Imaging Cancer: Multi-modal PET Technology Provides Novel Insights in Preclinical Oncology

Todd Sasser, Head of PCI Applications, Americas & Snr NMI Applications Scientist, Bruker BioSpin

Positron Emission Tomography (PET) is a widely-used imaging technique in nuclear medicine, for both clinical diagnostics and preclinical applications. PET provides three-dimensional (3D) functional imaging using radioactive tracers (radiotracers), showing the spatial distribution of biomolecular activity in the bodies of animal models and humans. Small animal studies are necessary to explore and validate imaging agents in the *preclinical* phase, before beginning clinical trials. In this context, PET imaging is increasingly being used by researchers in the drug development phase as it provides data that can be extrapolated from animal to human studies. Preclinical imaging facilitates the development and evaluation of novel therapeutic strategies, by providing important insights into disease mechanisms, from molecular to organ level. Small animal imaging deepens our understanding of disease development and the effect of potential treatments, and advances in PET technology are powering the translation of this research into the clinical setting.

The need to offer patients more personalized cancer treatment is driving advances in preclinical PET oncology research. The numerous different types of tumors – including those not yet well characterized – and their varying reactions to treatment, make the search for new effective cancer therapies incredibly challenging. Non-invasive *in vivo* imaging technologies such as PET enable researchers to better understand the course of tumor progression, by visualizing cancer-related processes in real-time. The combination of other imaging modalities, such as magnetic resonance imaging (MRI), computed tomography (CT), and single-photon emission computed tomography (SPECT), joins structural and functional imaging in one experiment. PET/CT, PET/MR and PET/SPECT/CT multi-modal systems are able to provide quantitative 3D tomographic images of radiotracers, bone, and soft tissue, furthering the growing knowledge of cancer biology and treatment.

Analysis of Tumor Biology

Preclinical *in vivo* imaging methods are helping to further our knowledge of tumor morphology, progression and biomarker expression. PET is able to provide information on the expression of receptors, energy metabolism, and other biomarkers of tumors, by imaging an intravenously injected radiotracer – a radioisotope, most commonly fluorine-18 (^{18}F), attached to a molecular probe that targets a specific molecule or metabolic pathway – and monitoring its uptake by tumor cells. A key characteristic of tumor cells is their elevated metabolic turnover, and ^{18}F-fludeoxyglucose (^{18}F-FDG) is often used as a radiolabelled glucose analogue tracer to analyze glucose uptake in tumors to track their progression and monitor aggressiveness.

"Conventional" PET tracers, such as ^{18}F- FDG or ^{18}F-Fluorothymidine (FLT), are considered the gold standard and monitor universal markers of tumor physiology, including altered metabolism and hypoxia, proliferation, and metastasis. More specific PET agents are capable of targeting the expression of one molecule or gene product, and have the potential to help researchers better understand and assess tumor biology and therapy responses.

For example, the PET radiopharmaceutical ^{68}Ga-PSMA has revolutionized prostate cancer imaging in recent years. The

Prostate-Specific Membrane Antigen found in cell membranes is highly expressed in the prostate and in particular prostate cancer cells, making ^{68}Ga-PSMA very effective in imaging. This tracer has the added advantage of using the PET isotope gallium-68 (Ga-68), which has comparatively lower production costs.

Tumor vascularization and blood flow is another indicator of tumor biology, and its visualization requires radiotracers than can diffuse freely throughout the vascular system and across tumor cell membranes, such as ^{15}O-water, ^{13}N-ammonia, and ^{82}Rb-chloride[1]. Chemotherapy can be used to target the tumor's microvascular system, a therapy which intravenous PET radiotracers can help monitor and optimize.

Development of PET Tracers

Imaging techniques such as PET and SPECT rely on molecular tracers to produce clear images, and the high sensitivity of these technologies is required to detect low concentrations of molecular tracers. In oncology, ^{18}F-FDG is commonly used to detect abnormal glucose metabolism, which might occur in rapidly growing tumors. There are hundreds of other PET radiotracers developed and evaluated for preclinical oncology research[2], with dozens of FDA-approved radiotracers currently available. Theranostics is an area of medicine that integrates diagnosis and therapeutics, and represents a powerful step towards more personalized treatment strategies. Recent developments in molecular biology, proteomics and genetics have significantly enriched understanding of tumor biochemistry and function, including identification of the receptors tumor cells express. In theranostics, these molecular targets can be used to access tumors, image the disease area and deliver targeted cytotoxic substances directly to tumor tissue. In PET and SPECT imaging applications, molecular targets can be combined with radiotracers for both diagnostic and therapeutic purposes. Diagnostic imaging is performed to determine tumor size, classification and stage so that localized radiation can be administered specifically to the diseased area, without damaging any surrounding healthy cells.

