British Journal of Pharmacology

DOI:10.1111/j.1476-5381.2010.01160.x
www.brjpharmacol.org

Themed Section: Imaging – the Interface with Pharmacology

REVIEW

Development of radiotracers for oncology – the interface with pharmacology

Rohini Sharma[1,2] and Eric Aboagye[1]

[1]*Comprehensive Cancer Imaging Centre, Imperial College London Hammersmith Campus, Du Cane Road, London, UK, and* [2]*Division of Experimental Medicine, Imperial College London Hammersmith Campus, Du Cane Road, London, UK*

Correspondence
Eric Aboagye, ICL-CCIC, Imperial College London Hammersmith Campus, Du Cane Road, London W12 ONN, UK. E-mail: e.aboagye@imperial.ac.uk

Keywords
positron emission tomography (PET); radioisotope; drug development; radiopharmaceutical

Received
21 July 2010
Revised
26 October 2010
Accepted
14 November 2010

There is an increasing role for positron emission tomography (PET) in oncology, particularly as a component of early phase clinical trials. As a non-invasive functional imaging modality, PET can be used to assess both pharmacokinetics and pharmacodynamics of novel therapeutics by utilizing radiolabelled compounds. These studies can provide crucial information early in the drug development process that may influence the further development of novel therapeutics. PET imaging probes can also be used as early biomarkers of clinical response and to predict clinical outcome prior to the administration of therapeutic agents. We discuss the role of PET imaging particularly as applied to phase 0 studies and discuss the regulations involved in the development and synthesis of novel radioligands. The review also discusses currently available tracers and their role in the assessment of pharmacokinetics and pharmacodynamics as applied to oncology.

LINKED ARTICLES

This article is part of a themed section on Imaging. To view the other articles in this section visit http://dx.doi.org/10.1111/bph.2011.163.issue-8 *BJP* has previously published an *Imaging in Pharmacology* themed section, edited by A Davenport and C Daly. To view this section visit http://dx.doi.org/10.1111/bph.2010.159.issue-4

Abbreviations

5-FU, 5-fluorouracil; ARSAC, Administration of Radioactive Substances Advisory Committee; BCNU, 1,3-bis-(2-chloroethyl)-1-nitrosourea; CT, computer tomography; DACA, N-[2-(dimethylamino)ethyl]acridine-4-carboamine; DOTA, 1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid; ECM, extracellular matrix; EGFR, epidermal growth factor receptor; eIND, Exploratory Investigational New Drug; EMEA, European Medicines Agency; ENT, equilibrative nucleoside transporter; EORTC, European Organisation for Research and Treatment of Cancer; FDA, Food and Drug Administration; GLP, Good Laboratory Practice; GMP, Good Manufacturing Practice; GRPP, Guidelines on Good Radiopharmacy Practice; IMP, investigational medicinal product; MCA, Medicines Control Agency; MHRA, Medicines Healthcare products Regulatory Agency; ML-10, 5-fluoropentyl-2-methyl-malonic acid; MTS, multicellular tumour spheroids; PD, pharmacodynamics; PET, positron emission tomography; PK, pharmacokinetics; RDRC, Radioactive Drug Research Committee; RGD, arginine-glycine-aspartic acid; SOP, standard operating procedure; TK, thymidine kinase; VEGF, vascular endothelial growth factor

Introduction

Drug development is a lengthy, complex and expensive process. Currently the development of a typical investigational agent takes 10–15 years and costs approximately US $800 million. Despite the time and financial commitment, attrition rates are high with less than 10% of novel anticancer drugs entering the market from phase I studies (Dimasi, 2001). This low success rate has been ascribed to two major causes; the lack of predictive preclinical models and inadequate, complex trial designs (Bergstrom *et al.*, 2003; Marchetti and Schellens, 2007). In parallel, the area of drug discovery and development is undergoing a rapid expansion in almost all therapeutic areas of medicine, particularly in oncology. This is in part fuelled by innovative technologies such as computer-aided drug design, combinatorial chemistry, and high-throughput methodologies for studying genomics and structural biology. The use of these tools has led to an increase in the identification of potentially druggable targets that drive pathological conditions. However, due to increasing costs and limited resources, it is not possible to develop all potential drug candidates and this has initiated

discussions as to how to more effectively drive drug development.

It is generally accepted that early information on exposure of target tissue to investigational drug, modulation of the desired target, and biological effect will assist in the prompt translation of agents from the laboratory to the clinic (Workman *et al.*, 2006). It is important to establish these measurements early in the drug development pathway, either prior to phase I studies (phase 0) or as part of phase I studies. To this end positron emission tomography (PET) can be used to obtain crucial pharmacokinetic (PK) and pharmacodynamic (PD) information non-invasively, and hence the role of PET imaging in drug development is increasing.

PET is a non-invasive imaging methodology that involves the administration of compounds labelled with positron-emitting radioisotopes (radiotracers or radioligands) that are formulated for intravenous injection (radiopharmaceuticals). Radiotracers are substrates of normal physiological pathways (activated probes) or localize to particular targets because of specific binding interactions (targeted probes). These radiolabelled probes are administered at tracer amounts so as to not perturb normal physiology but allows *in vivo* studies under physiological conditions. PET therefore allows the investigation of substrate-target interactions or physiological processes with the advantage of not having to administer an efficacious dose of a chemical compound as with classical pharmacological investigations. As radiotracers act as substrates for normal physiological processes they need to undergo extensive preclinical validation. The development of novel PET radiotracers is a complex process that requires a multidisciplinary research group including expertise in organic chemistry, radiochemistry, pharmacology, cell biology, physics, image analysis and oncology. In this review we initially describe the process by which novel cancer imaging tracers are developed, from conception to the clinic (Figure 1). The second part of the review will focus predominantly on PET for assessing PD end-points that illustrate the hallmarks of cancer, proliferation, apoptosis and angiogenesis studies in patients, and the utility of PET as an early biomarker of therapeutic response. We will also discuss the PET imaging in phase 0 and PK studies.

Radiosynthesis

Any biological target that is present at increased or decreased levels in cancer cells can be visualized by PET. Ideally the target should be as specific as possible for the disease process, and consideration should also be given to the clinical information that might be gleaned from imaging the target or pathway. The development of a targeted radiotracer involves the synthesis of an extensive library of potential compounds for a particular target, with the expectation that only a few imaging agents will successfully progress to clinical PET studies. This library can contain several analogues of the parent compound that have a known affinity for a target and often may be based on previously known compounds. For example, in the design of a PET imaging tracer for the detection of $\alpha_v\beta_3$-integrin as a marker of angiogenesis, a focused library of peptides containing the arginine-glycine-aspartic acid (RGD) sequence were screened and [^{18}F]Galacto-RGD, was taken forward into clinical development (Haubner *et al.*, 2001). PET tracers are manufactured by automated synthesis modules the first of which was designed for the manufacture of [^{18}F]fluorodeoxyglucose (FDG) (Iwata *et al.*, 1984). Within this module the radiochemical hardware was interfaced with a microcomputer that controlled the 32 steps involved in the radiosynthesis of [^{18}F]FDG (Fowler *et al.*, 1981). More recently, the synthesis of [^{18}F]FDG occurs via a high yield, one-pot sysnthesis reaction, which has become the synthetic pathway of most commercial [^{18}F]FDG automation modules (Hamacher *et al.*, 1986) (Figure 2).

To have clinical utility, these potential tracers must have subnanomolar or nanomolar affinity for the physiological target so as to not interfere with normal biological function. Other desirable characteristics of radiotracers for somatic tumours include:

- high specificity and/or selectivity
- high plasma clearance and low plasma protein binding
- neutral and hydrophilic to enhance elimination and reduce effective (radiation) dose
- limited or measurable metabolism and amenable to kinetic modelling
- low toxicity
- good *in vivo* stability because of low metabolic clearance

Of central importance, the compound must have a suitable site for labelling with radioisotopes such as carbon-11 or fluorine-18, the most commonly used radioisotopes for PET in oncology. However, research utilizing other radioisotopes including copper-64, yttrium-86 and iodine-124 is increasing. When labelling a drug or molecule of choice, an element in the target molecule is substituted by its radioisotope. Problems arise however with the substitution of hydrogen as this does not have a positron emitting isotope. In this case hydrogen is substituted by fluorine-18, which may result in altered PK compared with the native molecule but these substitutions are conservative, and in the past have yielded useful probes such as [^{18}F](FDG).

