Preclinical imaging: improving translational power in oncology

Imaging has long been indispensable in clinical practice, and researchers have for many years used the same toolbox of imaging modalities as a component of their preclinical and drug development work. Imaging continues to provide crucial datasets to scientists in neurology, cardiology and metabolic disease, for example. However, in this article we focus on the impact preclinical imaging is having in oncology – strengthening the options for *in vivo* visualisation of cancer-related processes over time. Importantly, technological developments both in instrumentation and in probe synthesis and labelling have resulted in imaging systems with increased potential for basic research, as well as for translational and clinical applications. In addition, more sophisticated models are now available to address cancer-related research and therapeutic questions.

By Dr Todd Sasser

Initially using clinical-scale instrumentation, imaging provided a non-invasive means of assaying biological structure and function *in vivo*. The development of dedicated small animal imaging systems followed and, more recently, techniques in molecular imaging have been established to allow imaging modalities to be combined into multi-modal methods. Among these, the combination of positron emission tomography (PET) and computed tomography (CT) is a successful imaging strategy and has become an important tool in clinical practice. Technological approaches that combine magnetic resonance imaging (MRI), optical modalities and PET have now been introduced. PET/MRI and the resulting combination of molecular, morphological and functional information will pave the way for a better understanding of physiological and disease mechanisms in the preclinical setting (**Figure 1**).

A convincing model

Small animal imaging provides quantitative, spatially and temporally-indexed information on normal and diseased tissues such as tumours. Importantly, because of the non-invasive nature of the technique, imaging allows longitudinal (serial) study of animal models of human cancer.

This allows monitoring of disease progression from inception to progression, as well as of treatment options over a period of time. Each animal acts as its own control, reducing biovariability. Not only does this minimise the number of experimental animals required, it also gives results in real time. Moreover, in contrast to cell or tissue culture-based experiments, studies in intact animals incorporate the interacting physiological factors present in a complex living system. Looking forward, as drug development continues on the path towards personalised medicines, such