A lack of affordable radiotracers has prevented widespread uptake of theranostics and the approach is currently mainly limited to neuroendocrine tumors. The development of new radiotracers is therefore at the forefront of preclinical imaging research, which aims to free up theranostics as a tool for many other types of cancer.

Folate-based Radiopharmaceuticals

One area of focus for preclinical oncology is folate-based radiopharmaceuticals. The folate receptor had limited expression in healthy cells, but is overexpressed in a variety of highly proliferating cancer cells, particularly ovarian and endometrial cancers, making it a key target for detecting and evaluating tumors *in vivo*. Certain properties of folate, including its low molecular weight and non-immunogenicity, make this type of B vitamin an ideal target for cancer imaging, and many radiolabelled folates have been developed for this purpose[3], particularly for SPECT imaging.

Folate-based radiopharmaceuticals for PET imaging are under development, but creating radiolabelled folates with high radiochemical yield, radiochemical purity and favorable pharmacokinetics is challenging. Studies on ^{18}F-alakyne folate indicate this radiofolate as a promising candidate for folate receptor PET imaging, but there remains a need to refine its metabolism profile[4].

Multi-modal PET Technology

Preclinical PET technology has undergone significant transformation since its initial emergence in the 1950s, notably propelled by the development of radiopharmaceuticals. The soft tissue morphological imaging from MRI and anatomical information provided by CT scanning are gaining popularity in preclinical imaging applications, evolving the functional imaging of PET as a standalone technique. PET/CT is a valuable tool in oncology research due to its ease-of-use, high-throughput capabilities and high resolution for bone and pulmonary applications, and has been used extensively to study cancer therapeutics and tumor biology, and for tracer development, since the mid-1990s. Tri-modal systems, such as Bruker's Albira Si, allow users to choose between PET, SPECT, PET/CT, SPECT/CT, PET/SPECT, and PET/SPECT/CT configurations, offering full field accuracy (FFA) and homogenous sub-millimetric volumetric PET resolution.

PET/MR, while less established in preclinical imaging, is gaining ground in oncology due to its ability to image without ionizing radiation, and its potential for multiparametric imaging. Another key benefit is the superior anatomical soft tissue contrast, which offers the unique ability to detect tumor margins, evaluate tracer distribution within individual tumors to generate volume of interest (VOI) and calculate standardized uptake value (SUV) in a range of preclinical models, improving the functional analysis of complementary PET data. Whereas PET and CT data are acquired consecutively, most PET/MR systems acquire data simultaneously, allowing for complex imaging workflows.

The combination of PET and MRI technology can be applied to many processes in tumor biology, such as the role of tumor microenvironment in tumor progression, and used to simultaneously investigate upstream, downstream, and parallel pathways of metabolism to fully characterize these changes and underlying biology.

The Rise of Immuno-oncology

Imaging, together with better understanding of cancer genomics and developments in molecular pathology, are key players in achieving personalized cancer treatment. Over the past 15 years, the field has seen explosive development of imaging technologies, beyond a simple anatomical approach,

towards more complex multi-modal systems. Such systems are expected to become the standard of care within the next few years. The overall aim is to provide patients with more precise cancer treatment, but this is compounded by inter- and intra-tumoral heterogeneity. The move from pure anatomical imaging to molecular imaging with PET has enabled researchers to visualize tumor heterogeneity, which is especially important with regards to administering combination therapies for cancer. PET imaging, in parallel with genomic profiling, could allow for visualization of drug-induced changes in a specific biochemical process, and could provide insights into drug target engagement or alterations in tumor phenotype.