Radioisotopes are produced by charged particle nuclear reactions within a cyclotron where a target vessel containing a gas or fluid is bombarded by protons or deuterons. These radioisotopes are obtained from the cyclotron in simple chemical forms (Table 1). For example carbon-11 could be obtained as [^{11}C]CO_2 or [^{11}C]CH_4 and fluorine-18 as either [^{18}F]fluoride or [^{18}F]fluorine. Once generated, radiochemistry is then used to incorporate these radioactive substrates into the compounds of interest. This process involves a number of steps, culminating in the conversion of the radioisotope to a reactive intermediate that is then used to label the compound of interest. The radiolabelled compound is isolated, for instance by high-performance liquid chromatography (HPLC), to eliminate radiochemical and chemical by-products that may have arisen during synthesis. The product is then formulated and can be used for aseptic injection.

Due to the short half-life of PET radioisotopes, the labelling of compounds involves considerable amounts of radioactivity, and therefore needs to be performed within remote-controlled lead-shielded 'hot' cells. The short half-life of radioisotopes also results in a time constraint for PET radiolabelling, and the introduction of the radioisotope at the latest possible stage in the synthesis is desirable. The

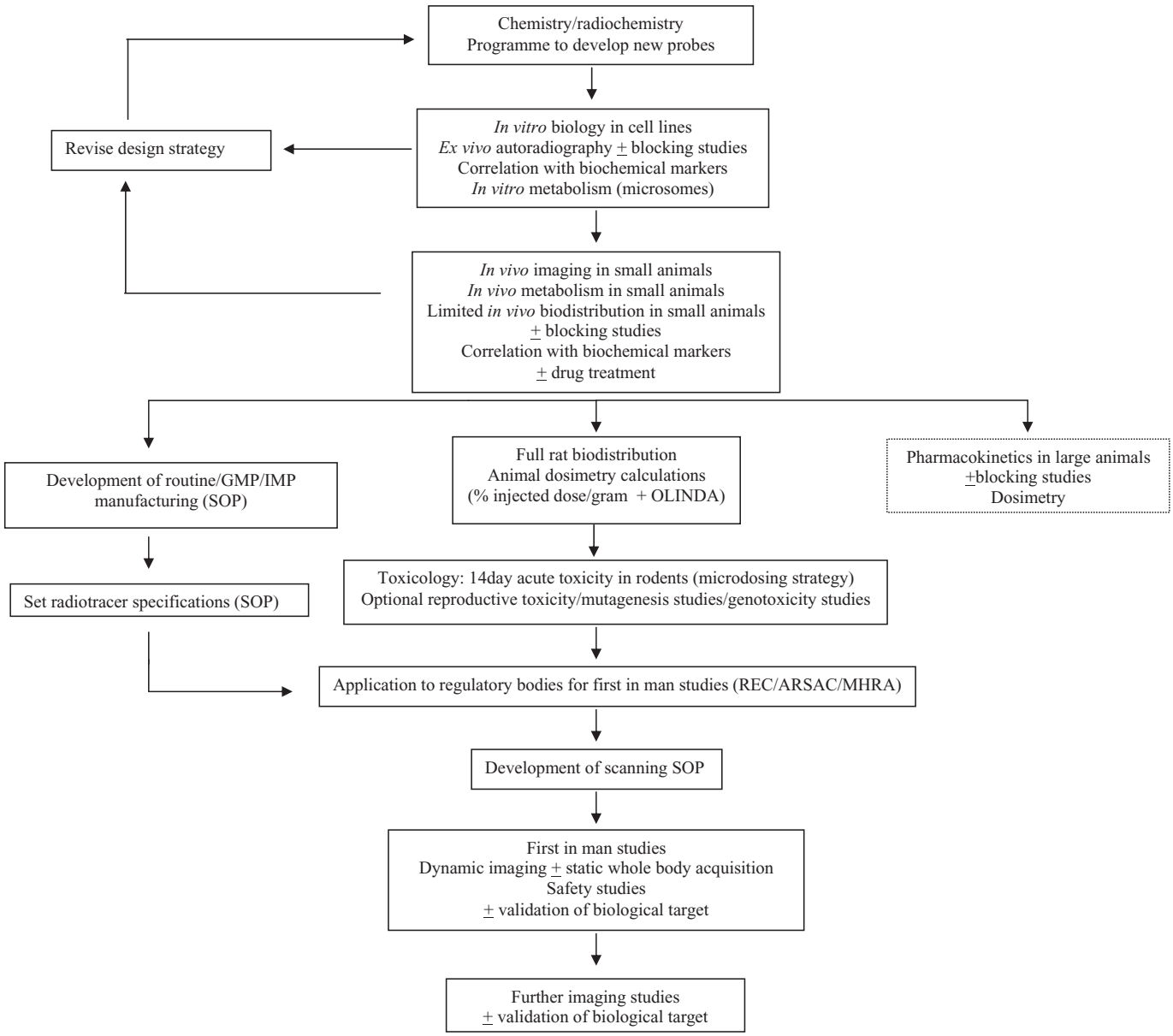

Figure 1

The developmental process involved in designing a new radiotracer for clinical use. OLINDA, organ level internal dose assessment.

development of novel synthetic procedures to improve this 'late labelling' is an active area of research.

Compounds labelled with carbon-11 or fluorine-18 are produced with high specific radioactivity, that is, these compounds have a high level of radioactivity and are associated with a very small amount of the stable compound or carrier. The amount of carrier present is usually sufficient to obtain a mass spectrum of the compound. To ascertain the position of the incorporated radioisotope, co-labelling experiments can be conducted. For example, for carbon-11 chemistry, very small amounts of carbon-13-labelled agents can be mixed with the carbon-11 equivalents and used in the radiosynthesis. The purified product is then examined by carbon-13 nuclear magnetic resonance and mass spectrometry.

The commonly used radionuclides in PET that are most appropriate for labelling of drugs are summarized in Table 1. Carbon-11 is an ideal nuclide for labelling molecules of biological interest, however, its short half-life (20.4 min), complex synthesis procedures and difficulties in obtaining high specific activities are limitations for routine manufacturing by PET centres. The metallic nuclides, gallium-68, copper-64 and yttrium-86 are useful for developing peptide- and protein-based radiotracers. Fluorine-18 is ideal for a number of reasons; it has a long half-life (110 min) allowing transportation from production to clinical PET imaging sites, it has acceptable dosimetry allowing multiple studies in a given patient, in terms of production it can be produced in large quantities in a cyclotron, has high labelling yields and

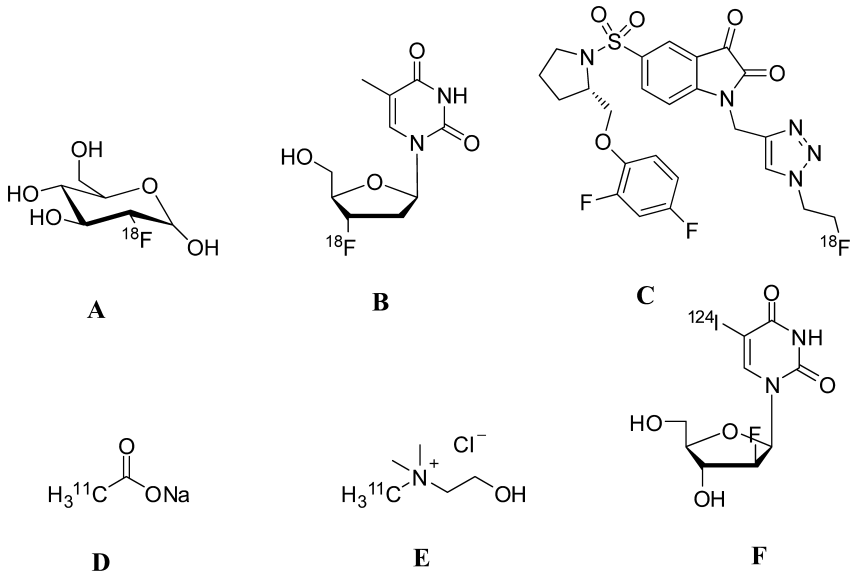

Figure 2

Chemical structures of common tracers involved in imaging. (A) [^{18}F]FDG (B). [^{18}F]FLT **C** isatin sulfonamide, [^{18}F]ICMT-11, (D) [^{11}C]acetate, (E) [^{11}C]choline, (F) [^{124}I] – 2′-fluoro-5-iodo-[beta]-D-arabinouridine (FIAU).

