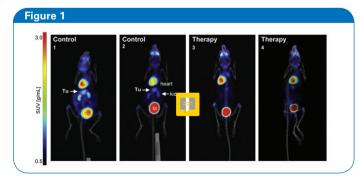# The Use of Positron Emission Tomography (PET) in Preclinical Pharmaceutical Research

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Positron emission tomography (PET) is a popular nuclear imaging technique used to gain information about specific metabolic functions. Used in clinical diagnostic and preclinical applications, PET is often combined with other imaging technologies, such as magnetic resonance imaging (MRI) and computed tomography (CT) scanning, to incorporate anatomical information. PET provides functional imaging, which shows the spatial distribution of biomolecular activity in living tissues and is therefore particularly useful for evaluating drug candidates in preclinical studies.

PET creates three dimensional (3D) images of the subject using radioactive tracers – molecules bound to a radioactive isotope – that are usually injected intravenously. The carrier molecule can interact with or bind to specific proteins, receptors, and biomolecular pathways in the body, to quantify a specific biological activity. The radioactive isotope, commonly fluorine-18 (^{18}F) or carbon-11 (^{11}C), produces positrons that interact with the surrounding electrons, resulting in the annihilation of both particles and the release of two photons (gamma rays). These photons speed off in opposite directions (~180°) and are picked up by detectors in the PET scanner to map the radionuclide distribution in the body.

The non-invasive, sensitive and quantitative nature of PET imaging is utilized in preclinical pharmaceutical research

Figure 1

Preclinical efficacy of fasudil in gastric tumors of transgenic mice with gastric carcinoma (CEA424-SV40 Tag). 3D PET/CT in situ detection of gastric tumors in mouse stomachs. Signals from ^{18}F-FDG uptake in fasudil-treated (4 weeks) and control mice presented as graded color code with standard uptake value (SUVbw) range of 0.5-10. (Tu = tumor, bl = bladder. Arrows show the gastric tumor between the two kidneys and below the heart. Reproduced from reference [1] under the Creative Commons License 4.0 https://creativecommons.org/licenses/by-nc-nd/4.0/.

to advance knowledge of diseases and drug activity in the body. Successful drug development relies on the ability to understand dynamic biological processes, gene expression, enzyme and protein activity, progression and treatment of diseases, biodistribution, and pharmacokinetics/pharmacodynamics of new drugs. Multi-modal PET imaging, such as PET/MR and PET/CT, provides a method to map the path of drugs throughout the body over time, to monitor efficacy and establish suitability for clinical use.

Preclinical Oncology Research

Evaluating Therapeutic Efficacy of Drug Candidates

PET imaging is ideally suited to preclinical cancer studies, as it can improve understanding of the mechanisms of tumor progression, response to cancer treatment, and drug toxicity. Glucose analogue tracers, such as ^{18}F-fludeoxyglucose (^{18}F-FDG), are used to monitor glucose uptake in tumors and detect molecular biomarkers, to distinguish and quantify tumor burdens.

PET/CT has been used in preclinical oncology investigations into the efficacy of potential novel treatments. For example, one study used small animal PET/CT imaging to confirm the preclinical efficacy of fasudil – an approved drug for cerebrovascular bleeding – for inhibiting tumor growth in gastric cancer (GC)[1]. Mice were injected with ^{18}F-FDG and imaged using Bruker's Albira II small animal PET/CT/SPECT system. Quantitative calculation of the 3D tumor volumes showed a reduction in ^{18}F-FDG uptake and signal intensity by fasudil, compared to non-treated control animals (Figure 1). The results of the study show that fasudil is a viable novel strategy for the treatment of GC.