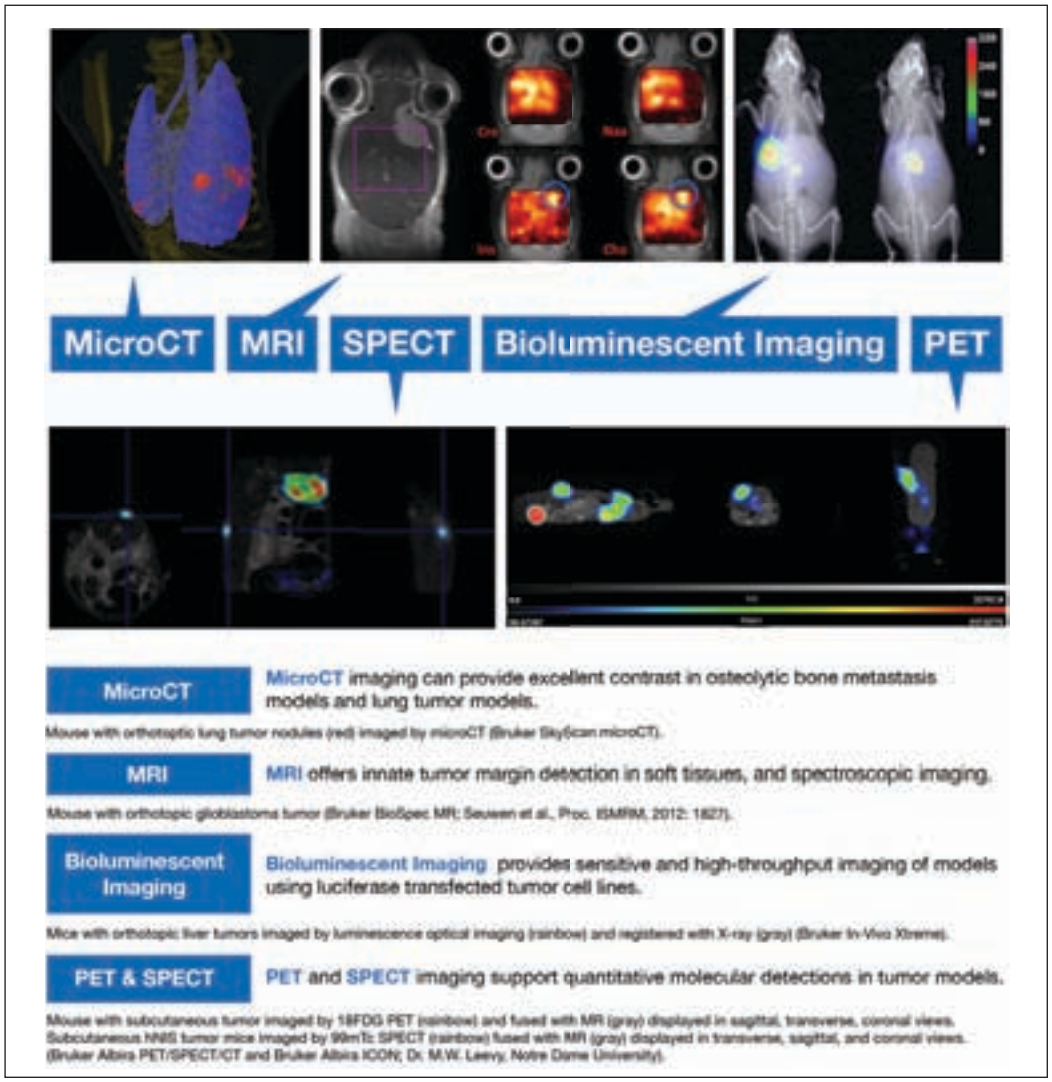

Figure 1
Overview of various types of currently commercially available *in vivo* imaging systems

longitudinal studies will offer scientists extremely valuable insights.

Advances in PET Technology

However, PET imaging is not without limitations, even with these advanced systems. Many available systems lack good spatial resolution[3]. Small anatomical structures cannot be distinguished or accurately analysed with low resolution PET. Additionally, PET imaging lacks anatomical context.

Technological advances have emerged to tackle these limitations, bringing significant improvements to the resolution issues faced by researchers. Using instruments that utilise innovative crystal technology and high sensitivity detectors, researchers can now obtain complete Full Field of View Accuracy (FFVA), which offers precise, homogenous sub-millimetre volumetric PET resolution in all three axes across the whole field of view. The latest breakthrough in PET detector technology enables rings to be located in-line with either MRI or CT, a design which also supports simultaneous PET and MR imaging. Advanced depth-of-interaction (DOI) detection enables precise 3D localisation of events without the constraint of having to work with conventional crystals configured in discrete layers. The result is the generation of an area of optimum resolution up to 10 times larger than conventional systems, providing unprecedented clarity.

This new technology has been successfully implemented into a tri-modal PET, SPECT, CT imaging system, and a bi-modal PET/MR system. Moreover, there is now the ability to transport a sedated subject to another instrument for further functional and/or anatomical study, for example to an MRI system. The use of multimodal animal

Imaging modalities explained

The most common preclinical modalities are PET, SPECT, CT, MRI and Optical imaging. Each of these offers distinct advantages, but arguably PET imaging is at the forefront of the revolution in functional imaging with direct potential for clinical translation. This is, in part, due to its ability to evaluate and quantify changes in drug biodistribution and pharmacokinetics, among others, which aid the assessment of drug efficacy[1]. The depth of imaging available by PET is another key strength of this technique, together with excellent temporal resolution[2]. In addition to these benefits, PET imaging is extremely sensitive to molecular details. PET imaging systems are becoming increasingly advanced and affordable, and multimodal imaging systems are now commonly found in large research laboratories, with functional modalities frequently combined with anatomical modalities for context.

PET – Positron Emission Tomography
A nuclear medicine, functional imaging technique that produces a highly quantitative 3D image of processes in the body.

SPECT – Single Photon Emission Computer Tomography
This method allows researchers to monitor the level of functional tracer activity at each place in the 3D region analysed.

CT – Computed Tomography
CT generates a 3D image of the inside of an object, from a series of 2D radiographic images taken around a single axis of rotation. CT provides innate contrast for skeletal, pulmonary and adipose tissues.

MR – Magnetic Resonance
MR generates an anatomical 3D image with excellent soft tissue contrast.

References

1 O'Farrall et al. Non-invasive molecular imaging for preclinical cancer therapeutic development. Br J Pharmacol 2013; 169(4): 719-735.
2 Massoud, TF, Gambhir, SS. Molecular imaging in living subjects: seeing fundamental biological processes in a new light. Gene Dev 2003; 17(5):545-80.
3 Wehrl, H et al. Preclinical and Translational PET/MR Imaging. J Nucl Med 2014; 55:11S-18S.
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beds removes any requirement to disturb the animal during study. This accurate positioning facilitates the layering of the three images to provide better understanding of the molecular mechanism or interaction of interest, with a correct anatomical reference. A further layer of imaging can therefore be added to the molecular visualisations achieved, with intelligent software ensuring precise automatic co-registration of images.