Table 1

Characteristics of radionuclides commonly used in the development of PET tracers

Radionuclide	Half-life (min)	Intermediate building blocks	Typical specific activity (GBq·μmol^{-1})
^{11}C	20	[^{11}C]CH_3I, [^{11}C]HCN, [^{11}C]CO, [^{11}C]CO_2, [^{11}C]CH_4	3.4×10^5
^{18}F	110	[^{18}F]F^-, [^{18}F]F_2	6.3×10^4
^{76}Br	972	[^{76}Br]Br^+,[^{76}Br]Br^-	7.2×10^3
^{13}N	10	[^{13}N]NH_4^+,[^{13}N]NH_4^-,[^{13}N]NH_2^-	7.0×10^5
^{15}O	2	[^{15}O]H_2O	3.4×10^6
^{124}I	40 (days)	[^{124}I]I^+, [^{124}I]I^-	1.15×10^3
^{68}Ga	68	[^{68}Ga]Ga	1.02×10^5
^{64}Cu	760	[^{64}Cu]$CuCl_2$	2.2×10^8–1.85×10^9

finally, fluorine-18 has low positron energy compared with the other nuclides, which improves the resolution of images obtained (Vallabhajosula, 2007). Radionuclides are administered to patients, where they decay by positron emission, which results in the release of two gamma rays. Gamma emission is detected by detectors placed around the subject and images obtained showing the distribution of radionuclide in the body. Improvements in anatomical localization is achieved by combining images obtained by PET with concurrent computer tomographic (CT) images obtained prior to scanning.

For a radiotracer to be developed further, a number of characteristics need to be fulfilled during the radiosynthesis phase (Pimlott, 2005). It must be possible to:

- Obtain sufficient yields (5–40% non-decay corrected, end of synthesis yields, depending on synthesis procedure) and rapid radiosynthesis. This optimization is particularly crucial in the development of a radiotracer for early clinical studies and may affect the cost of the radiotracer and logistics of these studies.
- Obtain high radiochemical purity adequate for human imaging studies (preferably 95% or higher).
- Obtain high specific radioactivity to allow injection of sufficient radioactivity into patients for imaging. The amount of compound present in a patient dose should be well below the amount that elicits a pharmacological response.
- Obtain the radiopharmaceutical in a form that is acceptable to regulatory bodies with respect to impurity profile [European Medicines Agency (EMEA) or Food and Drug Administration (FDA)].

Once radiosynthesis has successfully been developed, a standard operating procedure (SOP) can be initiated for the

synthesis of the radioligand, and to ensure that the radiopharmaceutical is sterile and endotoxin-free (Figure 1). The SOP will detail in full the synthesis and specifications for production and purity analysis of these tracers so that they are synthesized in a reproducible and reliable manner. The SOP will also indicate how scanning will be conducted. SOPs are central to both preclinical and clinical validation of a novel radiotracer before it is considered to be useful for imaging the target of interest. After synthesis, radiolabelled compounds must then undergo pharmacological characterization, initially in cell culture, and then *in vivo* to determine the biological action and affinity for the tumour target (Figure 1), a procedure that will be discussed in more detail later.

Production and quality control

The European Community Directive on Good Clinical Practice in clinical trials requires that the manufacture of investigational medicinal products be in compliance with Good Manufacturing Practice (GMP) standards within approved facilities, and that these facilities must be capable of meeting these standards. Within the UK, facilities involved in the production of radiopharmaceuticals must apply for a manufacturing license issued by the Medicines and Healthcare Products Regulatory Agency (MHRA), and these sites are regularly monitored. In the United States, the FDA acts as an equivalent regulatory body and is responsible for issuing guidance notes and regulations, and a summary of the regulations involved in radiotracer development is given in Table 2.

It is not always feasible to perform detailed quality control tests prior to administration of radionuclides, particularly as the half-life of the majority of radionuclides is short. Therefore, an ongoing quality assurance system is often established prior to initial clinical studies to ensure that quality analysis occurs. For radioligands with a high specific radioactivity, it is recommended to evaluate the stability of the ligand during test runs for up to 4 h (for carbon-11 and fluorine-18) after radiosynthesis to ensure that changes in the chemical composition of the radioligand has not occurred.

A number of in-house quality control procedures need to be assessed prior to administration of the radionuclide to the patient. These include assessment of purity, nature of any impurities, sterility of the solution, absence of pyrogens and suitable pH. With regards to impurities generated during the radiosynthesis, it should be emphasized that the parent compound is given at only tracer doses, and hence exposure to minor impurities is negligible. Minor impurities, however, are still considered and limits set; (EMEA, 2009). Consideration should also be given to levels of the other components in the formulation, such as anti-oxidants and residual solvents. These concepts should all be outlined within the SOP, which are adhered to by the manufacturing unit and must be readily available for audit purposes. At many centres a simple checklist is used to ensure that the radiopharmaceutical released adheres to strict criteria.

Chemical identity and purity. Prior to the administration of the radiopharmaceutical to patients it is essential, as part of the developmental process, to provide evidence that the radiopharmaceutical prepared via the radiosynthetic route is identical to the non-radiolabelled reference compound. This may not be the case for example with fluorinated compounds such as [^{18}F]FDG, where there are no known biological equivalent. If the radiosynthetic route differs from that of the non-radiolabelled compound then the radiosynthetic route should be documented and be available for review by an independent body. Following decay of the radiolabelled compound, the chemical identity should be determined by mass spectrometry and nuclear magnetic resonance, if possible. The purity of the radiochemical, radionuclide and radiopharmaceutical should be determined by HPLC-UV and HPLC-mass spectrometry. It is common to perform three to five runs of the radiosynthetic process once this has been determined to ensure the robustness and reproducibility of the manufacturing process and to ensure that the compound meets all clinical criteria. During this process batch numbers should be recorded including manufacturing records of the radiosynthesis and quality control analyses.

In vitro *and* in vivo *studies*

Prior to testing in human subjects, novel radiopharmaceuticals must undergo *in vitro* and *in vivo* validation in order to ascertain target specificity, biodistribution and toxicity. Radiotracers are initially assessed *in vitro* to assess target specificity, uptake and the impact on cellular responses. Previously, radioligands would then be transferred into animal models, now however, a number of authors have suggested the use of multicellular tumour spheroids (MTS) as a transitional model, as the cytology and the morphology of MTS resembles that of experimental tumours in mice (Fracasso and Colombatti, 2000; Kunz-Schughart *et al*., 2004). Therefore, the use of MTS would allow evaluation of dose and dosing regimen with less laboratory effort. Preclinical studies have shown MTS to be an appropriate *in vitro* system to investigate drug response to chemotherapy (Bates *et al*. 2000; Dubessy *et al*., 2000; Mueller-Klieser, 2000). A number of MTS of differing tumour types have been evaluated and shown to be good experimental models in which to investigate novel agents and PET imaging as a biomarker of response (Monazzam *et al*., 2006; 2007; 2009). However, MTS does not substitute the animal systems.

Animal studies are performed as part of the development of novel radioligands to determine biodistribution, reproductive toxicology, carcinogenic toxicity and genotoxicity. An integral part of early radiotracer development is a biodistribution study. These studies are often the first conducted and are important in determining whether binding of the radioisotope is tumour specific or if it binds to other sites outside the tumour, that is, non-specific binding. High non-specific binding of a radiotracer is a common reason for the failure of a radiotracer to reach clinical studies. Furthermore, knowing the degree of non-specific binding will assist in calculating the absorbed dose by tissues (dosimetry). Toxicology and biodistribution studies assure that the radiopharmaceutical is non-toxic at doses to be administered to patients.

