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The Role of Molecular Imaging in Personalised Healthcare

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Abstract: Functional molecular imaging provides a unique perspective on a disease. Methods including positron-emission tomography and magnetic resonance imaging allow us to interrogate spatial and temporal changes in biomarkers as well as probe the underlying biochemistry. When imaging is combined with molecular diagnostic tools, opportunities arise for measuring aberrant cellular signalling pathways with unprecedented detail. This brief commentary illustrates how radiotracers and nuclear imaging methods are being developed to monitor drug efficacy and simultaneously support the goal of personalised healthcare.

Keywords: Cancer · Molecular imaging · Oncogenic signalling · Personalised medicine · PET

Jason Holland is from Yorkshire in the United Kingdom and received a master's degree in Chemistry from the University of York (MChem, 2004) followed by a doctorate from the University of Oxford (D.Phil, 2008). He trained as post-doctoral scholar with Jason S. Lewis in radiochemistry and translational molecular imaging at Memorial Sloan-Kettering Cancer Center in New York (2008–2010). He was then awarded an ETH Fellowship and worked at ETH Zürich and the Paul Scherrer Institute (2010–2012). He was then appointed as Assistant in Chemistry in the Division of Nuclear Medicine and Molecular Imaging at Massachusetts General Hospital, Boston, with a joint faculty appointment as an Instructor in Radiology at Harvard Medical School (2012–2014). In 2015, he worked in the Department of Nuclear Medicine at the University Hospital Freiburg as a Visiting Scientist. In April 2016, Jason began his research group as an SNSF Professor in the Department of Chemistry at the University of Zurich, funded by the European Research Council and the Swiss National Science Foundation. Research activities focus on advancing radiochemical methods with various radionuclides (^{18}F, ^{64}Cu, $^{67/68}$Ga, $^{86/90}$Y, ^{89}Zr, ^{111}In, ^{177}Lu, *etc.*), and developing molecular imaging agents that target oncogenic signalling pathways in cancer.

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Introduction

Recent years have witnessed an increased scientific, clinical and political emphasis on delivering measureable benefits to individual patients, and to overall population health, through 'personalised medicine' (PM). Unprecedented advances in various '-omic' technologies have led to exponential growth in our understanding of the pathobiology of disease.[1] In turn, a deeper knowledge of disease mechanisms provides inspiration for developing innovative diagnostic and therapeutic solutions that promise to improve treatment efficacy and patient outcomes across medicine. In this setting PM, which is often considered synonymous with other terms including 'precision medicine', 'targeted medicine', or 'stratified medicine', provides the ideological framework around which many of the latest medical advances are being shaped.

It is important to note that PM is not a new concept. Hippocrates (c. 460 BC – c. 370 BC) famously postulated that, "*It is far more important to know what person the disease has than what disease the person has.*"[2] Many clinicians would also argue that medicine has always been 'personalised' – doctors treat patients on an individual basis and thus, at the point care, medicine is inherently personal. However, modern PM is evolving rapidly and goes beyond simply recognising patients as individuals. Perhaps the most scientifically rigorous definition of PM was provided by the National Institute of Health (NIH) which stated that PM, "*… uses information about a person's genes, proteins and environment to prevent, diagnose and treat disease.*"[3] A more approachable definition that captures the goals of PM was provided by the European Alliance for Personalised Medicine which noted that PM is about, "*The right prevention and treatment for the right patient at the right time.*"[4] Beyond these statements lies the current concept of PM which is to integrate vast amounts of patient-specific and population-based data with the aim of providing a more accurate and complete picture of not only the current status but the entire disease lifecycle (Fig. 1). In this regard, PM is a dynamic process. It facilitates informed decision-making, helping clinicians select the most appropriate therapeutic regimen at a given time, and simultaneously provides accurate, active monitoring to help tailor the treatment toward the most effective solution for a single patient.

Data integration lies at the heart of PM. Data sources include traditional medical records and family histories, through to population-based information on disease aetiology and epidemiology. During consultation and diagnosis, these physiologic data are usually combined with a range of standard *in vitro* diagnostic (IVDx) assays including biomarker measurements from serum or biopsy studies, and histological information from tissue samples that link disease symptoms with the underlying biochemistry. Nowadays, the latest '-omic' technologies provide increasingly detailed information on disease status at all levels from single-cell genetics through to

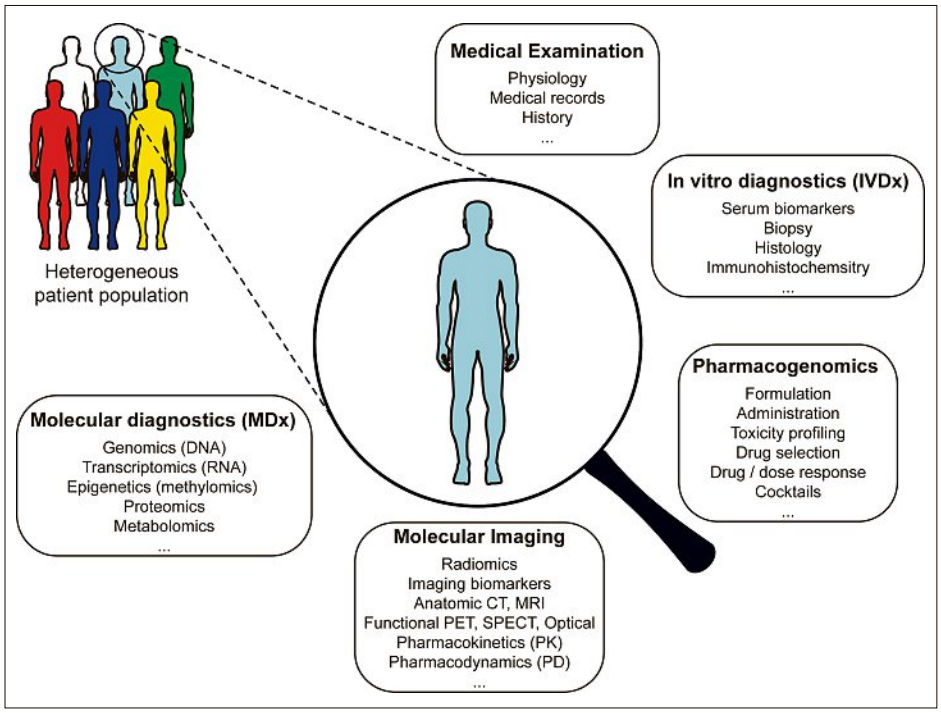

Fig. 1. Illustration of the key components that constitute practical application of PM. The concept is to integrate clinical data from many sources including medical records, patient histories, population studies, and *in vitro* diagnostic (IVDx) tools, with the latest information from molecular diagnostics (MDx) and imaging to provide clinicians with the most accurate pharmacogenomic picture for tailoring individual treatment.