ImmunoPET and Personalized Medicine

Another preclinical oncology study looked at imaging receptor expression in cancer models with microPET/CT, specifically for the treatment of triple-negative breast cancer (TNBC)[2]. The heterogeneity of TNBC and lack of actionable targets make treatment challenging, and there is therefore an unmet clinical need for new molecular targets for TNBC. The literature suggests that AXL – a member of

the receptor tyrosine kinase TAM subfamily – plays a role in TNBC and other cancers, and could be investigated as a potential therapeutic target.
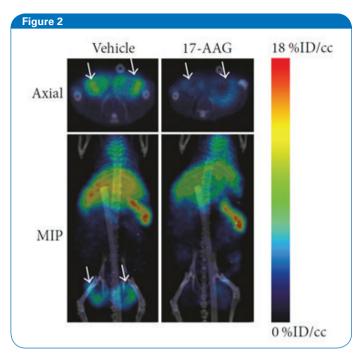
Using microPET/CT (Albira microPET/SPECT/CT, Bruker Bio-Spin), expression of AXL and its downregulation by 17-allylami-no-17-demethoxygeldanamycin (17-AAG) – a potent inhibitor of heat shock protein 90 (HSP90) – could be imaged and quantified using copper-64-labelled anti-human AXL antibody (^{64}Cu-an-ti-hAXL) as a radioactive probe, shedding light on therapeutic efficacy for AXL-targeted molecular therapies (Figure 2). Both PET imaging and radionuclide therapy are made possible by the short half-life (~13h) and β+ and β− emissions of ^{64}Cu, making it a desirable PET radionuclide for antibody and nanoparticle labeling.

The in vivo imaging experiment showed that ^{64}Cu-anti-hAXL had greater tumor uptake and accumulation than non-specific ^{64}Cu-IgG, suggesting that ^{64}Cu-anti-hAXL specifically binds to AXL-expressing tumor cells. By using ^{64}Cu-anti-hAXL as an imaging probe, the researchers demonstrated that non-invasive assessment of AXL expression using microPET/CT in TNBC may be used to predict drug resistance and response to therapies directed at AXL. Such results may be used to develop theranostic agents in the future, which enable a specific diagnostic test before delivering therapy to a particular molecular target on the tumor.

The combination of PET and CT imaging is a valuable tool in oncology research due to its ease-of-use, high-throughput capabilities and high resolution for bone and pulmonary applications. These studies place PET/CT at the forefront of cancer therapeutics development and tumor biology research, for cancers with high mortality rates such as GC and TNBC.

Monitoring Drug Activity

Ensuring a drug reaches the specific target tissue and exerts its localized effects is a key parameter for drug development. Absorption, distribution, metabolism and excretion (ADME) studies are designed to observe the journey of drugs and drug metabolites through the body, which must cross cellular membranes to act, and drug design and development researchers must strike a balance between potency, selectivity and ADME properties. In many cases, this membrane crossing is an active process that uses transmembrane transporter proteins belonging to either the solute carrier (SLC) or adenosine triphosphate-binding cassette (ABC) families. Many drugs are co-administered, and when the two drugs are recognized by the same transporters, drug-drug interactions (DDI) may occur where one drug alters the disposition of the other, often changing its tissue distribution. These transporter-mediated DDIs have significant implications for drug safety and efficacy, so methods for measuring drug tissue concentration levels in vivo are required.

Figure 2

MicroPET/CT images of MDA-MB-231 tumor xenografts in mice 24 hours after intravenous injection with ^{64}Cu-anti-hAXL. Treatment group received daily 17-AAG injections and showed lower tumor uptake of ^{64}Cu-anti-hAXL than the vehicle-treated control group. Reproduced from reference [2] in accordance with Creative Commons License 4.0 https://creativecommons.org/licenses/by/4.0/.

Drug interactions

Transporters can affect the ADME properties of drugs, therefore influencing their pharmacokinetics and pharmacodynamics. The importance of transporter-mediated DDIs has been acknowledged by the Food and Drug Administration (FDA) and although the draft guidance is targeted at the clinical study level, it highlights that clinically-relevant DDIs between an investigational drug and another drug should be defined during drug development[3].