Developing new imaging biomarkers

Researchers at the Institute of Cancer Research, London, led by Dr Gabriela Kramer-Marek, are focused on the development and characterization of imaging biomarkers to inform and guide cancer treatment management for individual patients. The group is studying predictive imaging biomarkers, and biomarkers that help to assess drug resistance and tumor response to drugs, with particular interest in the development of theranostic agents against receptors from the epidermal growth factor tyrosine kinase receptor (EGFR) family. The dimerization of these receptors promotes the activation of downstream signals that initiate and control a wide range of cellular processes, such as proliferation, survival and apoptosis. Overexpression of human EGFR receptors (HER) have been found in many human malignancies and have facilitated the development of target-specific drugs, some of which are currently in routine clinical practice and others in clinical trials.

Among these drugs are signal transduction inhibitors, namely monoclonal antibodies (mAbs) that directly block ligand binding or block receptor dimerization. However, the outcome of many clinical trials have been disappointing given the promising results of preclinical assessment. This could be due to inappropriate selection of potential responders, insufficient inhibition of the receptor, acquired resistance, or activation of parallel pathways. Evaluation of HER status is commonly carried out by immunohistochemistry (IHC), but this method requires invasive biopsies or post-operative tissue sampling, and can be affected by tumor inter- and intra-heterogeneity of receptor expression.

The group is aiming to develop HER-specific molecular imaging probes that could guide innovative therapy plans and rapidly identify potential responders. Different PET tracers have been developed for imaging these receptors, the most common being labelled antibodies. However, their clinical application is limited due to their large molecular size (~150 kDa), long blood circulation, slow clearance, and insufficient tumor penetration. In contrast, small molecules suffer from rapid *in vivo* metabolism, high level of non-specific binding and rapid dissociation from the receptor of interest. The development of Affibody® molecules combats these challenges and is a viable alternative to bulky antibodies as targeting agents. Their small size (~6.5 kDa), simple structure, stability and solubility makes Affibodies ideal candidates for *in vivo* imaging, and there are several Affibody binders available, not limited to HER receptors, that have been radiolabelled for PET and SPECT purposes.

In one recent study, the group used EGFR-specific radioligands to measure EGFR expression in mice with head and neck squamous cell cancer (HNSCC), with the aim to define a predictive biomarker to stratify patients for treatment. Cetuximab is currently the only approved anti-EGFR mAb used for the treatment of HNSCC, and the ability to monitor and assess the drug's efficacy and any cetuximab-mediated changes in receptor expression could help inform appropriate dosing with anti-EGFR antibodies.

The group used a radiolabelled Affibody molecule ($Z_{EGFR:03115}$) to non-invasively measure differences in EGFR expression, using a ^{89}Zr-labeled conjugate to assess tumor-to-organ ratios at different time points, and a ^{18}F-labeled analog to measure the response to cetuximab treatment *in vivo*. To evaluate whether ^{89}Zr-deferoxamine (DFO)-$Z_{EGFR:03115}$ could distinguish between tumors with varying levels of EGFR expression, mice bearing CAL27 (EGFR +++), Detroit562 (EGFR ++), and MCF7 (EGFR +) xenografts received the radiotracer and were imaged three hours after injection using PET/CT (Albira PET/SPECT/CT, Bruker BioSpin). The quantified PET imaging data indicated that the highest levels of radioconjugate accumulation were in CAL27 tumors (Figure 1), which correlated with receptor expression measured *ex vivo* by Western Blot and IHC staining.

Figure 1

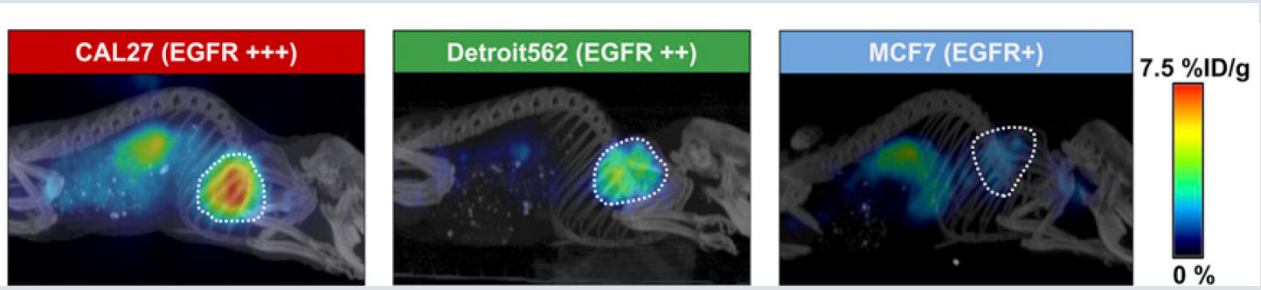

Figure 1: Radioconjugate uptake in xenografts with varying EGFR expression. Representative whole-body sagittal PET/CT images acquired 3 hours after injection. This research was originally published in JNM. Author(s). Title. J Nucl Med. Year;vol:pp-pp. © SNMMI.

