Multi-modal PET drives interdisciplinary preclinical imaging

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Tomography is an imaging technique used across a wide variety of fields, ranging from radiology and nuclear medicine, to geophysics and materials science. It provides three-dimensional information about a subject based on its sections or projections, and common examples include X-ray computed tomography (CT), positron emission tomography (PET) and single-photon emission computed tomography (SPECT). CT scanning provides information on the anatomy of the subject, while PET provides functional imaging which shows the spatial distribution of biomolecular activity in the body. PET was developed as a technology for both clinical diagnostic and preclinical purposes in the 1950s, the scope of which was expanded by the development of radiopharmaceuticals – a group of pharmaceutical drugs which emit radiation and commonly includes radiotracers.

Preclinical imaging (PCI) plays a vital role in understanding the biological processes behind disease states at the organ, tissue, cell and molecular level. Elucidating how the body responds to physiological or environmental change is important in the search for therapeutic agents to fight disease. PCI is also critical to the evaluation of new treatment effectiveness and safety, by informing researchers of drug distribution patterns in tissues. PET in preclinical studies enables users to conduct repeat experiments on the same animal subjects, providing strong statistically valuable data and therefore reducing the number of animals required for a study. For this reason, it has becoming increasingly important to use non-invasive in vivo imaging techniques to optimize the use of each animal used.

Multi-modal tomographs, such as PET/CT, allow the correlation of the functional imaging obtained using PET with the anatomic imaging obtained with CT scanning. PET can also be combined with other technologies, such as magnetic resonance imaging (MRI), to bring functional imaging together with soft tissue morphological imaging. PET/MR is gaining ground in preclinical imaging applications, as it offers superior soft tissue contrast, imaging without the CT's ionizing radiation risk, and multiparametric data.

Preclinical PET applications

PET, PET/CT, SPECT/CT, PET/MR and PET/SPECT/CT multi-modal imaging techniques are used across a number of life science applications, including oncology, neurology, cardiology and pneumology. Total body PET imaging is also possible, and enables researchers to determine the pharmacokinetics of new drugs in all the body's organs and tissues at low masses.

Oncology

Preclinical researchers are interested in understanding the biology of tumor development, response to cancer treatment, and drug toxicity. There are various types of tumors, some of which have not yet been well characterized, so imaging technologies such as PET can shed light on the mechanisms of progression for many different tumor types, and how treatment affects them.

PET 101

Positron emission tomography (PET) is an imaging technique that creates 3D images of the subject using radioactive tracers, usually injected intravenously, which consist of molecules that are bound to a radioactive isotope. The carrier molecule can interact with or bind to specific proteins, receptors, and biomolecular pathways in the body, for quantifying a specific biological activity. The molecule used depends on which tissue is being investigated, but the most common radioisotope used is fluorine-^{18} (^{18}F).

The isotope produces positrons that interact with the surrounding electrons, resulting in the complete annihilation of both particles and the release of two photons (gamma rays), which speed off in opposite directions (~180°). The detectors in the PET scanner measure the photons and use them to map the radionuclide distribution in the body.

Many cancers are associated with a higher metabolic turnover than normal cells so, using PET and an injected radiolabelled glucose analogue tracer such as fluorine-18 (^{18}F)-fludeoxyglucose (^{18}F-FDG), glucose uptake can be quantified and tumor burdens detected. This method can also be used to detect molecular biomarkers to contribute to cancer detection and treatment response assessment. PET/CT and, more recently, PET/MR are used to determine the accumulation regions of ^{18}F-FDG, to obtain a semiquantitative standardized uptake value (SUV) to assist in the diagnosis of tumor malignancy. Tumor blood flow is another important marker evaluated in order to investigate tumor vascularization, to potentially discriminate between non-neoplastic and neoplastic lesions.

Combination cancer therapies are often desired for their ability to address multiple molecular targets, as well as a reduced chance of drug resistance. A relevant study used preclinical PET/CT imaging to monitor ^{18}F-FDG tumor uptake for different treatment combinations: radiotherapy (Rad) alone, Rad + Temozolamide (Tmz), Rad + Mifepristone (Mife), and Rad + Mife + Tmz. Rad+ Tmz is the typical treatment regime for glioblastoma, but the study found that using Mife as a priming agent suppressed tumor growth more than the other treatment combinations[1] (Figure 1). The mechanism of this chemo-radio-sensitizing effect of Mife is yet to be fully characterized, but studies such as this help researchers make important steps towards improving available cancer treatments.