A key aspect of the *in vivo* assessment of radioligands for use in oncology is selection of appropriate tumour models. This is of particular importance with reference to biodistribution studies; if the tumour model does not express a receptor

Table 2

Regulatory guidelines pertaining to the manufacture and clinical use of radioligands

Body	Locality	Regulation name	Process	Type of regulation
Medicines and Healthcare products Regulatory Agency (MHRA)	UK	Medicines Control Agency (MCA) 'Specials' License (MHRA, 2007)	Ensures manufacture of investigational medicinal products are in compliance with Good Manufacturing Practice (GMP) standards	Legislative and binding
			Issue guidance notes and regulations	Non-binding
		Investigational Medicinal Product 'IMP' or equivalent (UK 'Specials') (MHRA, 2010)	Ability to undertake human studies with medicinal products	Legislative and binding
Food and Drug Administration (FDA)	USA		Issue guidance notes and regulations	Non-binding
		Radioactive Drug Research Committee (RDRC) approval mechanism: Investigational new drug (IND)	Safety evaluation for entirely new radiolabelled compounds for which there has been no prior human exposure	Legislative and binding
		Radioactive Drug Research Committee (RDRC) approval mechanism: Exploratory Investigational New Drug (eIND)	Safety evaluation for situations where there has been some prior human exposure to the radiolabelled compound	Legislative and binding
		Critical Path Initiative	Action plan to assist investigators along the approval path towards IND or eIND	Encourage investigators to submit new tracers for approval. Non-binding
Radiopharmacy Committee of European Association of Nuclear Medicine (EANM)	EU (incl UK)	'Guidelines on Good Radiopharmacy Practice (GRPP)' (Committee, 2007)	Production of radiopharmaceuticals, specific GMP issues such as compliance to GRPP, self-inspection, acceptance criteria and ensuring appropriate staff qualifications.	Non-binding
Administration of Radioactive Substances Advisory Committee (ARSAC)	UK	Certificate for the administration of radioactive medicinal products	Certification allows specific radiolabelled compounds of pre-defined effective dose to be administered to study subjects for a specified time interval	Legislative and binding

of interest for example then any proof of concept study will be negative. In a study by Ullrich and colleagues [^{18}F]fluorothymidine ([^{18}]FLT) was investigated as an early response marker to erlotinib, an epidermal growth factor receptor (EGFR) inhibitor in animal models of non-small cell lung cancer (Ullrich *et al.*, 2008). The authors report a reduction in [^{18}]FLT by PET in erlotinib-sensitive EGFR-dependent tumours; in contrast [^{18}]FLT-PET changes were attenuated in tumours harbouring T790M secondary EGFR mutations that confer resistance to EGFR tyrosine kinase inhibitors. Similarly, in a study investigating [^{18}]FLT-PET as a marker of response to mTOR inhibitor, rapamycin, reductions in [^{18}]FLT-PET were not seen in PTEN wild-type resistant tumours but occurred in sensitive cell types (with PTEN loss), again highlighting the need for the target of interest to be present for proof of concept studies (Wei *et al.*, 2008 p. 140).

Carcinogenicity studies are not required unless there is a specific concern. This is particularly true for tracers being developed for use in oncology where, in general, patients have limited life expectancy. In these patients, long-term repeat dose toxicology, genotoxicity and carcinogenicity studies are usually omitted. Similarly, reproductive toxicology studies are not required as long as patients of reproductive age will be advised to take appropriate precautions to avoid pregnancy, and pregnant and lactating patients will be excluded from any clinical studies.

In terms of toxicology studies, single-dose studies are performed in animals using the formulation to be used in patients. Single-dose toxicology studies are deemed appropriate in animals, as in the clinical setting, patients will usually receive only a single, low dose of the radioligand, and if repeated administrations are delivered, the doses are usually

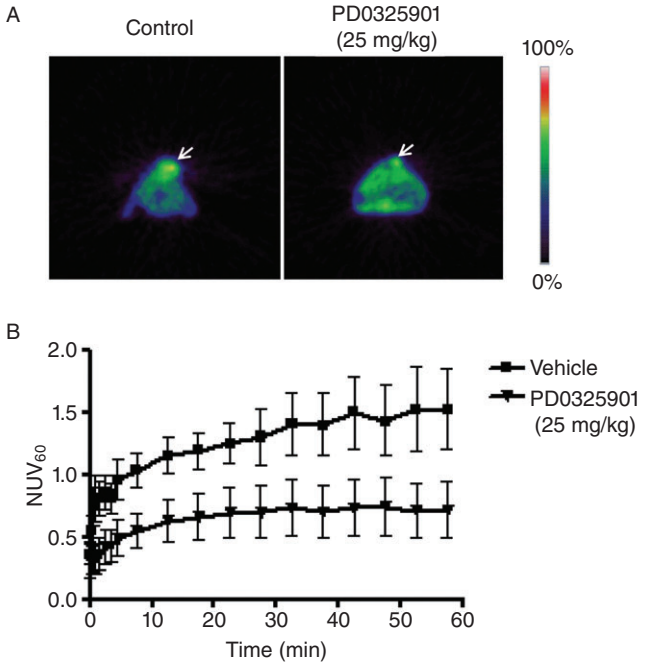

Figure 3

Use of [^{18}F]FLT in positron emission tomography (PET) studies (A). Transverse [^{18}F]FLT-PET images of mice bearing the SKMEL-28 human melanoma tumour, 10 days after inoculation with either vehicle or PD0325901 treatment. (B) [^{18}F]FLT-PET time (min) versus radioactivity curve [as measured by normalized uptake value at 60 min postinjection (NUV_{60})] for the vehicle or PD0325901 treatment group (courtesy of Julius Leyton).

administered weeks apart and, from a toxicological standpoint, are considered equivalent to a single dose. All animal toxicology studies are performed under the highest quality standards possible [equivalent to Good Laboratory Practice (GLP)].

Initial dosimetry studies are performed in animals over a number of time points depending on the biologicalal and physical half-lives of the radioisotope. The radioactivity in blood, urine and tissues, expressed as a percentage of injected radioactivity, is determined to enable calculation of the predicted human effective radiation dose (mSv) to each tissue. Human dosimetry often tends to be the first human study of new radiotracers. The dose to critical organs, such as the bladder and liver, can limit the amount of radioactivity that can be injected in human studies and therefore has the potential to limit the success of the tracer.

PK studies, mainly in rodents but where possible in larger animals, play important roles in the prediction of human radiation organ dosimetry and toxicokinetics (Figure 3). This is an important concept as metabolism can affect not only the amount of tracer that is able to bind to the target, but the generation of radiolabelled metabolites may interfere with image analysis. Furthermore, rapid metabolism of the radiotracer during the imaging time frame can limit the potential usefulness of a tracer, and thus needs to be considered.

The potential immunogenicity of the radiopharmaceuticals is also of importance, particularly if the tracer is to be administered more than once to the same patient. This issue should be addressed by inspecting the structures of compounds for any possible alerts for immunogenicity by analogy with related compounds, for example, the presence of peptides or the presence of a penicilloic acid moiety.

Once animal toxicology and biodistribution studies are complete and the tracer is deemed suitable for clinical use from a scientific perspective, the radiotracer must undergo validation in the clinical setting. There are a number of regulatory bodies that manage the clinical development of radioligands to eusure that trials are run in a safe and consistent manner. These agencies tend to have similar remits in various countries, and the approval process is outlined in Table 2.

Role of imaging in early clinical trials

Pre-phase I or phase 0 studies

In 2003, the EMEA published a concept note followed by a Position Paper on the non-clinical safety studies required to support human clinical trials with a single dose of pharmacologically active compound using microdose techniques (http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002941.pdf). These guidelines affect PET in two ways: firstly in terms of the development process of radioligands, but also use of PET as clinical trial end-points (Marchetti and Schellens, 2007). As sub-pharmacological single doses of the investigational agent are administered, ultrasensitive analytical methods such as PET are required to assess the drug distribution and PK. The paper raises the possibility of a reduced preclinical safety package for microdose studies and facilitates the development of novel radioligands (VanBrocklin, 2008).