To monitor response to cetruximab, ^{18}F-aluminium fluoride (AlF)-NOTA-$Z_{EGFR:03115}$ was administered intravenously to mice bearing HN5 tumors (EGFR ++++), and the group observed significantly lower uptake in ceuximab-treated mice than in control HN5 tumors (Figure 2).

Figure 2

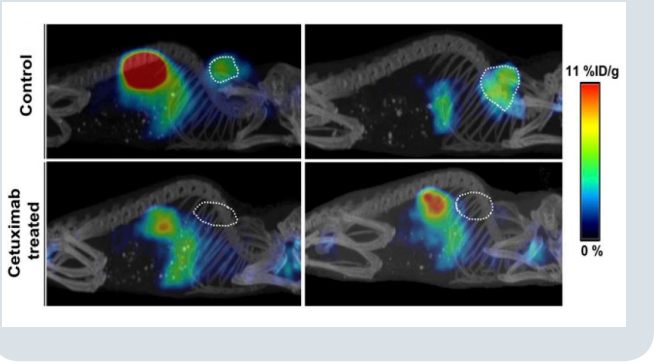

Figure 2: ^{18}F-AlF-NOTA-$Z_{EGFR:03115}$ uptake assessed 1 h after injection. Representative sagittal whole-body PET/CT images of mice bearing HN5 tumors (outlined on image) with or without treatment with cetuximab. This research was originally published in JNM. Author(s). Title. J Nucl Med. Year;vol:pp-pp. © SNMMI.

These results, together with an insignificant change in tumor volume during treatment, highlight the potential for using EGFR imaging as a tool for assessing cetruximab efficacy based on receptor level, rather than relying purely on anatomical imaging, and provide image-guided therapeutic strategies for the clinic.

Continuing Preclinical Oncology Research

The ongoing development of multi-modal PET technology will continue to drive preclinical oncology research. The benefits of PET, PET/MR, PET/CT and PET/SPECT/CT for tracer development, therapy monitoring and studying tumor biology are changing the way cancer is treated, moving towards a personalized medicine approach. The growing importance of immuno-oncology is augmented by the sophisticated PET imaging systems available on the market. Cutting-edge research, such as that at the Institute of Cancer Research, is bringing the field one step closer to personalized treatment by using Affibody molecules as novel PET agents to target specific molecular pathways in tumor progression. Such studies are vital to achieve the ultimate aim of optimizing cancer treatment and patient care.

For more information on Bruker's PET imaging solutions for preclinical oncology research, please visit https://www.bruker.com/applications/preclinical-imaging/oncology.html.

About Bruker Corporation (NASDAQ: BRKR)

Bruker is enabling scientists to make breakthrough discoveries and develop new applications that improve the quality of human life. Bruker's high-performance scientific instruments and high-value analytical and diagnostic solutions enable scientists to explore life and materials at molecular, cellular and microscopic levels. In close cooperation with our customers, Bruker is enabling innovation, improved productivity and customer success in life science molecular research, in applied and pharma applications, in microscopy and nanoanalysis, and in industrial applications, as well as in cell biology, preclinical imaging, clinical phenomics and proteomics research and clinical microbiology. For more information, please visit: www.bruker.com.

References

[1] Croteau E, Renaud JM, Richard M, Ruddy TD, Bénard F and deKemp RA (2016) PET Metabolic Biomarkers for Cancer, *Biomarkers in Cancer*, 8(S2): 61-69.

[2] Molecular Imaging and Contrast Agent Database (MICAD) [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2004-2013 (https://www.ncbi.nlm.nih.gov/books/NBK5923/).

[3] Muller C, Schibli R (2011) Folic acid conjugates for nuclear imaging of folate receptor-positive cancer. *J Nucl Med.* 52:1-4.

[4] Schieferstein H, Ross TL (2014) A polar ^{18}F-labeled amino acid derivative for click labeling of biomolecules. *Eur. J. Org. Chem.* 17: 3546-3550.

www.bruker.com ● **Bruker BioSpin**
info@bruker.com