There is growing interest in combining PET imaging with MRI in this manner, as MRI provides superior soft tissue contrast, one of the key challenges for PET imaging. MRI techniques have also advanced with developments including a new 3T cryogen-free magnet, and now incorporate functions such as diffusion weighted imaging (DWI) that can be included into preclinical studies. These technology combinations are being applied in a number of clinical areas including oncology, neurology, cardiology and metabolic disease. In addition to cross-platform methodologies, integrated PET/MR solutions are employed to streamline workflows.

In practice – cross-platform MRI/PET or MRI/SPECT imaging and co-registration

To illustrate the power of multimodal imaging using cross-platforms, here we report results from a study that employed the Bruker Multimodal Animal Bed (MMAB)[4]. In this instance preclinical imaging was undertaken using PET or SPECT together with MRI.

To achieve cross-platform imaging, researchers frequently employ makeshift animal transports. While this approach is generally useful, there are typically limitations with animal care, anaesthesia, stable positioning and image registration between scans. Nelson et al (2011) reported on an immobilisation bed for cross-platform (PET/CT) imaging in a tumour xenograft model. Interestingly, when the immobilisation bed was used, inter-user variability for SUV analysis fell from 9.4% to 0.7%. This illustrates the importance of stable animal positioning and registration.

To ensure the highest quality results, scientists conducting this study employed MMAB which is equipped with a snug immobilisation shell that maintains the specimen animal positioning. Fiducial markers were employed for simple image registration.

This cross-platform imaging method was first tested for 18FDG-PET/MR using a Foxn1nu mouse without tumour grafting or other experimental treatments. Cross-platform PET/MR images were neatly registered using the MMAB solution (**Figure 2**).

Next, the protocol was evaluated for cross-platform 99mTc-SPECT/MR imaging in a HCT 116-hNIS-NEO tumour model. This imaging protocol and registration method resulted in excellent SPECT and MR tumour signal/contrast registration (**Figure 3**). These results provide for excellent tumour margin contrast with MRI and reliable cross-platform PET or SPECT registration. This protocol should also allow for accurate production of volume-of-interest (VOIs) based on tumour margins identified in MR images and application to functional PET or SPECT images.

Conclusion

It is apparent that there are considerable benefits of preclinical imaging – and the new PET technology in particular – aiding researchers as they seek to

translate their work from *in vivo* models into the clinical situation. The sub-millimetre spatial resolution now available is a key improvement in comparison with conventional imaging systems, which will allow researchers to produce much higher quality images for analysis. This is particularly valuable for scientists looking at small anatomical features.

In addition, the introduction of a multimodal animal platform allows for considerably better image co-registration when using cross-platform imaging protocols. This again, adds significantly to the quality of data now available.

There are further benefits in cross-platform imaging; it maximises access to equipment, and has the potential to minimise down-time for maintenance. But perhaps most importantly – given that

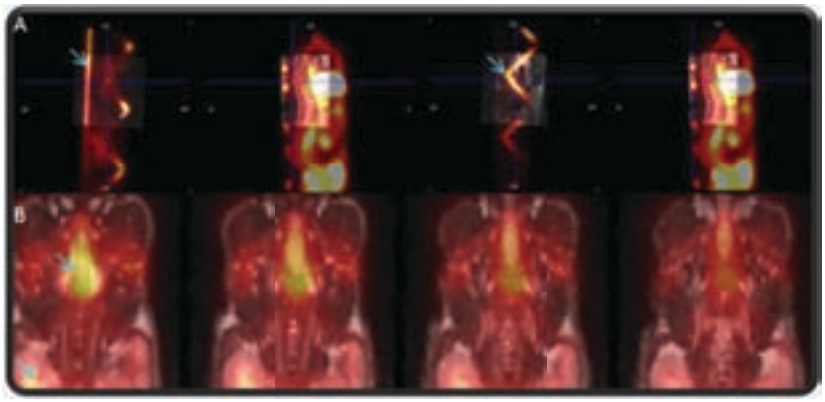

Figure 2: Cross platform 18FDG-PET/MR image registration of healthy mouse imaged using the MMAB. (A) Image registration between 18FDG-PET (fire) and MR (gray). Fiducial marker points used to confirm image registration are shown in two slices of coronal view. (B) Four slice sequence of 18FDG-PET/MR coronal view of hind region of mouse with kidney (lower left arrow) and spine (upper arrow) visible

Setting up a preclinical *in vivo* imaging laboratory

Considerations for fitting-out a preclinical imaging laboratory vary for single and multimodality facilities – by the choice of specific imaging systems, by the physical constraints of the space available and by the anticipated workflow of studies that will be made. For example, some instruments have specific requirements for room engineering, such as heat sinks and shielding etc, while institutional, local and national regulations on health and safety must be followed for systems that use or produce ionising radiation.