tissue and whole organisms phenotypes. Categorised as a broad spectrum of molecular diagnostics (MDx), these new technologies span the fields of genomics (DNA), transcriptomics (RNA), and epigenetics[5] (Me-DNA, methylomics), through to proteomics and metabolomics.[1] Anatomic imaging in the form of computed tomography (CT) or magnetic resonance imaging (MRI), as well as functional molecular imaging (MI) using single-photon emission computed tomography (SPECT), positron emission tomography (PET) and various optical methods, are vital tools that provide a visual link between changes at the molecular, tissue and organism levels.[6] Collectively, these diagnostic data offer an improved understanding of the pharmacokinetic (PK) and pharmacodynamic (PD) profiles of drug response. Thus, by combining IVDx, MDx and imaging with pharmacogenomics, it is (theoretically) possible to monitor the entire course of therapy and to respond actively in a way that leads to the optimum outcome for a patient.

PM, and indeed molecular imaging and radiochemistry, are too broad to be covered in detail in this commentary. Therefore, the following sections focus on the role that functional molecular imaging plays in advancing the practical application of PM in the clinic. Emphasis is placed on illustrating the use of PET for detecting changes in oncogenic signalling pathways in various cancers.

Molecular Imaging and Personalised Medicine

Molecular imaging plays a vital role in PM.[7–9] Images are inherently personal and at a basic level can assess the location, size and severity of a lesion. Imaging data can also be recorded (and sometimes processed) in real-time to provide spatial and temporal information about the way in which the body interacts with a drug (PK) and how the drug affects the biochemistry of the recipient (PD). Imaging methods including PET, SPECT and functional MRI can be used as a companion diagnostic (CDx) for staging a disease/lesion, as a prognostic indicator of disease progression, and for stratifying patients by predicting likely response to therapy. Further, imaging provides a non-invasive way of monitoring the outcome of therapy.

The quality of information provided by imaging is highly dependent on i) the choice of imaging biomarker, and ii) the physical, biochemical, and pharmacological characteristics of the imaging agent.

Serum Biomarkers versus Imaging

To highlight the power of imaging, consider a patient undergoing therapy to treat a disseminated disease (Fig. 2). After treatment, responders typically present with an overall decreased disease burden including fewer and/or smaller lesions, with a lower grade or less aggressive phenotype (green). In the ideal case, responders are completely disease-free after treatment. Non-responders show either no change in disease status or may exhibit higher disease burden with an increased number of lesions, increased lesion size, or transformation to a more aggressive phenotype (red). Partial responders, including patients who develop subsequent resistance to the therapy or those who relapse, can exhibit a heterogeneous response. This can include complete loss or presentation of new disease foci, as well as lesions that decrease in size and/or become less aggressive, dis-

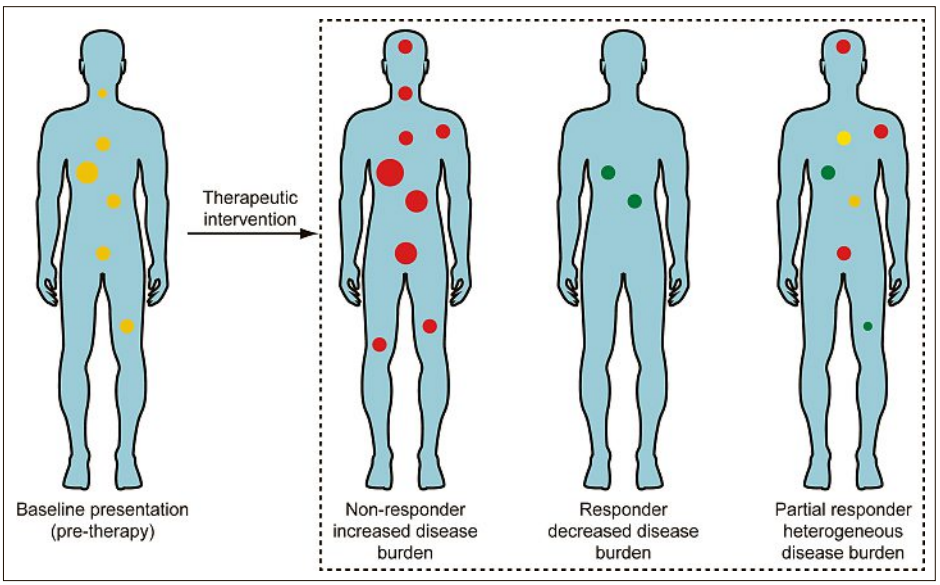

Fig. 2. Illustration of the potential range of response for a patient undergoing therapy to treat a disseminated disease. Partial responders exhibit a heterogeneous response to therapy which may include lesions that decrease in size and/or become less aggressive (green), lesions that display no change (yellow), lesions that increasing in size and/or transform to a more aggressive phenotype (red), complete loss and/or presentation of new disease foci.

play no change, increasing in size and/or transform to a more aggressive phenotype.

Concentration levels of serum biomarkers are commonly assessed using a range of IVDx tools. For example, blood pool concentrations of prostate-specific antigen (PSA; kallikrein III) are commonly used as a risk factor for prostate cancer (PCa). Although still experimental, PSA concentrations generally show a positive correlation with PCa staging (Gleason score).[10,11] PSA concentrations below 4.0 ng/mL are considered normal. Measurements between 4–10 ng/mL are suspicious and these patients are usually referred for additional testing. PSA values between 10–20 ng/mL indicate an intermediate risk factor, whereas values >20 ng/mL represent high risk and often correlate with Gleason score ≥8. Consider a patient that exhibits a partial response to chemotherapy (*e.g.* using antiandrogens like non-steroidal flutamide, nilutamide or enzalutamide, *etc.*); hypothetically, individual tumour lesions that respond to treatment could correlate with decreased PSA expression. In contrast, lesions that do not respond, grow and/or transform to a more aggressive phenotype could correlate with either no change or elevated PSA (Fig. 3). For partial responders that have a heterogeneous disease burden, serum biomarker measurements can be misleading. Put another way, it is impossible for single measurement of a blood pool biomarker to discriminate between a patient in which 100% of the tumours show a 50% response, from a patient in which 50% of the tumours show a 100% response. This situation is where whole-body, non-invasive molecular imaging presents an advantage over classical IVDx assays.