As there has been a significant decline in the number of new molecules submitted to the FDA over the past decade, the FDA responded to this by devising an action plan called the 'Critical Path Initiative' to facilitate the drug discovery and development process from inception to approval (FDA, 2004; 2010). A recent initiative implemented by the FDA is the establishment of the Exploratory Investigational New Drug (eIND) application, a characteristic of which are microdosing/phase 0 studies. These studies involve the administration of a single 'micro-dose' or 'tracer-dose' of a novel agent or radioligand, either to healthy volunteers or patients. The aim of a phase 0 study is to obtain information on PK and/or distribution properties or receptor selectivity prior to phase I. The studies do not provide information about the safety or tolerability of the radiopharmaceutical. The definition of a microdose is 'less than 1/100 of the dose of a test substance calculated (based on animal data) to yield a pharmacological effect of the test substance with a maximum dose of ≤100 μg or, in the case of biological agents, ≤30 nmol' (CHMP, 2004). The translational research questions that justify a pre-phase I study include whether the agent distributes to the tumour, to what extent the agent distributes to normal tissues, and whether the metabolite profile in rodents and humans are similar. These studies are used to establish whether the radiopharmaceutical behaves in

human subjects as expected from preclinical studies. Theoretically, microdose trials should allow an early discrimination between those lead compounds to take forward into further development and those to be discarded. The biodistribution of a compound is also important to determine the extent of non-specific binding. Pre-phase I studies can also yield information regarding PK modulation and scheduling. N-[2-(dimethylamino)ethyl]acridine-4-carboxamide (DACA) is a DNA intercalating acridine derivative that was labelled with carbon-11 in order to determine the metabolic profile of DACA, the distribution to the tumour – as a predictive marker of response – and distribution to normal tissues – as a predictor of toxicity (Saleem *et al.*, 2008). Twenty-four patients were enrolled in the study and [^{11}C]DACA administered at 1/1000 of the phase I dose. This PK study illustrated that [^{11}C]DACA undergoes extensive metabolism and its metabolites distributed well to the tumour. Low peak concentrations of [^{11}C]DACA-derived radioactivity were obtained in the vertebrae and brain compared with other normal tissues suggesting that myelosuppression and neurotoxicity were unlikely to be dose-limiting toxicities. There was uptake within the myocardium suggesting the possibility of myocardial toxicity in the phase I study. In a similar study LY2181308, an antisense oligonucloetide to survivin, was labelled with carbon-11 and administered concurrently with therapeutic doses of unlabelled drug (Saleem *et al.*, 2009). [^{11}C]LY2181308 uptake in both normal tissue and tumour was assessed. The authors demonstrated the utility of microdosing experiments illustrating that active drug concentrations could be achieved within the tumour and that unlabelled LY2181308 saturated normal tissue kinetics resulting in increased tumour uptake. These studies establish the feasibility of performing PET studies with radiolabelled anticancer drugs in a phase 0 setting.

PET can also been utilized to provide proof of principle for mechanism of action of the labelled drug. The effect of eniluracil, a drug that inhibits dihydropyrimidine dehydrogenase, an enzyme responsible for the degradation of 5-fluorouracil (5-FU), was studied using PET (Ahmed *et al.*, 1999; Saleem *et al.*, 2000a,b). The study illustrated that in eniluracil-naïve patients, [^{18}F]FU-derived radioactivity localized more strongly to the normal liver than in metastases. In addition there was distinct localization of radioactivity in the gall bladder consistent with hepatobiliary clearance of [^{18}F]FBAL that disappeared in the presence of eniluracil. In patients receiving eniluracil, there was increased plasma uracil and unmetabolized [^{18}F]FU. There was also an increase in the half-life of [^{18}F]FU-derived radioactivity in tumours, providing proof of principle for mechanism of action of eniluracil. More recently Rosso and collegues utilized a PET imaging approach to generate kinetics of temozolamide following administration of labelled drug to patients with gliomas. The authors illustrate that the utilization of impulse response function to predict drug exposures in both tumours and healthy tissue, assuming linear drug kinetics (Rosso *et al.*, 2009).

Following these studies a novel radioligand will undergo formal clinical evaluation prior to licensing for clinical use. A list of PET tracers currently used in the clinical setting, either as research tools or for diagnostic purposes, is given in Table 3.

Drug PKs

As discussed earlier, a successful imaging agent must be retained by the target but must not affect the target, nor can it be too toxic for use with other treatment agents. In contrast, therapeutic agents are designed to interact and affect the target of interest. Labelling drugs may therefore be useful in understanding the PK of an agent particularly as part of the drug development process (Figure 4). Parameters including drug absorption, distribution, metabolism and excretion, as well as the maximum plasma concentration and area under the plasma concentration versus time curve are major determinants of toxicity and efficacy of a drug, and can be achieved by labelling drugs of interest with radioisotopes such as carbon-11, fluorine-18 or nitrogen-13 (Osman *et al.*, 2001; Saleem *et al.*, 2001; 2008). PK parameters are obtained through mathematical kinetic modelling, a process that involves comparing the concentrations of radioactivity in tissues with that in arterial plasma and calculating the plasma-tissue exchange rates to derive tissue PK parameters (Saleem *et al.*, 2000a,b). This allows the investigator to ascertain what happens to the tracer within the body and, in particular, within the target organ.

The interpatient variability in the PK of anticancer drugs is generally high and these drugs have a narrow therapeutic index. Steady state plasma PK often poorly reflect the levels of drug within normal or tumour tissues. PET PK studies may aid in determining intra-tumour drug exposure. For example when comparing uptake of [^{11}C]-1,3-bis-(2-chloroethyl)-1-nitrosourea (BCNU) within the tumour after both intravenous and intra-arterial administration it was shown that intra-arterial administration resulted in 50 times higher intratumour concentrations (Tyler *et al.*, 1986). Examples of drugs that have been evaluated by PET include [^{11}C]temozolamide, [^{18}F]5FU, [^{18}F]paclitaxel, [^{13}N]cisplatin and [^{11}C]DACA (Ginos *et al.*, 1987; Kissel *et al.*, 1997; Dimitrakopoulou-Strauss *et al.*, 1998; Saleem *et al.*, 2001; 2003; Kurdziel *et al.*, 2003). PK studies may also allow for the investigation of the mechanism of action of novel therapies prior to phase I. For example, the proposed mechanism of action of temozolamide was studied in patients using [^{11}C]temozolmide tracers labelled at the methyl and carbonyl positions. The study demonstrated that temozolamide undergoes tissue-specific ring-opening in humans, although this conformational change was not tumour specific (Saleem *et al.*, 2003).

On labelling the drug it is important to ensure that no modification has occurred to the molecule, as even a slight change can alter its physicochemical properties. Initial exploratory studies of hydrogen-3 (tritium) and carbon-14 labelled compounds in animals are often informative in determining if imaging in patients will be useful. For example, if a large number of degradation products are rapidly generated, imaging may add little value in determining response. On the other hand, imaging the distribution of a drug may provide valuable insight into further development and choice of tumours to be treated.

The PK of 5-FU has been successfully studied by labelling the drug with ^{18}F, allowing enough time for kinetic studies to be performed with good reproducibility (Harte *et al.*, 1998). Up to 80% of 5-FU is degraded through catabolism. A PK study in 13 patients with colorectal liver metastases showed a

Table 3

Common PET tracers used in oncology

Isotope	Radiotracer	Target	Measured effect
^{11}C	[^{11}C]choline	Phosphocholine	Choline kinase activity, membrane function
	l-Methyl-[^{11}C]methionine	Amino acid transporter	Protein synthesis, amino acid transport, tumour cell growth
	L-1-[^{11}C]tyrosine	Amino acid uptake	Amino acid metabolism
	2-[^{11}C]thymidine	DNA	DNA synthesis. Tumour cell proliferation
	N-methyl-[^{11}C]temozolamide, Carbonly-[^{11}C]temozolamide, *N*-[2-(dimethylamino) ethyl]acridine-4-carboxamide, [^{11}C]-1,3-bis-(2-chloroethyl)-1-nitrosourea		Drug pharmacokinetics
^{18}F	[^{18}F]fluorodeoxyglucose	Glucose metabolism	Glucose transport. Aerobic and anaerobic glycolysis, glucose consumption or metabolism
	5-[^{18}F]fluorouracil, [^{18}F]fluorotamoxifen		Pharmacokinetics
	[^{18}F]fluoride	Incorporation in the hydroxyapatite crystals in bone	Bone metabolism; Bone blood flow and osteoblastic activity
	1-[^{11}C]acetate [^{18}F]fluoroacetate	Tricarboxylic acid cycle via acetyl coenzyme A	Lipid synthesis
	3-[^{18}F]fluorothymidine	Activity of thymidine kinase-1	DNA synthesis. Tumour cells proliferation
	[^{18}F]fluoroazomycin arabinoside, [^{18}F]fluoromisonidazole	Hypoxic cells	Tumour hypoxia
	16α-[^{18}F]fluoro-17β-oestradiol	Oestrogen receptors in breast cancer	Receptor binding, response to anti-oestrogen therapy
	16β-[^{18}F]-5α-dihydrotestosterone (FDHT)	Androgen receptor (ARs)	Functional status of tumour ARs
	[^{18}F]AH111585/fluciclatide, [^{18}F]Galacto-RGD	Angiogenesis	Integrin receptors ($\alpha_v\beta_3$) on endothelial cells of neovasculature
	[^{18}F]oligonucleotide	Gene expression	*In vivo* hybridization with mRNA
	9-1-[^{18}F]fluoro-3-hyroxy-3-propoxy-methylguanine	Gene expression	Substrate to herpes virus thymidine kinase
^{68}Ga	[^{68}Ga]DOTATOC, [^{68}Ga]DOTANOC	Specific binding to somatostatin receptor SSTR-II; SSTR-II, III, V	Presence of SSTRII; SSTRII, III, IV
^{124}I	Na[^{124}I]	Gene expression	Sodium iodide symporter expression
^{15}O	[^{15}O]H_2O	Perfusion	Blood flow/perfusion
	[^{15}O]CO	Blood volume	Antivascular response
others	^{124}I-, ^{64}Cu-, ^{86}Y-Labeled antibodies	Binding to tumour antigens	Specific binding to tumour associated antigenic binding sites (such as CEA, PSMA, CD20 and CD22)
	[^{124}I]Annexin V, [^{64}Cu]Annexin V, [^{18}F]-Annexin V	Apoptosis	Specific binding to Phosphatidylserine (PS) on cell membrane

relationship between uptake of 5-FU and tumour response; the likelihood of disease stabilization was higher and survival was longer in patients whose tumours had a higher uptake of the radiolabelled drug (Moehler *et al.*, 1998).