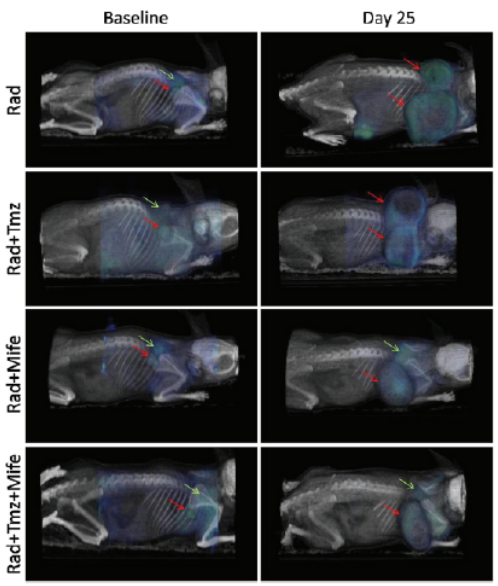
Preclinical laboratories are increasingly aware of the benefits of PET/MR for oncology research. Thanks to MRI's unique ability for imaging soft tissue, users can visualize the true tumor margin and evaluate tracer distribution within individual tumors, to generate a desired volume of interest (VOI) and calculate SUVs more accurately. Tumor margin detection is a unique and significant enhancement to preclinical cancer PET studies.

Neurology

Neuroscientists benefit from PET imaging technologies to obtain metabolic information about the brain, for example to detect changes in brain metabolic activity that might be indicative of abnormalities, potentially leading to conditions such as Alzheimer's Disease (AD), Parkinson's Disease (PD), stroke, memory loss and cognitive decline. PET imaging is also used for the study of addiction and psychiatric disorders. By using PET in preclinical studies on the brain's impact on behavior and cognition, researchers can advance their understanding about nervous system processes in healthy individuals, in addition to various disease states, and elucidate abnormalities in its organization and connectivity.

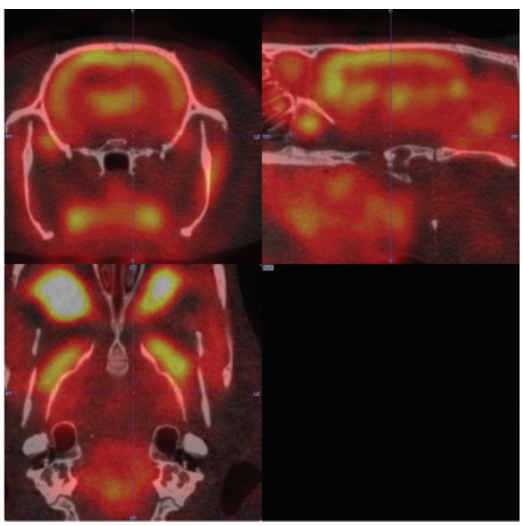
PET/MR is particularly useful in neurology as it provides synchronized soft-tissue images with metabolic imaging, enabling scientists to investigate the brain's anatomical structures, pathologies and metabolic abnormalities in preclinical animal models (figure 2). Researchers can use PET and MRI to localize molecular markers in brain tissue, and image brain microstructure, connectivity, vasculature and activity at high resolution.