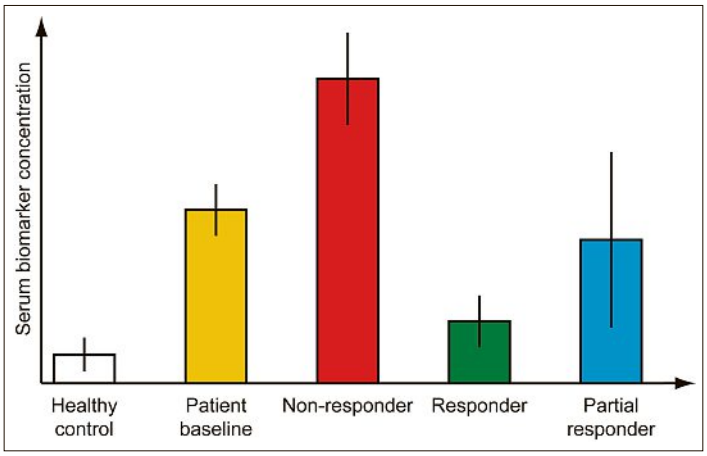

Fig. 3. Schematic bar chart presenting a hypothetic change in the concentration of a serum biomarker that exhibits a rapid change and strong positive correlation with disease burden and/or aggression in non-responders (red), responders (green) and patients that display partial response to therapy (blue).

Imaging Biomarkers

It should be noted that while overlap exists between biomarkers used in IVDx, MDx and imaging, in general, optimal imaging biomarkers do not necessarily have the same characteristics as those used in other experiments. Identification of new 'imageable' biomarkers is a priority in basic science and clinical research.

Biomarkers can usually be classified as either prognostic, predictive or pharmacodynamic based on the type of the information that their detection provides.[12,13] Prognostic biomarkers indicate the risk of disease or likelihood of progression. For instance, many genomic mutations can be considered as prognostic risk factors toward the development of different cancers.[14] Examples include mutated BRCA1 and BRCA2 genes in breast, ovarian and prostate cancers, RB1 in retinoblastoma, mutations in the Von Hippel-Lindau (VHL) gene in renal cancers, or Lynch syndrome (also called hereditary non-polyposis colorectal cancer) which is caused by faults in the MLH1, MSH2, MSH6 and PMS2 genes and correlates with increased risk of developing several cancers including (among others) ovarian, uterus endometrial, colorectal, gastric and renal.

Predictive biomarkers indicate the likely response of a lesion toward therapy. For example, it is estimated that between 20–30% of breast cancer patients overexpress the human epidermal growth factor receptor 2 (HER2).[15,16] Imaging trials using ^{89}Zr-trastuzumab[17] and other PET radiotracers show promise for identifying patients that display elevated HER2 expression and may potentially benefit from immunotherapy using Herceptin® (trastuzumab) or Perjeta® (pertuzumab).[18,19]

In 2011, Yeh *et al*. reported an elegant example of developing a PET radiotracer, [^{18}F]F-PEG6-IPQA, for detecting the L858R/T790M dual mutant epidermal growth factor receptor (EGFR) in non-small cell lung cancer (NSCLC).[20] The L858R/T790M dual mutation is located in EGFR kinase domain and confers resistance to EGFR inhibitors (*i.e.* gefitinib). At the same time, the T790M mutation prevents the irreversible binding of [^{18}F]F-PEG6-IPQA to EGFR. Thus, PET imaging with [^{18}F]F-PEG6-IPQA can potentially predict response or resistance to anti-EGFR therapies.

A portion of our work is dedicated toward the development of radiotracers that image changes in oncogenic signalling in various cancers.[21] To achieve this, we target PD biomarkers.[13] A schematic representation of cellular signalling, and possible sources of targets for radiotracer imaging is shown in Fig. 4. In general, PD biomarkers are usually proteins that show differential expression during disease progression and/or therapeutic intervention. Targets can be selected from either upstream or downstream components of the signalling pathway of interest. For example, if the concentration of a predictive biomarker (usually chosen as an overexpressed, upstream component of a signalling pathway) changes during the disease lifespan or treatment, we can potentially image and correlate this change with alterations in tissue biochemistry and drug efficacy. Inhibition of a particular signalling pathway can alter DNA transcription and protein expression which, *via* positive or negative feedback loops, can modulate biomarker concentration. In this context, the predictive biomarker also doubles as a PD biomarker. As a practical example, PET imaging of human epidermal growth factor receptor 2 (HER2/*neu*) has been used as a PD biomarker correlating with the efficacy of different heat shock protein 90 (HSP90) inhibitors in breast cancer models.[17,22]

Alternatively, PD biomarkers can be selected from proteins that are expressed downstream of a particular signalling pathway. The key criterion is that the expression level of the downstream PD biomarker should correlate with the parameter of interest. These biomarkers can be used as surrogates for detecting changes in the activation or inhibition of enzymes, signal transduction, or transcription factor activity. Similarly, PD biomarkers can correlate with broader cellular processes like growth and proliferation, survival, or apoptosis, *etc*. Essentially, the only prerequisites for surrogate imaging of PD biomarkers are, i) developing specific and sensitive imaging agents that allow for quantitative detection, and ii) understanding the biochemical mechanisms of how target expression correlates with the parameter of interest. If these conditions are met, then imaging data can provide a sensitive, lesion-specific readout of whether the drug has hit the target and if the dose administered was sufficient to elicit a pharmacologic response. Imaging PD biomarkers can also show early onset of resistance thereby allowing clinicians to adapt the treatment regimen accordingly.