General requirements

Importantly though, there are a number of key considerations that are common across all preclinical laboratories, regardless of the specific modalities present. Solutions for animal welfare and effective transport must be taken into account for any *in vivo* imaging facility, while efficient access to the resulting data and tools to enable effective analysis are of prime importance to all laboratories. The growing importance of imaging techniques in the preclinical setting brings these considerations to the fore for laboratories seeking to maximise the value of these techniques to their research.

Animal transport

Access and transportation of animals into an imaging facility is an important consideration for any imaging laboratory, particularly where animals are to be transported from a dedicated animal housing facility that employs barrier and isolation policies. Many imaging laboratories designed for institute-wide use are established within the facility's animal housing area, which, to some extent, overcomes some of the barrier and transport logistical issues.

Even when animals remain housed within such a facility, however, solutions for isolating subjects during transportation and imaging may be required. SPF-compatible animal chambers equipped with high-efficiency particulate arrestance (HEPA) filters are ideal for such animal handling requirements. For transport to and from staging areas and the imaging systems, quick gas connectors to such chambers are a prerequisite.

An adjacent location for animal housing and preparation is of particular importance when using PET imaging. This room should have a dedicated 'hot' rack to store radioactive animals before and after PET acquisitions as well as a 'cold' rack to allow investigators to store their animals for the duration of the study. A biosafety cabinet should also be available for the investigators to perform animal care, small surgery or other preparation steps before imaging.

Increasingly, institutional imaging systems are installed in a centralised location, with multiple platforms available in a close proximity. Multimodal animal beds facilitate cross-platform imaging by ensuring accurate animal positioning throughout, and remove any requirement to disturb the animal during study.

Animal care

Animal monitoring and welfare are prime considerations for optical imaging research, particularly during longitudinal studies. Multimodal animal beds are designed to facilitate monitoring of respiratory and cardiac functions and body temperature, both during preparation and imaging.

Optimum configurations for delivery of gas anaesthesia for animal preparation and imaging, as well as end-user safety, should also be determined. Uniformity in animal treatment is critical when imaging *in vivo* bio-distribution and/or kinetic reactions, given that enzymatic reactions can be disrupted in hypoxic environments and variable anaesthetic conditions. The use of systems designed to distribute gas anaesthesia equally to each subject will ensure not only equivalent anaesthetic doses, but also similar oxygen (or air) flow rates.

Scavenging and ventilation solutions, storage and access of anaesthetic supplies that conform to regulatory and legal requirements, as

Setting up a preclinical *in vivo* imaging laboratory (continued)

well as proper mounting solutions for carrier gas (eg oxygen or medical air) cylinders should be included in the anaesthesia set-up design.

In addition, supporting laboratory infrastructure must be in place to ensure efficient workflows. For example, optical imaging reagents commonly require refrigeration and/or light protection prior to use, therefore many optical imaging laboratories include a small refrigerator/freezer so that *in vivo* fluorescent and luminescent substrates can be stored in close proximity. Compartments for waste items, such as syringes, decontaminated materials and other laboratory consumables, should also be included. Autoclaves, sterile hoods, interim animal cages including sterile ventilation and exhaust, cold storage of tracers (4° and -20°C) as well as sample preparation materials and space (eg for tissue preparation and immunohistochemical tissue sampling) should be considered as well.

Data

In many laboratories, personnel that are not imaging specialists can be quickly trained to acquire and analyse optical imaging data, although ongoing training is recommended. However, for some imaging modalities, including PET, a moderate level of expertise is required for basic applications, while a high degree of expertise is necessary for a number of applications, including those related to tracer development, kinetic imaging and kinetic modelling, and detailed cardiac functionality. Similarly, low field MRI systems (ie <3T) have been designed to be convenient solutions for users with low to moderate technical expertise, while higher field systems (ie >4.7T) require greater expertise due to instrument complexity. Expertise requirements for CT imaging/analysis vary in complexity depending on specific application, from basic image segmentation to applications including complex cardiac function analysis.