Figure 1



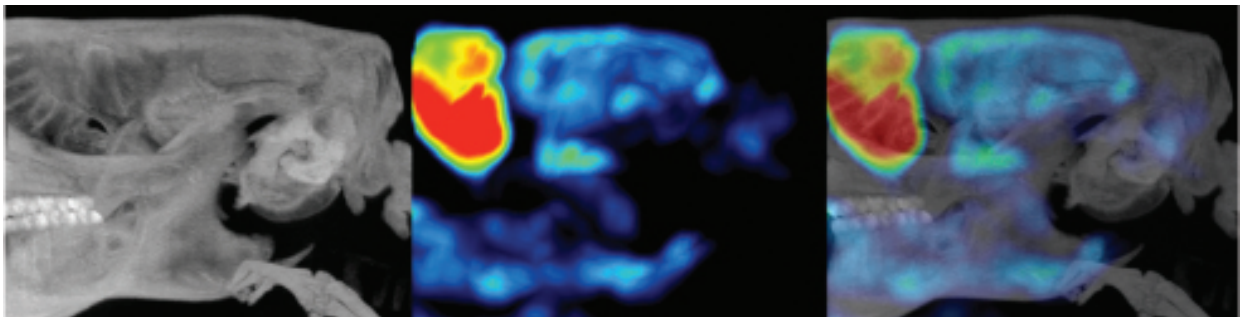
PET/CT images showing ^{18}F-FDG tumor uptake, in four treatment combinations, at the beginning of treatment and 25 days later. Red arrows indicate tumor location at baseline and day 25, green arrows show sites of typical ^{18}F-FDG uptake in brown adipose tissue (BAT). Reproduced from reference [1] in accordance with the Creative Commons License (https://creativecommons.org/licenses/by/2.0/).

Figure 2



PET/CT of a normal rat, showing fluorine-18-fludeoxyglucose (^{18}F-FDG) metabolic activity of cerebral cortex, Harderian glands and jaw muscles clearly resolved.

Figure 3



MicroCT (50 µm) of well-defined rat skull, sinuses and jaw structures.

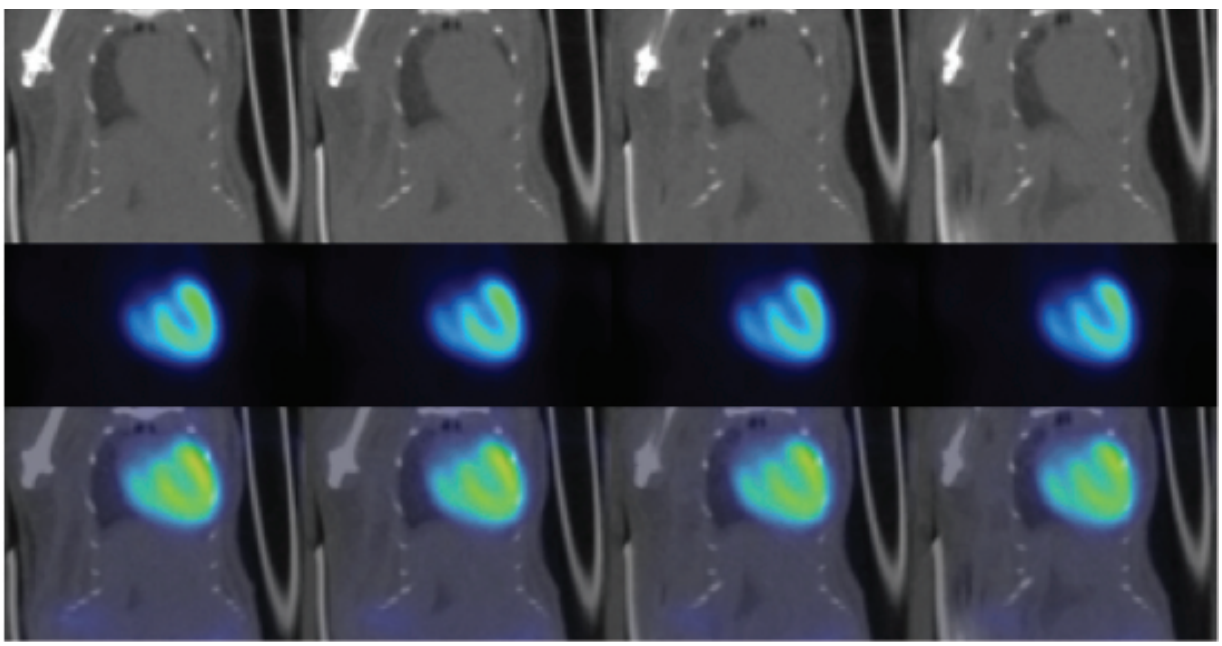
Neurodegenerative diseases, such as AD, PD, multiple sclerosis and Huntington's disease are a key focus of preclinical research, with MRI technology providing the high spatial resolution *in vivo* imaging requires to determine the structure and function of central nervous system tissues. Used in tandem with MRI, PET imaging enables scientists to investigate the pathological hallmarks of neurodegenerative disease, such as amyloid-β (Aβ) and tau protein depositions in AD models.