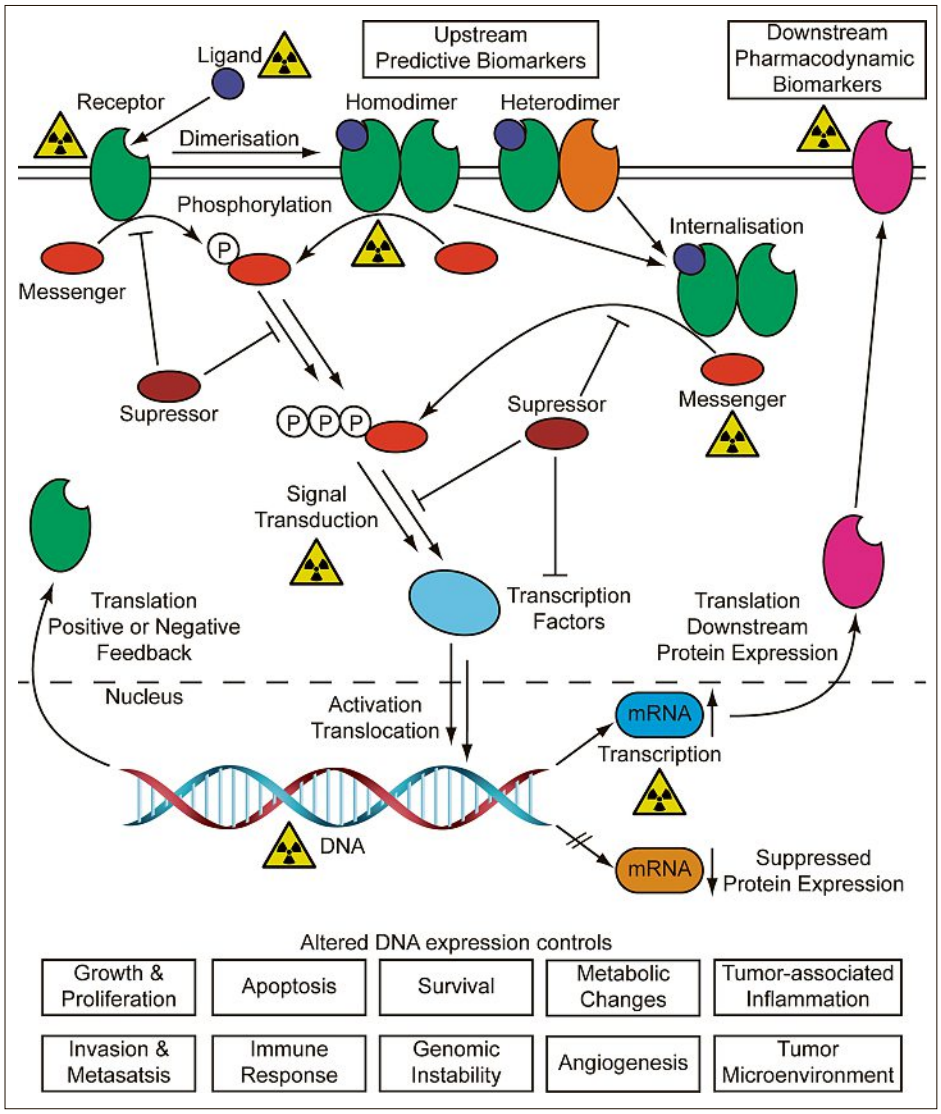

Fig. 4. Schematic flow of information during intracellular signalling from ligand-receptor interactions, messenger and transcription factor activation, regulation of DNA expression and protein translation. Potential sources of 'imageable' predictive and PD biomarkers that can be used in the development of radiotracers are highlighted with a radioactive symbol.

Imaging Pharmacodynamic Biomarkers in Prostate Cancer

This section highlights two examples that show how surrogate imaging of PD biomarkers is being used to monitor disease status and treatment in prostate cancer (PCa).

Imaging Androgen-receptor Signalling and Antiandrogen Therapy in PCa

Together with colleagues at Memorial Sloan-Kettering Cancer Centre in New York, we are developing an imaging protocol for measuring response to antiandrogen therapy in PCa patients (Fig. 5). Androgen-receptor (AR) signalling is a key driver of PCa. Prior to transformation toward a castrate-resistant (or hormone refractory) phenotype, antiandrogen therapies (*e.g.* flutamide, enzalutamide, *etc.*) have an increased likelihood of working. Therefore, the goal was to develop a PET imaging protocol that could be used to measure both AR levels (in the sense of a predictive biomarker for estimating response to therapy and monitoring drug-target engagement) and AR activity using a PD biomarker to measure if the drug concentration delivered to the target is sufficient to elicit a pharmacologic response.

In preclinical work, Evans *et al.* demonstrated that activation of AR signalling by natural ligands including testosterone (Test.) or dihydrotestosterone (DHT) leads to suppression of cellular surface biomarker prostate-specific membrane antigen (PSMA) expression.[23] Conversely, AR-inhibition using antiandrogens including MDV3100 (enzalutamide) increases PSMA expression (Fig. 5A). Preclinical immuno-PET imaging using an antibody-based radiotracer administered to mouse models bearing subcutaneous, AR-positive, human xenografts confirmed that, in comparison to the non-treated (No Tx) vehicle control, PET signals in tumours decreased by ~35% in animals that received supplementary testosterone. In contrast, treatment with MDV3100 led to an approximate 80% increase in radiotracer accumulation. Subsequently, we developed and translated the immuno-PET radiotracer, ^{89}Zr-DFO-J591 for measuring PSMA expression (Fig. 5B).[24–26] In combination with the well-established radiotracer, ^{18}F-FDHT[27] (16β-^{18}F-fluoro-5α-dihydrotestosterone; Fig. 5C), we are evaluating a novel molecular imaging algorithm (Fig. 5D) for measuring AR expression, drug binding and efficacy. In this protocol, baseline ^{18}F-FDHT PET can be used to predict if a patient (or a specific lesion) will respond to antiandrogen treatment. During treatment, further ^{18}F-FDHT scans can monitor drug-target engagement. Here, a high PET signal on a post-treatment ^{18}F-FDHT scan is potentially indicative of either sub-optimal dosing, phenotypic transformation or the onset of resistance toward antiandrogens. Antiandrogen dose can (theoretically) be adjusted based on the imaging data. However, simply hitting the target does not mean that the desired pharmacologic response (*i.e.* inhibition of the AR-signalling pathway) has occurred. PET imaging of PSMA can be used as a PD biomarker that correlates with AR-signalling, allowing clinicians to address the question, "*Is the drug working?*". In these PSMA scans, the inverse correlation between AR-signalling and PSMA expression means that a high PET signal intensity suggests that the drug is working. In contrast, a low PET signal indicates that the AR-pathway remains active. This mechanism for monitoring antiandrogen response has recently been studied in a pilot human image using the small-molecule radiotracer ^{68}Ga-PSMA-11.[28] Notably, we have also extended the technology to include ^{89}Zr-DFO-5A10 immuno-PET imaging of AR-signalling *via* measuring the expression of tissue-localised PSA.[21,29]

In practice, a molecular imaging algorithm that involves multiple PET scans acquired using two or more different radiotracers is technically challenging. However, as with IVDx tests, no single image/radiotracer can be expected to provide all the information required to characterise disease status in full. Our ability to detect and interpret temporal changes in several biomarkers is crucial to the future of PM. In this regard, imaging AR expression *via* ^{18}F-FDHT PET, and AR-signalling *via* PSMA represents a positive step toward multi-parametric imaging protocols.