When used in a PK context, labelled macromolecules, for example, antibodies that target cell surface receptors may show specific targeting to receptors as well as significant non-selective distribution in intracellular and extracellular pools related in part to their slow systemic clearance (Verel *et al.*, 2005). This characteristic of radiolabelled macromolecules poses a challenge when they are used in a PD context. In the case of IgG entities, this requires labelling with long-lived radioisotopes, for example, copper-64 and imaging typically at 24–48 h.

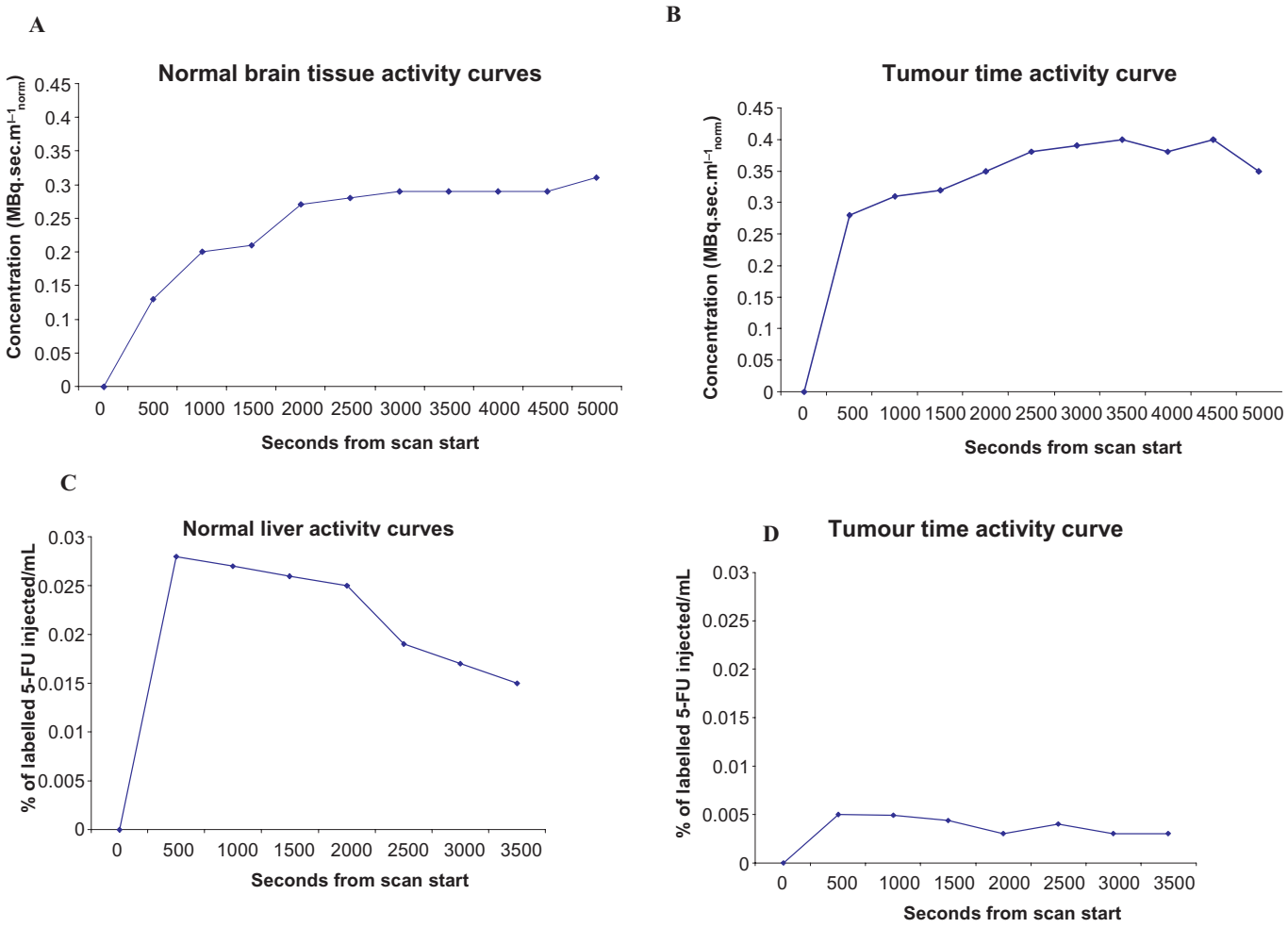

Figure 4

Uptake of tracers varies between normal and tumour tissue. (A, B) Time activity curves demonstrating normal brain and tumour uptake of [^{11}C*methyl*]temozolomide. (C, D) Time activity curves demonstrating normal liver and tumour uptake of [^{18}F]5-fluorouracil.

PDs

PD endpoints in PET imaging can be considered as either generic or specific end-points. Generic end-points image physiologic processes such as cellular proliferation, glucose metabolism, apoptosis, hypoxia and angiogenesis. Imaging of specific end-points provides proof of principle for a proposed mechanism of action such as the use of 16α-^{18}F-fluroro-17β-oestradiol to assess oestrogen receptor status in breast cancer (Lee *et al.*, 2009). We will discuss a number of these probes that are currently undergoing preclinical and clinical development or in routine clinical use.

Tumour metabolism and proliferation. [^{18}F]FDG is the commonest clinically available tracer and assesses the generic end-point of glucose utilization, as a measure of metabolism. Despite the increasing number of potential radiotracers, PET is generally still synonymous with [^{18}F]FDG. Metabolic imaging with [^{18}F]FDG relies on differential utilization of glucose in cancer cells compared with normal cells (Kelloff *et al.*, 2005). [^{18}F]FDG enters the cell and is phosphorylated by hexokinase to [^{18}F]FDG-6-phosphate. [^{18}F]FDG-6-phosphate cannot undergo any further metabolism and is trapped within the cell, with the rate of accumulation of [^{18}F]FDG-6-phosphate being proportional to the rate of glucose utilization. Therefore, the more metabolically active the cell the more glucose is utilized. The major limitation with [^{18}F]FDG is the false positivity seen within areas of inflammation (Subhas *et al.* 2005; Rosenbaum *et al.*, 2006). Furthermore, as [^{18}F]FDG relies on increased cellular proliferation compared with surrounding tissues, it has low sensitivity in slow growing, well-differentiated and less metabolically active tumours (Shreve *et al.* 1999; Burton *et al.*, 2004). Moreover, [^{18}F]FDG distributes within the renal collecting system obscuring pelvic lesions.