Similarly to oncological applications, glucose metabolism is a useful biomarker for many neurological disorders. Normal brain glucose metabolism is modified by many disease states, and can be easily monitored by ^{18}F-FDG-PET. Currently, most AD treatments are targeted towards the disease's symptoms, rather than the underlying causes. Some studies have investigated the possibility of halting AD progression with therapeutics that target the early stage causes of the disease. For example, one experiment found that a new candidate therapeutic synthetic cannabinoid, JWH, a selective CB^2 agonist, significantly increased metabolic activity in the hippocampus and cortical regions of mice, measured by ^{18}F-FDG-PET^2. The mice also showed reduced neuroinflammation and an increase in Aβ clearance when administered with JWH, improving overall cognitive performance.

Cardiology

Preclinical researchers use multi-modal PET imaging to gain a deeper understanding of cardiovascular metabolism, inflammation and perfusion. High spatial and temporal resolution

Figure 4



4 slice coronal plane PET/CT of normal mouse. Fluorine-18-fludeoxyglucose (^{18}F-FDG) heart right ventricle clearly resolved. MicroCT (200 µm) with lung and heart shadow.

cardiac imaging is crucial for resolving features of cardiac disease, including plaques and ischemic lesions, in small animals. The primary assessment parameter for cardiac preclinical imaging is myocardial metabolic viability and perfusion, but others such as inflammation, innervation, apoptosis and neovascularization are also attainable through PET[3].

MRI, PET and microCT imaging are well-established in the study, diagnosis and treatment of heart disease. Myocardial PET is used to separate cardiac disease stages from normal physiological stages, which is important in the evaluation of therapeutic strategies and the determination of imaging biomarkers of coronary artery disease (CAD). Resolution is an important factor in cardiac imaging, because blood concentration is often obtained from the left ventricle. The higher the resolution, the lower the partial volume effect and, therefore, the lower the chance of contamination from the myocardium in to the blood concentration derived from imaging. PET scanners that maintain resolution regardless of the animal's position in the field of view (FOV), such as Bruker's PET systems, open up the possibility of multi-animal imaging, enabling researchers to obtain results with low or ultra-low tracer activity, at high diagnostic accuracy, in the shortest possible time frame.

Sophisticated preclinical PET scanners can achieve sub-millimeter resolution and allow accurate detection of small lesions, as well as better characterization of the right myocardial physiology. These scanners are also able to image smaller animals, such as mice, in models that were previously only performed in rats, adding the valuable use of genetically modified rodent strains. The CT capabilities of state-of-the-art PET/CT scanners provide complementary information to PET imaging, such as calcification area and fine shape of vessel and cardiac structure in murine models. Although PET/CT is considered the "gold standard" for cardiac imaging, PET/MR is gaining more ground in this area of preclinical research. MRI information can be used for retrospective cardiac/respiration gating (IntraGate, Bruker BioSpin), without the use of electrocardiogram (ECG) electrodes, and PET/MR fingerprinting can provide information on tissue metabolic state, structural integrity, perfusion, global/local function and molecular pathways in the same subject.

PET/MR is particularly beneficial for plaque imaging and the molecular characterization of inflammation, making this technology useful for characterizing disease states associated with these factors. PET/MR is also suitable for the *in vivo* monitoring of new drugs being developed to target inflammatory diseases. PET is one of the few technologies capable of delineating cardiac autonomic denervation, the degree of which has been shown to identify those at risk of cardiovascular events[4].