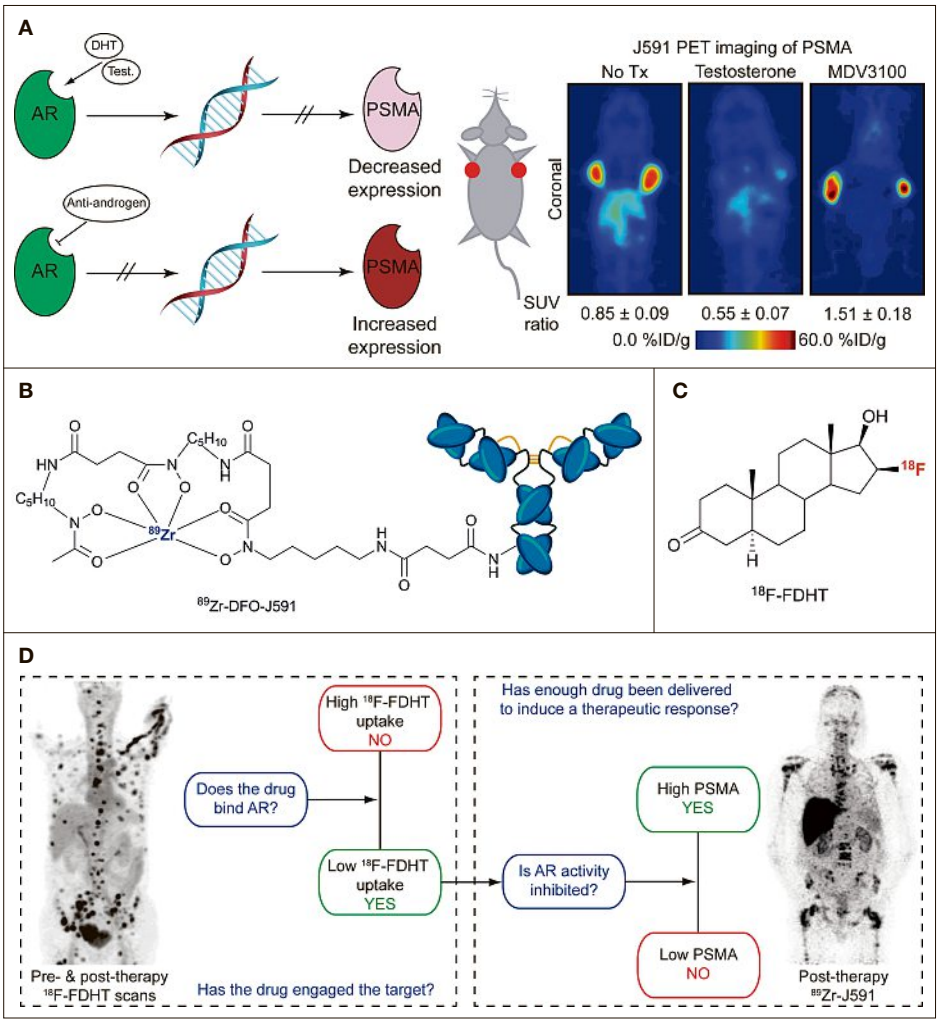

Fig. 5. Imaging AR signalling and antiandrogen therapy in PCa. (A) Schematic of AR-signalling in relation to inverse PSMA expression and proof-of-concept PET images showing the effects of MDV3100 (Enzalutamide) on PET radiotracer uptake. (B) Structure of ^{89}Zr-DFO-J591. (C) Structure of ^{18}F-FDHT. (D) A molecular imaging algorithm for measuring AR-inhibition and drug efficacy in PCa patients.

Imaging MYC Transcription Factor Activity

Studies have revealed that 30–70% of PCa patients display loss of the PTEN tumour suppressor gene and elevated signalling *via* PI3K/Akt.[30] Similarly, ~30% of PCa patients are found to have a gain in copy number and activity of the transcription factor MYC.[31] These signalling pathways play vital roles in cell survival, growth, proliferation and metastasis.[21,32]

Since the 1970s, many attempts to target MYC met with limited success, primarily because transcription factors are in low abundance and MYC lacks any recognisable ligand binding sites as a basis for drug discovery. However, in 2010 Bradner and co-workers reported the structure and activity of JQ1, a first-in-class inhibitor of the bromodomain and extra-terminal domain (BET) protein, BRD4 (Fig. 6).[33] BET proteins (like BRD4) regulate gene expression by binding to acetylated histones and alter chromatin structure. JQ1 inhibition of BRD4 (and potentially other BET proteins [BRD2-4]) was found to induce down-regulation of MYC transcription *via* the loss of BRD4 at the MYC promoter site.[34] Consequently, reduced levels of MYC also led to reduced expression of MYC target genes including the transferrin receptor (CD71 or TRFC).[31]

Recognising the central role of MYC and PI3K/AKT signalling in various cancers, we developed ^{89}Zr-transferrin (^{89}Zr-Tf) to detect changes in these signalling pathways *via* detection of downstream TRFC (Fig. 7). Proof-of-concept studies using a transgenic mouse model that displays high levels of MYC expression/activity in the dorsolateral prostate demonstrated that ^{89}Zr-Tf imaging of TRFC correlated with changes in MYC-signalling in PCa.[35] In addition, we found that ^{89}Zr-Tf can detect differences in PI3K signalling in gliomas.[36] Collectively, these studies provided convincing evidence that TRFC can be used as a PD biomarker of MYC and PI3K-signalling, and also suggested that ^{89}Zr-Tf could be used to monitor therapies that target these pathways. In 2016, this hypothesis was confirmed by Doran *et al.* who showed that ^{89}Zr-Tf imaging provides a diagnostic measure of JQ1 activity in lymphoma.[37] Efforts are now underway to translate this technology to the clinic.