Regardless of imaging technique or specific application, access to appropriate image analysis tools is critical to ensure robust data acquisition and examination. Specialised software packages for PET image analysis are available – for example, for brain studies, kinetic modelling and specific disease models – but can require specialised training. Frequently, laboratories have designated system operators to facilitate imaging and provide expertise for study design and analysis.

Imaging analysis may be extensive for some applications and may occupy several hours of processing. Depending on the anticipated level of use for equipment, it may be advisable to configure separate workstations that are dedicated for analysis only.

Modality specific requirements

In addition to the common considerations noted above, there are a number of specific additional requirements for each imaging modality.

Space

Technological advances mean that laboratories may face space restrictions when trying to house a growing number of instruments. The increase in use of multimodal imaging techniques means that the physical dimensions of instruments have become increasingly important for laboratories wishing to use multiple complementary techniques.

Although benchtop PET systems are available, these can have significant limitations including low resolution imaging, high dead-time errors and small axial fields of view (FOV). More space will typically be required for systems with good performance specifications, such as sub-millimetre resolution, >9% sensitivity and large FOV. Instruments are now commercially available which integrate imaging techniques, such as a bi-modal PET/MR system and tri-modal PET/SPECT/CT imaging system, all within a compact footprint.

Peripheral devices

Peripheral devices and solutions are necessary for some imaging modalities and applications. Use of a dose calibrator may be required to provide an accurate starting dose measurement, for example in order to perform SUV calculations in a PET imaging study. Additionally, cardiac and respiratory studies can require ECG and respiratory monitoring and gating. For studies in drug and tracer kinetics, accessories and/or methods for precision tracer injection and blood sampling may also be required.

Health and safety

For multimodal systems that offer x-ray and/or radio isotopic imaging, radiation safety regulations may be relevant. Laboratories that intend to use SPECT and/or PET radionuclides will be required to comply with relevant institutional radiation regulations. Some multimodal systems have been designed to comply with such regulations, equipped with fail-safe interlocks and other safety mechanisms including cabinets designed to shield the end user. As such, these instruments can be operated in typical laboratory environments without the need for any additional external shielding.

Similarly, modern CT scanners are self-shielded cabinet systems, with multiple layers of safety interlocks and emergency stops to prevent accidental radiation exposure to operators. These features enable active system operation with personnel in the direct area, obviating the need for remote operator rooms.

Consideration must also be given to storage, handling and managing isotopes, including radioactive waste and animals. Applications in PET laboratories may have specific requirements. 18FDG is one of the most common PET isotopes used in preclinical research, for example, and is relevant to many studies in oncology. 18F has a 110-minute half-life, therefore laboratories using 18F compounds will need to be in proximity to suitable sources.

There are several commercially-available turnkey solutions for end-user shielding for isotope storage, for shielded dose and animal preparation, and for shielded storage of active animals. Many aspects of radionuclide safety will be addressed through radiation safety programmes managed by an institutional RSO. Typical components include training to minimise exposure, radiation surveys using wipes or counters where appropriate, and personal dosimetry hardware may also be employed to ensure that personal exposure limits are not exceeded.

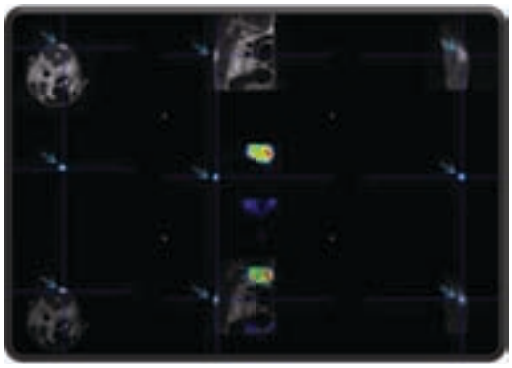

Figure 3: Cross-platform 99m Tc-SPECT (rainbow) and MR (gray) imaging of HCT 116-hNIS-NEO tumour mouse with image registration facilitated by the MMAB with the FML. All rows show transverse, sagittal and coronal views. Top row is MR only. Middle row is SPECT only. Bottom row is registered MR/SPECT

all-modality imaging systems are not currently available – is how the developments described here, that allow full integration of all modality combinations, are advancing the state of the art and bringing more powerful data to the research community as they search for ways to combat cancer and improve patient outcomes. DDW

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