[^{18}F]FDG has been extensively studied both for the staging of tumours and response to cytotoxic therapy having an overall average sensitivity of 84% and a specificity of 88% in cancer (Gambhir *et al.*, 2001). More recently [^{18}F]FDG has been used to monitor responses to targeted therapies particular those that affect glucose homeostasis such as the mTOR and Akt inhibitors (Contractor and Aboagye, 2009) (Table 4). The most impressive results with [^{18}F]FDG were seen in the

Table 4

Selected clinical studies of the use of [^{18}F]FDG for monitoring therapy response

Tumour type	Therapy	Change in FDG uptake with therapy	Reference
Lymphoma		Reduction after 7 days correlates with progression free survival and overall survival	Romer *et al.*, 1998
		Reduction after two cycles correlation with complete response, progression free survival and overall survival	Haioun *et al.*, 2005
Gastrointestinal stromal tumour	Imatinib	Reduction after 7 days; correlated with response	van Oosterom *et al.*, 2001
	Imatinib	Reduction at 1 month correlated with response	Holdsworth *et al.*, 2007
Biliary tract cancer	Bevacizumab, gemcitabine and oxaliplatin	Reduction at 6 weeks; correlation with progression-free survival and overall survival	Zhu *et al.*, 2010
Non-small cell lung cancer	Gefitinib	Reduction on day 2, associated with improved progression free survival	Sunaga *et al.*, 2008
Breast cancer	Cyclophosphamide, doxorubicin, vincristine, and prednisolone or docetaxel	Reduction after first cycle predictive of response	Smith *et al.*, 2000
	Epirubicin, cyclophosphamide or epirubicin, paclitaxel	Reduction after first cycle predictive of response	Schelling *et al.*, 2000
	Lapatinib	Reduction after 1 month correlated with response	Kawada *et al.*, 2007
Prostate cancer	Goserelin	Reduction 1 after–5 months of therapy	Oyama *et al.*, 2001
Gastric cancer	Cetuximab, irinotecan, 5-fluorouracil	Reduction correlated with response, time to progression and overall survival	Di Fabio *et al.*, 2007
Gastro-oesophageal cancer	Epirubicin, cisplatin and 5-fluorouracil	Reduction at 14 days correlates with response and overall survival	Ott *et al.*, 2006

early trials of imatinib mesylate, a c-kit inhibitor used in the management of gastrointestinal stromal tumours (GISTs). In these tumours, response on [^{18}F]FDG-PET was seen as early as 24 h after therapy, with the response on PET imaging correlated with progression-free survival (Stroobants *et al.*, 2003). This is of particular importance in early phase clinical trials as standard radiological assessment of these tumours often fails to show any change in tumour size. Currently [^{18}F]FDG is approved for the staging and response assessment of a number of tumour types. A number of guidelines have been developed for the assessment of response including the European Organization for Research and Treatment of Cancer (EORTC) and the National Cancer Institute guidelines (Young *et al.* 1999; Shankar *et al.*, 2006). More recently the PERCIST guidelines were suggested by Wahl and colleagues but remain to be adopted (Wahl *et al.*, 2009).

Proliferation

Proliferation is one of the hallmarks of malignancy (Hanahan and Weinberg, 2000) and the majority of chemotherapeutic agents act to inhibit proliferation of cancer cells. It has been shown in a number of tumour types that the rate of proliferation, as indicated by Ki-67 and PCNA expression and S-phase fraction of tumour samples, is predictive of both survival and prognosis (Stuart-Harris *et al.*, 2008; Yerushalmi *et al.*, 2010) There are inherent difficulties in patient acceptability of routine biopsies, particularly if performed serially. Furthermore, there may be issues of tumour accessibility and, because of tumour heterogeneity, the biopsy sample may not be representative of the proliferative activity of the whole tumour. There is a need therefore to assess tumour proliferation, non-invasively.

Thymidine is a nucleoside utilized in DNA replication in proliferating cells, and both thymidine and its analogues have been extensively studied as potential tracers of cellular proliferation. Thymidine was first labelled with tritium, which is still used for *in vitro* work. During the process of development, thymidine was labelled with carbon-11. However, [^{11}C]thymidine undergoes rapid metabolism resulting in significant levels of labelled metabolites within the blood (Shields *et al.*, 1992). The fluorinated analogue, [^{18}F]FLT, has a suitable nuclide half-life (109.8 min) for clinical use and stability against phosphorylase-mediated degradation *in vivo* and is now extensively used as a marker of proliferation (Shields *et al.*, 1998).

After injection, [^{18}F]FLT enters the cell by both Na^+-dependent active nucleoside transporters and by passive diffusion. [^{18}F]FLT follows the salvage pathway of DNA synthesis and like thymidine undergoes phosphorylation by thymidine kinase–1 (TK1) to [^{18}F]FLT-monophosphate (Seitz *et al.*, 2002).

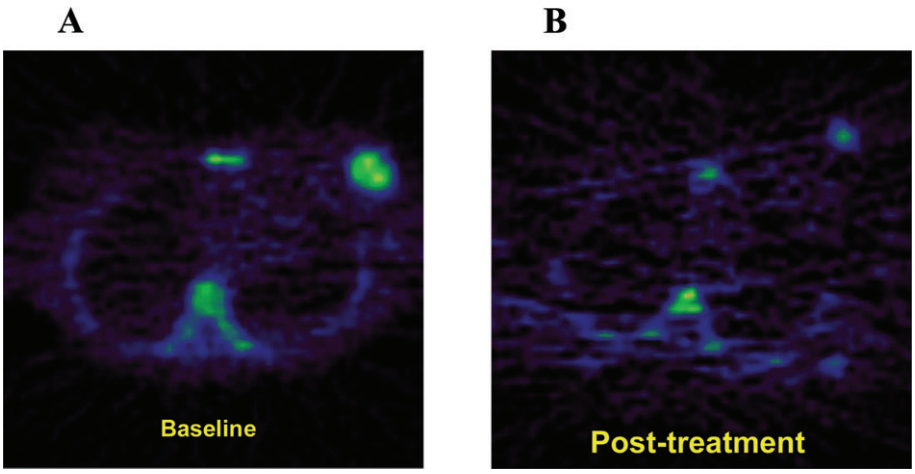

Figure 5

[^{18}F]FLT-positron emission tomography images to assess early response (1 week) to epirubicin, cyclophosphamide and 5-fluorouracil chemotherapy in a patient with T3N0M0 breast cancer. (A) Pretreatment uptake is seen in the right breast and (B) post-treatment response is observable as a reduction of [^{18}F]FLT uptake (courtesy of Dr. Laura Kenny).

[^{18}F]FLT is a selective substrate for TK1 whereas thymidine is also phosphorylated by TK2. TK1 is virtually absent in quiescent cells but is increased in proliferating cells (Sherley and Kelly, 1988; Munch-Petersen *et al.*, 1995). Phosphorylated [^{18}F]FLT is not significantly incorporated into DNA and is trapped within the cytosol. The rate limiting step for [^{18}F]FLT accumulation is the initial phosphorylation by TK1; it is also the rate limiting step in the salvage pathway of DNA synthesis, therefore the handling of [^{18}F]FLT reflects cellular proliferation (Shields *et al.*, 1998).

In a paper by Barthel and colleagues the authors investigated the ability of [^{18}F]FLT-PET to monitor response to chemotherapy in mice bearing sarcomas injected with 5-FU (Barthel *et al.*, 2003). The authors reported reduced uptake of [^{18}F]FLT following treatment that correlated with the PCNA labelling index within the tumour and a reduction in tumour volume. The authors then considered the role of TK1 in [^{18}F]FLT-PET imaging by contrasting the uptake of [^{18}F]FLT in TK1 knock-out and functional heterozygote lymphoma models (Barthel *et al.*, 2005). They reported higher uptake in TK1 heterozygote lymphoma compared with the knock-out model, which correlated with higher TK1 protein expression within the tumours and accumulation of [^{18}F]FLT. These studies were then reproduced in a clinical setting. In the seminal paper by Kenny and colleagues, [^{18}F]FLT-PET was reported as being an early response marker in patients receiving chemotherapy for breast cancer when compared with RECIST assessment (Figure 5) (Kenny *et al.*, 2005; 2007). Furthermore, the [^{18}F]FLT uptake parameters within the breast tumours correlated with Ki-67 expression within surgical resection specimens lending further support of [^{18}F]FLT as a PD marker for early clinical response.