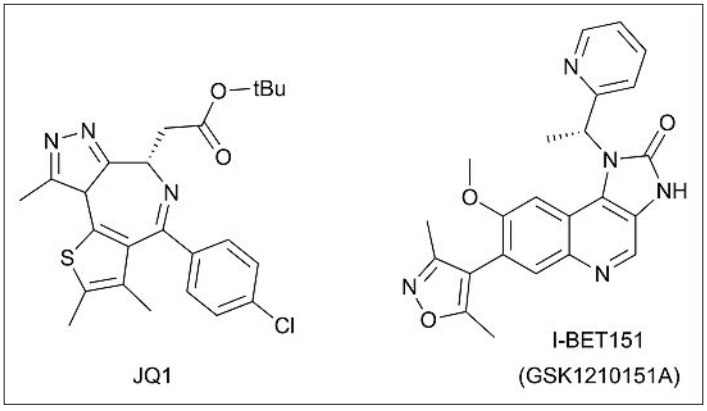

Fig. 6. Structures of BET inhibitors JQ1 and I-BET151 (GSK1210151A).

Methods in PET Radiochemistry

This section provides a flavour of the types of chemistry used in the synthesis of PET radiotracers. The discussion is restricted to ^{18}F and radiometal nuclides, but it should be noted that work with ^{11}C, ^{13}N and ^{15}O are also active areas of PET research.[38] A schematic showing the process of positron (β^+) emission and subsequent annihilation to give two antiparallel γ-rays is shown in Fig. 8. Coincident detection of two 511 keV γ-rays is the physical basis of PET imaging. However, the true value of PET resides in our ability to synthesise positron-emitting radiotracers that bind to specific targets with high affinity.

Fluorine-18 Radiochemistry

The range of chemical reactions that can be used to incorporate a radionuclide into a molecule is very diverse.[38] Yet in radiochemistry with PET nuclides such as ^{18}F ($t_{1/2}$ = 109.7 min) or radiometals like ^{64}Cu ($t_{1/2}$ = 12.7 h), ^{68}Ga ($t_{1/2}$ = 67.7 min) and ^{89}Zr ($t_{1/2}$ = 78.41 h), limitations arise that are not encountered in traditional non-radi-

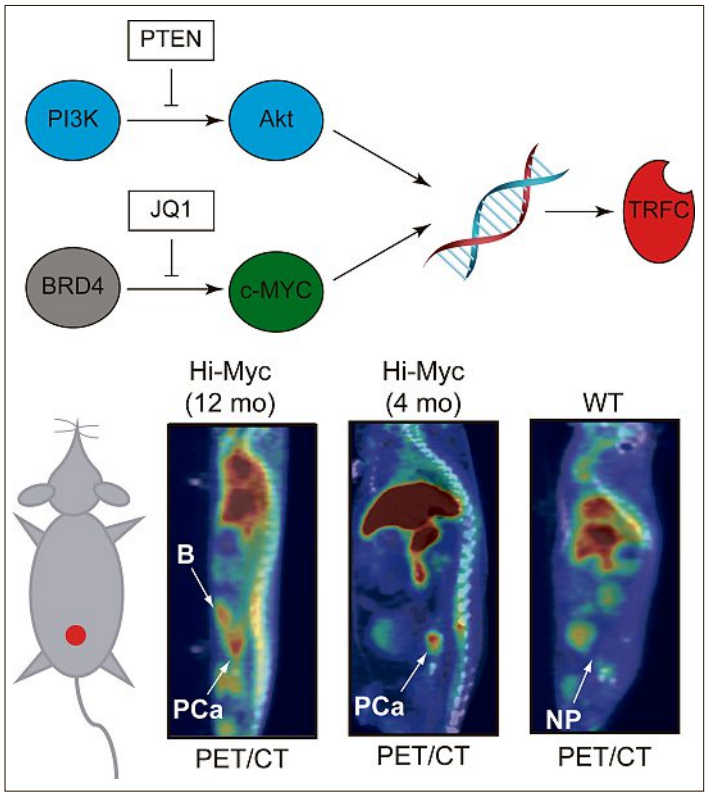

Fig. 7. Schematic showing how PI3K/AKT and BRD4/MYC signalling relates to changes in expression of the transferrin receptor (CD71 or TRFC). PET images demonstrate proof-of-concept for imaging changes in MYC transcription factor activity in a transgenic mouse model that displays high levels of MYC signalling in the dorsolateral prostate.

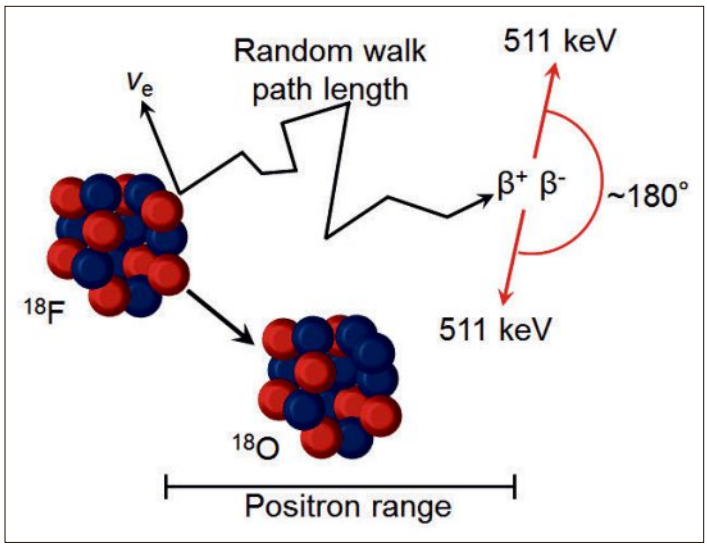

Fig. 8. Schematic showing the decay of an ^{18}F nucleus releasing the daughter nuclide ^{18}O, a neutrino (υ_e) and a positron (β^+). Positron-electron annihilation emits two 511 keV γ-rays at approximately 180°. Coincident detection of these two γ-rays is the basis of PET imaging.