A recent study investigated the role of transporters in the disposition of erlotinib – a drug used for the treatment of advanced, metastatic non-small cell lung cancer (NSCLC) and advanced, metastatic pancreatic cancer – using PET combined with MRI[4]. Erlotinib is a reversible tyrosine kinase inhibitor (TKI) of the epidermal growth factor receptor (EGFR), and is a substrate of breast cancer resistance protein (BCRP/ABCG2), P-glycoprotein (P-gp/ABCB1), organic ion transporter 3 (OAT3/SLC22A8) and organic cation transporter 2 (OCT2/SLC22A2). The drug is extensively metabolized in humans and is primarily excreted via the hepatobiliary pathway, leading to high concentrations in the liver and bile[5].

PET/MR imaging can be achieved either by software fusion, where subjects are scanned in two stand-alone scanners in a serial manner and images are fused, or by hardware combination in simultaneous preclinical PET/MR systems. This experiment used a standalone MRI system (Bruker BioSpin)

to image mice before being moved to a microPET system. The whole imaging chamber was moved from the MRI to the PET scanner, minimizing movement of the subject within the chamber, therefore simplifying image fusion. A custom adapter and mounting plate for the PET scanner were created, and images co-registered using specialist software.

The study used serial PET/MR to assess the effect of transporters on tissue distribution and excretion of ^{11}C-erlotinib in four groups of mice: (1) wild-type with microdose of ^{11}C-erlotinib, (2) wild-type with microdose of ^{11}C-erlotinib and co-injection of a pharmacological dose of unlabeled erlotinib, (3) wild-type with microdose of ^{11}C-erlotinib pretreated with elactridar, a dual P-gp/BCRP inhibitor, (4) P-gp/Bcrp knockout (Abcb1a/b(−/−) Abcg2(−/−)) mice with microdose of ^{11}C-erlotinib.

BCRP, P-gp and SLC uptake transporters were confirmed to influence the *in vivo* disposition of ^{11}C-erlotinib, affecting its distribution to normal and potentially to tumor tissue. It was also found that erlotinib could cause transporter-mediated DDIs when combined with other drugs that use the same transporters, which could potentially lead to changes in hepatic disposition of the co-administered drug. Previous studies have shown that efflux transport by P-gp and BCRP at the blood brain barrier (BBB) results in reduced brain distribution of erlotinib[6]. This, together with the results of the PET/MR study, indicate that brain distribution of erlotinib could be enhanced by inhibiting P-gp and BCPR at the BBB, ultimately increasing the drug's efficiency in treating NSCLC brain metastases.

The addition of a MRI system to PET imaging allowed the delineation of the organs of interest (brain, lung, left ventricle of the heart, kidney, liver, gall bladder, intestine and urinary bladder), which was particularly beneficial for defining the urinary bladder in the erlotinib co-injected group, where there was no radioactivity uptake. Establishing the volume of interest (VOI) using MRI data led to more precise extraction of PET information, generating reproducible quantitative data. The PET/MR experiment enabled the researchers to create an organ atlas template based on MR images, which could then facilitate the analysis of PET-only image data.

Evaluating Drug Delivery Systems

Preclinical studies could benefit from easily prepared formulations with different dose strengths, rather than the often used restricted dose-range units, in order to develop more patient-specific therapeutics. A number of 3D printed solid dosage forms are now being developed, many of which are commercially available, with innovative release characteristics and the capability to combine more than one drug in a single formulation.

Multi-modal PET imaging is now being used for novel pharmaceutical applications, such as visualizing the *in vivo* behavior of 3D printed formulations, to improve the delivery of drugs to the desired site of action. A recent study investigated the movement and disintegration of four different devices, with different formulation release characteristics, in the gastrointestinal tracts of rats[7]. The devices, Kollicoat IR (immediate release), Klucel EF (water soluble), Aqualon N7 (insoluble polymer) and Aquasolve LG (pH dependent), were radiolabelled with 18F-FDG and imaged using microPET/CT (Albira, Bruker BioSpin). CT scanning enables the direct imaging and differentiation of soft tissue structures and, due to short scanning times, can be used for all anatomic regions (including those susceptible to motion and breathing). In combination with CT, PET imaging obtains radiopharmaceutical release data along with the anatomical position of the formulation at different times.

