – drive tissue fibrosis, has led to development of imaging biomarkers for lung, kidney and liver fibrosis.

Small Molecules to Biologic Therapies

A further driver for the increasing role of imaging technologies in therapeutics research has been the shift in the last 10-20 years to biologic molecule and cell-based therapies. Tomorrow's cures and medicines hinge on the success of tailored antibodies, antibody fragments, proteins, peptides, antibody-drug conjugates, nanoparticles, genes and cells. This transition has been related to the ability to more precisely target biologic entities to diseased cells and tissues, in parallel with the ability to manufacture them.

The move has also necessitated the development of methods to detect

Ex-Cancer Imaging Example: Quantitative MRI of Kidney Fibrosis

Over the last decade, fibrosis has become a major target for the drug industry. A current unmet need in this area of medical research is for reliable, non-invasive and translational biomarkers for assessing fibrosis progression and related therapeutic interventions.

Non-invasive imaging is being used to fill this gap. In particular, MRI and CT – being highly sensitive to tissue structure and density – can provide quantitative readouts for fibrosis in organs, among them kidney, lung and liver. For example, kidney fibrosis is a complication that stems from a variety of common diseases including chronic kidney disease and lupus. The pathogenesis of chronic kidney disease is characterised by progressive decline of renal function and accumulation of extracellular matrix, which leads to a diffuse fibrosis.

While gross tissue density changes in kidney fibrosis can be subtle, MRI-based apparent water diffusion mapping – as shown in studies conducted by Togao and colleagues – can sensitively detect the presence of hypercellularity that progresses in the rodent unilateral ureteral obstruction surgery model of chronic kidney disease (2).

The work by Togao, as well as research using microCT quantification of pulmonary fibrosis by Scotton and his team (3), are excellent testimonies to the power of *in vivo* preclinical fibrosis imaging and the ability for non-invasive imaging biomarkers to facilitate discovery of future medicines. Importantly, these same biomarkers can be translated for clinical use, enabling rapid end-points that provide better disease and treatment specificity than traditional measures.

MRI-Based Cell Biodistribution Imaging Example

The promise of cell-based therapies – such as stem cell-based regenerative medicine – is stronger than ever, with the technology for isolation, *in vitro* expansion and storage/manufacturing of potentially therapeutic cells having advanced substantively over the last decade. There is now an expanding list of companies with commercial cell therapeutics. Furthermore, the advent of T cell-based therapeutics, including chimeric antigen receptor T cells against cancer, has acutely intensified the field of cell therapeutics (4).

One major challenge that may limit translation and approval of cell-based therapies is the ability to detect and track cells in the body after administration. This is critical for assessing properties comprising *in vivo* viability, and targeting the precision and tissue engraftment that drive efficacy, as well as for evaluating fate and the potential for off-target toxicity. Non-invasive imaging is addressing this hurdle through *in vitro* image reporter cell labelling and associated image-based tracking of administered cells.

Cell Detection

One example that has shown considerable promise is MRI cell detection via labelling using MRI-detectable particles, or gene reporters. MRI cell detection has typically been based on either super paramagnetic iron oxide (SPIO) that creates significant signal perturbation in proton MRI, or 19F-based detection using 19F MRI. 19F MRI-based cell biodistribution has the important benefit of the detected signal correlating directly with cell concentration, and this is facilitated by the lack of 19F background in the body – making the method highly exact. The first report demonstrating 19F MRI-based cell tracking in the clinic was recently published by Ahrens and colleagues (5). Methods based on cell-infecting organelles that create intracellular SPIO particles are also in development, with a key advantage being the ability for these organelles to replicate with cells, precluding dilution of the label as the therapeutic cells divide (6).

In all cases, therapeutic cells are cultured *in vitro* with SPIO, 19F reagents or organelles, and manipulated if needed to promote labelling. After confirming key properties are unchanged, the labelled cells can be detected using standard MRI protocols, leading to maps of cell biodistribution that can be overlaid on high-resolution anatomical images. These maps can be acquired repeatedly over time in order to quantify cell viability, targeting, tissue residence and engraftment, clearance pathways and kinetics, helping to ensure the success of future cell therapies.

the biodistribution and related kinetic and targeting precision of these biologics. Imaging methods are meeting this demand, as it has been broadly possible to label large biologic molecules, nanoparticles and cells with imaging reporters, without disrupting their critical properties and intended ability to combat disease. This labelling approach provides a powerful method to understand where, and over what time period, large therapeutic molecules and cells persist after administration in animal models or human patients.

Tomorrow's Capabilities

In summary, state-of-the-art imaging technologies are enabling discovery and optimisation of tomorrow's therapeutics. Today's imaging technologies enable a broad range of non-invasive and translational disease-specific biomarkers. Imaging reporter molecules have increased our ability to quantify molecular processes that critically underly disease progression, with high sensitivity and specificity.

Additionally, the successful translation of future biologics, cell and gene therapies depends heavily on technologies such as imaging, which can precisely detect and track the biodistribution of these therapeutics. Through careful integration of technologies that can quantify critical properties and effects of new medicines in discovery – for example, imaging readouts – next generation therapeutics can be optimised and selected in a way that mitigates patient risk and maximises benefit.

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