oactive synthesis.[39] These challenges derive from the radioactive decay properties of the nuclide.[39] Considerations including the half-life, particle energies and type of emissions, radiolysis, specific activity, available chemical forms of the nuclide, and slow reaction kinetics at pico-molar concentrations, often transpire as restrictions in the chemical methods that may be utilised in radiosynthesis. To illustrate, after more than four decades of development, synthesis of ^{18}F-radiotracers is dominated by late-stage incorporation of the nuclide using mainly one-step nucleophilic (aromatic S_NAr and aliphatic S_N2) substitution reactions (Fig. 9).[38] For molecules where direct labelling is not practical, prosthetic group methods can be used but typically at the expense of performing time-consuming, multi-step reactions which compromise yields and reduce specific activities.[40] Use of bulky, lipophilic prosthetic groups also incurs the risk of altering the radiotracer properties (binding affinity, specificity or PK/PD profiles, *etc.*) through excessive modification. Recent advances in ^{18}F-radiochemistry include the use of *p*-block elements as scaffolds such as boron trifluorides,[41] silicon fluorides[42,43] or even metal-mediated 'AlF' chemistry where ^{18}F reacts with Al^{3+} ions, followed by chelation using macrocyclic chelates conjugated to a biological vector (peptide or protein) to give the corresponding 'Al^{18}F'-complex.[44] Metal-based catalysts for ^{18}F-labelling of substituted aromatics have been reported including Pd(IV)[45] and Ni(II)[46] agents for 'electrophilic' labelling and copper-catalysed substitutions using iodonium(III) salts.[47–49] Studies have also explored the potential of iodonium(III) ylide chemistry for nucleophilic ^{18}F-labelling of challenging drug-like synthons containing electron-rich (deactivated) aromatic substrates.[50–53]

Metal-based Radiochemistry

In many ways, the radiochemistry of metal nuclides remains underdeveloped. With few exceptions, production of metal-based radiotracers entails the use of an organic chelating ligand.[54,55] For example, the ubiquitous *aza*-macrocycles DOTA and NOTA, as well as bicyclic versions like CB-TE2A are useful for complexing various nuclides including ^{64}Cu, ^{68}Ga, ^{177}Lu and ^{225}Ac, *etc.*; EDTA and DTPA are powerful ligands for the SPECT and Auger electron emitter ^{111}In, and desferrioxamine B (DFO) is the ligand of choice for complexing $^{89}Zr^{4+}$ ions (Fig. 5B *vide supra*).[39,55] Typically, the chelate is first conjugated to the biological vector (through standard coupling reactions like peptide bond synthesis, maleimido-chemistry or 'click'-reactions, *etc.*), and then after purification, the radiometal ion is introduced in the final step.[40] State-of-the-art research on radiometals is focused on developing new radiochemical reactions, accessing alternative nuclides (like the positron-emitter ^{90}Nb, or alpha-emitters ^{223}Ra, ^{213}Bi and ^{225}Ac), and designing new ligands with improved coordination properties, increased metal ion selectivity, and/or increased stability *in vivo*.

Nanoparticles in Radiochemistry and Imaging

As macromolecular scaffolds for developing drugs and MI agents, nanoparticles offer unparalleled opportunities for exploiting their chemical and structural features. Nanoparticle characteristics include enhanced rigidity, controlled shape and size, defined electromagnetic properties, high surface area, variable porosity, resistance to metabolism *in vivo*, and tuneable chemical reactivity at the surface, on coatings and inside the particle core.[56–64] The majority of current methods for labelling nanoparticles derive from well-established ^{18}F-labelling or radiometal chelation chemistries (Fig. 10).[65,66] Available methods can be broadly classified as either surface-based or coating-based modifications. Briefly, surface modifications include physisorption through electrostatic interactions, direct chemisorption with discrete chemical bond formation (*c.f.* As(III)/As(V) chemistry[67]), direct attachment of a radiolabelled species (*e.g.* $-SCH_2CH_2CH_2{}^{18}F$), and pre-surface modification with subse-

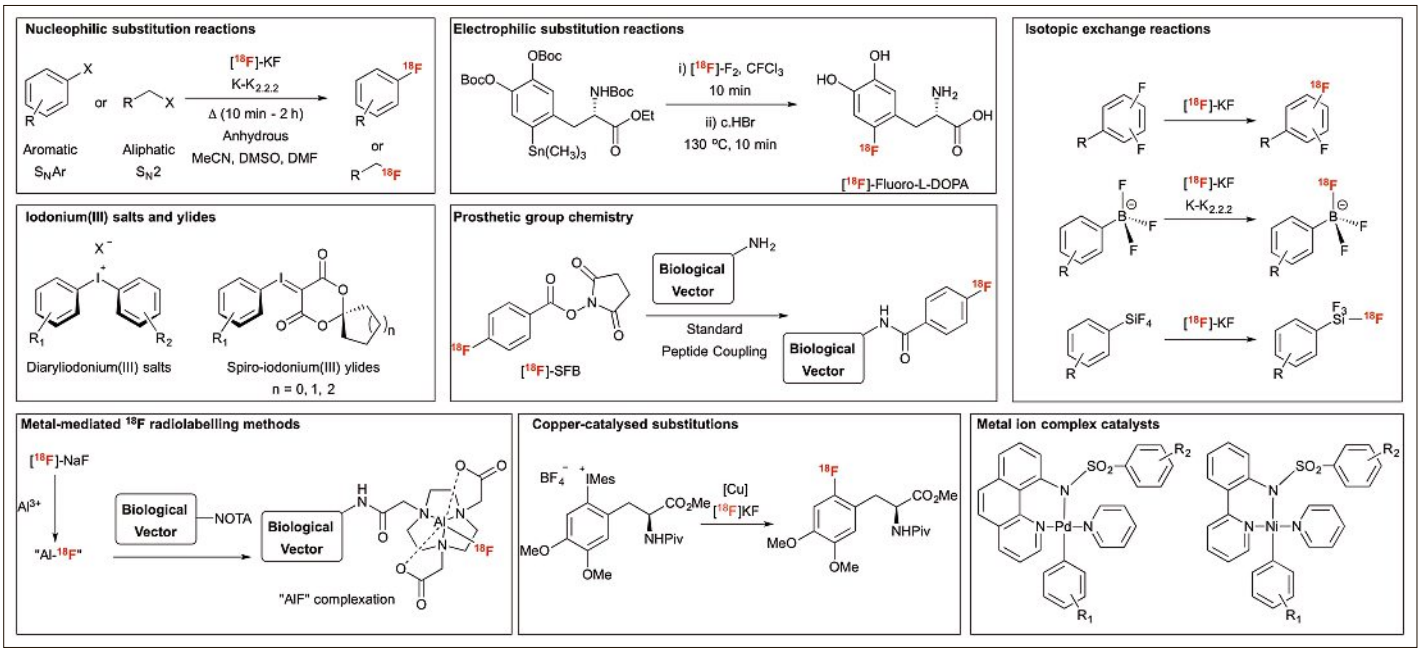

Fig. 9. Chemical scope of ^{18}F-radiolabelling methods used in current preclinical and clinical syntheses. Note: the figure is not intended to be a comprehensive. The examples presented highlight some of the modern techniques employed by the radiochemical community in synthesising PET radiotracers.