Case study: University of Genoa

One example of multi-modal PET in use is in the Department of Health Sciences at the University of Genoa, Italy. Leading the Nuclear Medicine Laboratory is Dr Gianmario Sambuceti, who is studying cancer biology and therapeutic targets, as well as neurodegenerative and cardiac diseases using PET imaging technology. The university is affiliated with the IRCCS San Martino Hospital, which is one of the largest hospitals in Europe, and the National Institute of Cancer, bringing together expertise in the field of molecular imaging and oncology.

Dr Sambuceti's laboratory has built up a body of research on metformin – the most widely prescribed drug for type 2 diabetes – which works by modifying normal glucose metabolism. Studies have shown that patients treated with metformin have a much higher background level of abdominal FDG signal than normal patients, which can cause issues for PET imaging protocols where diagnosis for other disorders of the abdomen is ongoing. A secondary effect of the drug, which was discovered by mistake but for which there is growing evidence, is that it suppresses tumor growth, with patients displaying improved clinical outcomes. Although still the subject of debate, one of the key mechanisms for metformin's action as an anti-cancer agent is through AMP-activated protein kinase (AMPK), which plays a major role in the regulation of metabolism and growth in both normal and cancer cells[5].

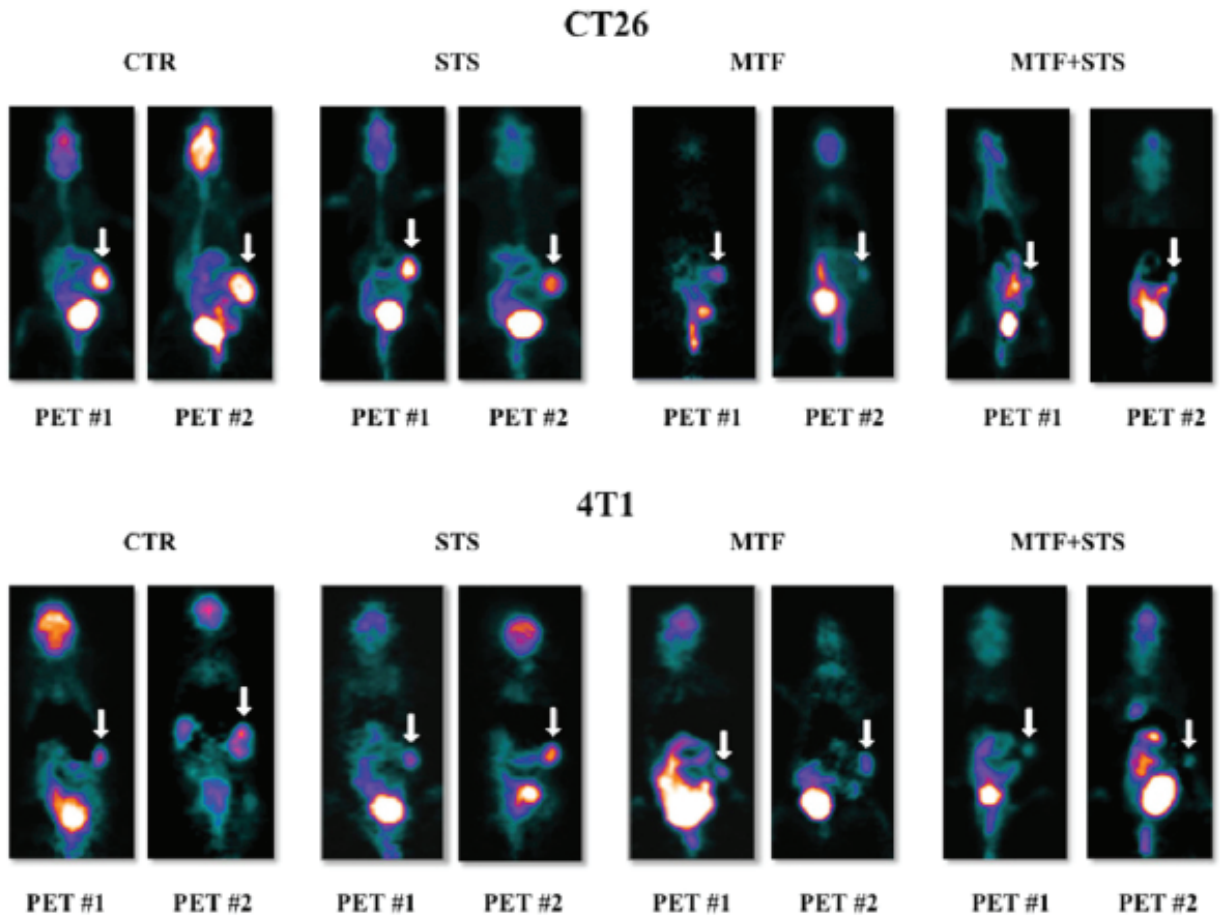
Targeting energy metabolism is therefore considered an important tool in designing cancer therapies. Dr Sambuceti's group investigated the impact of short-term starvation (STS) – which is known to significantly impair cancer growth but leads to toxic reactive oxygen species (ROS) production – combined with metformin treatment on tumor development. The group observed changes in tumor growth in murine models of colon (CT26) and breast (4T1) carcinoma using *in vivo* FDG-PET imaging, which showed that tumor progression was halted most significantly when metformin and STS were combined (Figure 5)[6].