Results showed that Kollicoat IR and Klucel EF devices disintegrated within 1-2 hours, Aquasolve-LG devices took over 7 hours, and Aqualon N7 devices did not disintegrate or release the tracer. Gastric emptying was not observed for any of the devices. The study confirmed that microPET/CT can be successfully used to obtain *in vivo* data of the behavior of 3D printed devices in rat models, which helps develop the design of formulations suitable for preclinical drug testing, furthering work on targeted release dosage forms and personalized dosing.

Advanced Imaging Technology

There are a number of advantages offered by PET imaging for preclinical pharmaceutical research. The high sensitivity enables measurement and very low drug concentrations, while the high biochemical resolution allows differentiation of physiological alterations that occur nanometers apart[8]. Combined systems are available on the market that integrate PET with MRI or CT imaging in one instrument, which saves laboratory space while ensuring seamless co-registration between PET and MR/CT images. However, many laboratories favor separate systems, where images are integrated following the experiment. Although the subject must be moved between two scanners, the advantage of this method is the option for standalone PET, MRI or CT imaging if required.

Bruker's Solutions

Bruker's PET portfolio covers a range of modalities, ideally suited to preclinical pharmaceutical studies:

Albira Si (PET/CT/SPECT)

- Choose any configuration from PET, SPECT, PET/CT, SPECT/CT, PET/SPECT, and PET/SPECT/CT.
- Silicon photomultiplier (SiPM)-based PET technology.
- Bruker's Si detectors provide submillimetric resolution across the entire field of view (FOV) and imaging (15 min. acquisitions) at a Wide Activity Range.
- Universal Si PET detectors are MR compatible.

PET/CT Si78

- SiPM PET technology.
- High performance total body PET and CT for mice and rats.
- Homogeneous, high-resolution and quantitative PET/CT imaging with large FOV of 80x up to 200 mm.
- Unique low dose X-ray technology combined with ultra-fast full 3D CT scanning for longitudinal studies.
- Automated co-registration, image fusion and image analysis.

PET/MR 3T

- Homogeneous, constant PET resolution over the whole FOV.
- Newly developed 3T cryogen-free magnet and motorized animal transport system.
- Consistent quantification with attenuation correction based on high quality MRI data.
- Automatic co-registration of images.
- Whole body scans with a total FOV of >285 mm.

PET Insert

- Enables simultaneous high-field MR and PET imaging.
- Designed to be used with new and existing BioSpec high-field MR systems to expand system functionality.
- Option for standalone PET.
- Up to 0.7 mm resolution with Full Field of View Accuracy (FFA) and 12% sensitivity.
- SiPM design compatible with high-field MR.

PET Inline

- Module can be added to all high-field MRI systems.
- Homogenous, high-resolution imaging with FFA PET quantification with attenuation correction based on MRI attenuation maps.
- Large PET FOV of 80 mm diameter, 150 mm in a single acquisition.
- Fully integrated software interface for controlling both modalities.
- Fully automatic high-precision PET/MR image coregistration.

Future developments

There is a wealth of research documenting the potential of multi-modal PET imaging for pharmaceutical drug discovery and development. The studies presented in this paper outline the ability of advanced instrumentation to determine the mechanism of action of a drug, monitor biomarkers to assess a drug's therapeutic efficacy, and track novel drug delivery devices in the body. Developments in PET, MRI and CT imaging, as well as ongoing research into new radiopharmaceuticals for in vivo imaging, have enabled the non-invasive evaluation of new potential therapies, for a broad range of diseases.

The physiological, pharmacological and biochemical measurements that are made possible by multi-modal PET systems are propelling drug development in preclinical studies. The ability to radiolabel drugs and monitor tissue delivery over time can be used to determine appropriate administration schedules, establish efficacy and predict possible toxicity. Harnessing the power of nuclear molecular imaging with advanced instrumentation, such as Bruker's PET/CT Si78, PET/MR 3T, and Albira Si systems, allows pharmaceutical researchers to progress potential drugs through preclinical studies and secure the development of tomorrow's therapies.

For more information on Bruker's preclinical imaging solutions for drug development, please visit https://www.bruker.com/applications/pharma-bio-pharma/drug-development/preclinical-imaging.html.

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