quent radiolabelling *via* a prosthetic group (*e.g.* use of [^{18}F]-SFB, or ligand conjugation followed by radiometal chelation). Labelling the nanoparticle coating can be accomplished by using similar direct methods, prosthetic group chemistry, or metal complexation strategies.[64,68,69]

Particle- or surface-specific reactions have only recently been exploited for radiolabelling. In 2014, Goel *et al.* noted the importance of the emerging area of intrinsically labelled nanoparticles.[70] A handful of articles have now reported the use of particle-specific chemical reactions to radiolabel nanoparticles *without* the use of a chelate or prosthetic group. In their pioneering work on chelate-free radiochemistry, Chen *et al.* reported labelling USPIO particles with arsenic radionuclides *via* a surface-specific reaction.[67] Other examples include the development of USPIO-based multi-modality PET/MRI agents using intrinsically labelled $^{68/69}$Ge nanoparticles,[71] synthesis of dextran-coated ^{64}Cu-doped iron oxide nanoparticles,[72] ^{64}Cu-labelled quantum dots,[73] CuS sulphide nanoparticles,[74] Au nanorods,[75] MoS_2 nanosheets[76] and various ^{89}Zr-labelled compounds.[77–80] Recent work has also demonstrated intrinsic labelling of silica particles with a range of metal radionuclides.[81,82] In many of these reports, the researchers highlighted that these agents showed promise for use in PET and/or dual-modality PET/MRI imaging. However, most of these approaches lack generality or have important drawbacks when labelling *pre-fabricated* nanoparticles.[83]

To address the challenges of radiolabelling pre-fabricated nanoparticles, we developed a novel, heat-induced, chelate-free labelling reaction.[83] Remarkably, we found that metal ions in various oxidation states and taken from across the *p*-, *d*- and *f*-block groups can bind to the magnetite crystal core without using a chelate. Detailed studies on the mechanism of binding are underway and it is likely that metal ion affinity is mediated *via* binding to oxides on the surface of the ultra-small super paramagnetic iron oxide (USPIO) core. The combination of facile radiolabelling chemistry with USPIO-based nanoparticles opens the potential for developing multi-modal agents for simultaneous imaging with both PET and MRI.

Summary and Outlook

It is safe to say that personalised healthcare and molecular imaging go hand-in-hand. Economic and social pressures mean that early-stage validation of drug efficacy is crucial in clinical trials, and in assessing response in patients re-

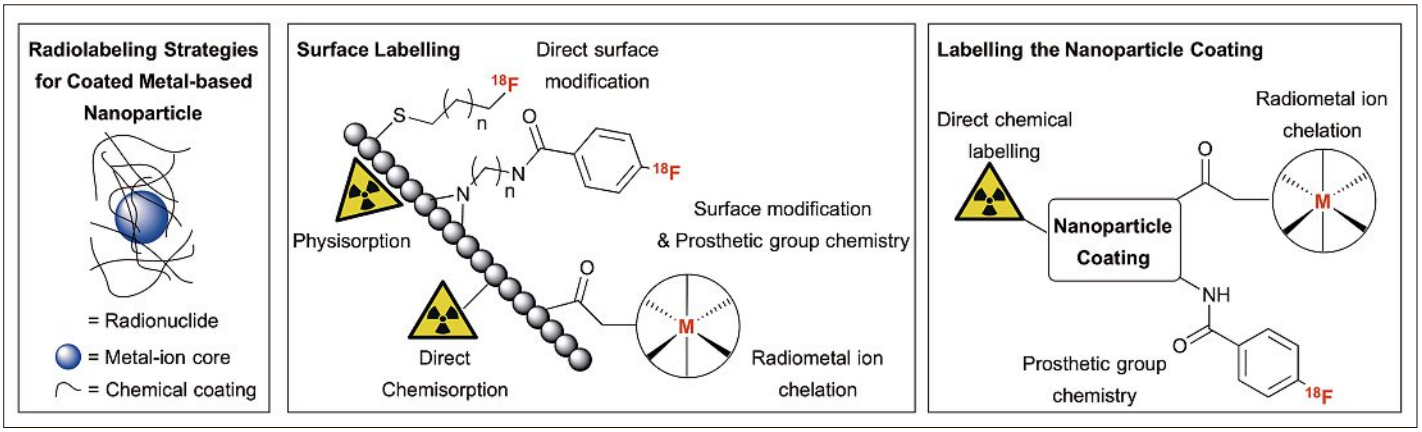

Fig. 10. Chemical approaches for labelling the surface or coating of nanoparticles.

ceiving approved therapies. The gamut of '-omic' technologies provide valuable *in vitro* data that complements classical IVDx tools. MDx methods are analogous to taking photographic snapshots – they provide high-resolution data and can characterise many biomarkers simultaneously, but have inherent limitations measuring spatial and temporal dynamics. On the other hand, molecular imaging offers the advantage of measuring lesion-specific, spatial and temporal changes in throughout the whole body, but is often limited to the detection of a single biomarker. Nevertheless, the examples presented here highlight the power of using imaging to correlate changes in oncogenic signalling pathways with target-specific therapies. The stage is now set for combining '-omic' technologies with molecular imaging tools for exploiting disease-specific signatures in cancer and beyond. In addition, combined imaging modalities like PET/MRI will advance our understanding of pathobiology and pharmacogenomics. For radiochemists, the challenge remains to identify new chemical reactions and develop radiotracers that maximise the information available from functional molecular imaging.

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