The group's experiments confirmed that combining metformin and STS significantly depletes cancer energy, and found that FDG uptake shows a dose-dependent decrease under metformin treatment, which was further amplified by STS in CT26 and 4T1 cell lines. In order to transfer these findings into a clinical setting, researchers must now find a way to carry the drug directly to the tumor, at a lower concentration.

Advances in PET technology

There are a number of performance criteria that PET systems must meet to provide the highest quality imaging results. Sensitivity and resolution depend in part on the material in the detector stopping the gamma rays (the scintillator) and the detector design. A very dense material is needed to stop as many gamma rays as possible. Dense and thick scintillator crystals which convert the gamma energy

Figure 5



PET maps of glucose consumption. Both metformin (MTF) and short-term starvation (STS) affected murine colon cancer (CT26) and breast cancer (4T1). Under combined treatment (MTF+STS) tumor progression was almost completely abolished. PET#1 = 1 week after treatment, PET#2 = 2 weeks after treatment.

into light are therefore the material of choice in PET detectors. Diameter of the ring and depth of interaction (DOI) correction must also be optimized for high resolution imaging. Crystal technology also underpins PET sensitivity, and has undergone a number of advances in recent years. The industry has been dominated by pixelated crystals, tightly packed together, but the use of continuous crystals has shown to better measure light distribution and, therefore, provide greatly improved resolution and sensitivity.

The Bruker PET portfolio combines continuous crystal scintillators with novel light detection technology for superior imaging capabilities. Rather than using traditional avalanche photodiodes (APD) or photomultiplier tubes (PMTs), continuous crystals are coupled with silicon photomultiplier (SiPM) photosensors to allow the accurate determination of all three spatial coordinates of the gamma photon interaction within the detector crystal. The result is sub millimetric spatial resolution, regardless of the position, a term known as Full Field Accuracy (FFA). FFA results in more reproducible data, independent of variable sample positioning, and more reliable imaging of large samples or multiple animals across the FOV, to facilitate accuracy and throughput in preclinical imaging. SiPM technology is also becoming increasingly popular for its compatibility with strong magnetic fields employed by MR.

Advanced electronics are also required to meet the standards of state-of-the-art PET scanners. Smart data acquisition systems enable the scanner to acquire many events without compromising DOI resolution or count rate, which is another key performance criteria for PET systems.

Preclinical PET in the future

Multi-modal PET imaging is enabling scientists to break new ground in preclinical research spanning a number of fields.

As a highly sensitive, non-invasive technique, PET can help improve the understanding of the underlying causes of disease, improving methods of detection and treatment. Preclinical PET studies facilitate the development of imaging biomarkers, with the goal to translate these to the clinic to identify patients at risk of, or in the early stages of disease. Multi-modal systems such as the Albira Si PET/SPECT/CT, PET/CT Si78, and PET/MRI Insert and Inline systems make it simple for researchers to combine the benefits of PET imaging with CT, SPECT and MRI technology, for optimal imaging results.

For more information on Bruker's preclinical imaging solutions, please visit https://www.bruker.com/products/preclinical-imaging/nuclear-molecular-imaging.html

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