However, [^{18}F]FLT has several limitations (Barwick *et al.*, 2009). Firstly, [^{18}F]FLT uptake in tumours depends on TK1 activity, including therapy-induced activation of the salvage pathways (Perumal *et al.*, 2006; Pillai *et al.*, 2008; Kenny *et al.*, 2009). Therefore, agents that only inhibit *de novo* DNA synthesis such as fluoropyrimidines may increase FLT uptake. Other therapeutic agents inhibit both pathways and subsequently reduce [^{18}F]FLT uptake. Moreover, tumours with low TK1 expression or low proliferative activity may not be readily identified (Bading and Shields, 2008). High physiological uptake of [^{18}F]FLT in liver and bone marrow also limits the assessment of disease in these organs. However, the recent introduction of kinetic filtering has led to significant signal reduction from the liver, improving visualization (Gray *et al.*, 2010). There is evidence to suggest that membrane localization of nucleoside transporters can be enhanced in tumours in response to a number of chemotherapy agents, which may result in a 'flare' response with [^{18}F]FLT. Perumal and colleagues investigated the role of [^{18}F]FLT-PET in monitoring intra-tumour inhibition of thymidylate synthase *in vivo* 1–2 h following 5-FU injection into mice bearing RIF-1 fibrosarcomas (Perumal *et al.*, 2006). The authors reported an increase in the 60 min [^{18}F]FLT tumour/heart radioactivity ratio in drug treated mice compared with vehicle controls. Plasma and tumour deoxyuridine levels increased significantly, but TK1 expression and ATP remained unchanged. Cellular assays for membrane uptake suggested a functional role of type-1 equilibrative nucleoside transporter (ENT-1). The authors hypothesized that [^{18}F]FLT uptake is mediated by redistribution of ENT-1 transporters to the plasma membrane 1–2 h after treatment. This was utilized clinically by Kenny and colleagues in breast cancer patients receiving capecitabine therapy where the authors suggested that PET retention parameters obtained following capecitabine therapy could be utilized as a PD marker for TS inhibitors (Kenny *et al.*, 2009). There have been a number of studies considering the uptake of [^{18}F]FLT in both the preclinical (Table 5) and clinical (Table 6) setting, with differing results according to the type of therapy utilized and at differing time points, both of which need to be considered in the utilization of [^{18}F]FLT as a response marker.

The choline pathway can also be imaged as a marker of cell growth, and clinical response biomarker in malignancy. Phosphocholine is a biosynthetic product of choline kinase

Table 5

Preclinical studies of the use of [^{18}F]FLT for monitoring therapy response

Tumour	Therapy	Change in FLT uptake with therapy	Reference
High grade lymphoma	Doxorubicin	Reduction at 24 h	Graf *et al.*, 2008
Lymphoma	Cyclophosphamide or rapamycin	Reduction after 2 days with cyclophosphamide. Rapamycin: initial reduction after 2 days then increase at day 7	Brepoels *et al.*, 2009
Glioblastoma	Rapamycin	Reduction at 48 h	Wei *et al.*, 2008
Mammary MCaK tumours	Radiotherapy	Reduction at 24 h	Pan *et al.*, 2008
Follicular lymphoma	Cyclophosphamide/immunotherapy/ radioimmunotherapy	Uptake reduced 48 h post-cyclophosphamide No change with other treatments	Buck *et al.*, 2007
Squamous cell cancer	Radiotherapy	Reduction after 4 days	Molthoff *et al.*, 2007
Squamous cell cancer	Radiotherapy	Reduction at 24 h	Yang *et al.*, 2006
Epidermoid carcinoma	ErbB-selective kinase inhibitor PKI-166	Reduction after 1 week	Waldherr *et al.*, 2005
Fibrosarcoma	Cisplatin	Reduction at 48 h	Leyton *et al.*, 2005
Squamous cell cancer	Radiotherapy	Reduction between 6 h and 3 days	Sugiyama *et al.*, 2004
Oesophageal cancer cells	5-FU Methotrexate Gemcitabine Cisplatin	Increase at 24 h with 5-FU and methotrexate. Moderate increase with gemcitabine. Reduction with cisplatin	Dittmann *et al.*, 2002
Oesophageal cancer	Docetaxel and radiotherapy	Reduction at 2 days	Apisarnthanarax *et al.*, 2006
Small cell lung cancer	Fibroblast growth factor receptor (FGFR) inhibitor PD173074	Reduction 7–10 days	Pardo *et al.*, 2009
Breast cancer MMTV/HER2 and BT474	Trastuzumab	No change in MMTV/HER2. Reduced in BT474 after 1 week	Shah *et al.*, 2009
Non-small cell lung cancer	Erlotinib	Reduction after 2 days	Ullrich *et al.*, 2008
Melanoma	mitogenic extracellular kinase 1/2 inhibitor, PD0325901	Reduction at 1 and 10 days	Leyton *et al.*, 2008
Fibrosarcoma-1	5-FU	Increase at 1 h	Perumal *et al.*, 2006
Colon cancer	Histone deacetylase inhibitor LAQ824	Reduction at 4 days	Leyton *et al.*, 2006a
α-FR–positive KB xenograft	BGC 945, an FR-targeted antifolate TS inhibitor; the nontargeted antifolate BGC 9331	Increase 1–4 h after BGC 945 Increase 4–24 h after BGC 9331	Pillai *et al.*, 2008
Ovarian cancer	Adenovirus	Reduction at 48 and 168 h	Leyton *et al.*, 2006b
Glioma	Prodrugs	Reduction at 3 days	Rueger *et al.*, 2010
Fibrosarcoma	Patupilone	Reduction at 24 h	Ebenhan *et al.*, 2009
SKMEL-28 xenografts	MEK inhibition	Reduction at 1 week	Solit *et al.*, 2007

Table 6

Clinical studies of the use of [^{18}F]FLT for monitoring therapy response

Tumour type	Therapy	Change in FLT uptake with therapy	Reference
Lung	Gefitinib	Reduction after 7 days; correlated with response	Sohn *et al.*, 2008
	Chemo/radiotherapy	Reduction at day 2, 8 and 29	Everitt *et al.*, 2009
Gliomas	Bevacizumab and irinotecan	Reduction at 6 weeks; correlation with progression-free survival	Chen *et al.*, 2007
	Temozolamide	Correlation with tumour response	Galldiks *et al.*, 2010
Germ cell tumours	Combination chemotherapy	Reduction; no association with response	Pfannenberg *et al.*, 2010
Non-Hodgkin's lymphoma	CHOP, R-CHOP, rituximab	Reduction after 2 days; correlated with response. No change with rituximab	Herrmann *et al.*, 2007
Breast cancer	Various chemotherapy and hormone therapy	Reduction at 2 weeks; correlated with response	Pio *et al.*, 2006
	Combination 5-FU, epirubicin, cyclophosphamide	Reduction at 1 week; correlated with response	Kenny *et al.*, 2007
Colorectal	Combination 5-FU and radiotherapy	Reduction at 2 weeks; no correlation with response	Wieder *et al.*, 2007
Oropharyngeal cancer	Radiotherapy	Reduction at 1 week	Troost *et al.*, 2010
	Radiotherapy	Increase 2–3 months compared with baseline; correlation with presence of disease	Been *et al.*, 2009
Melanoma	CTLA4 blockade	No change in tumour uptake	Ribas *et al.*, 2010
Colon (COLO205 cells)	Checkpoint kinase 1 (Chk1)inhibitor and docetaxel	Reduction with docetaxel and combination treatment at day 2 + 16	Zhang *et al.*, 2009

and is a precursor for membrane phosphatidylcholines, a class of phospholipids and a major component of biological membranes. The expression and activity of phosphocholine is up-regulated in a number of tumour types including prostate, lung, breast and colon cancer (Ramirez de Molina *et al.*, 2002; 2007; Glunde *et al.*, 2005). This increase in phosphocholine levels seen in malignancy is largely attributable to the up-regulation of choline kinase that occurs with malignant transformation. However, increased phosphocholine levels may also be due to increased breakdown via phospholipase C (Glunde *et al.*, 2004). Choline can be radiolabelled with either carbon-11 ([^{11}C]choline) or flurorine-18 ([^{18}F]fluorocholine, or [^{18}F]FCH). As [^{11}C]Choline has reduced excretion by the urinary collecting system it has been used clinically in the imaging of pelvic tumours.

[^{11}C]Choline has been most extensively used in the assessment of nodal disease in prostate cancer (Hara *et al.*, 1998; de Jong *et al.*, 2003; Reske *et al.*, 2006; Scher *et al.*, 2007). However, the utility of [^{11}C]choline as an early response biomarker for the management of prostate cancer is yet to be established. Promising preclinical work by Krause and colleagues with [^{11}C]choline showed utility for early therapeutic response to docetaxel in a prostate cancer xenograft model (Krause *et al.* 2010). Kenny and colleagues report on a subgroup of patients treated with trastuzumab who had early responses measured by [^{11}C]choline-PET (Kenny *et al.*, 2010). These findings require further investigation. [^{18}F]-fluoromethyl-choline (FCH) has the advantage of longer half-life compared with [^{11}C]choline, but these tracers undergo oxidation, with metabolites being detected within the plasma soon after administration. Thus the relative contribution of both the parent compound and the metabolites are difficult to ascertain when a late imaging protocol is used. Leyton and colleagues have recently reported data with a deuterated analogue,[^{18}F]-fluoromethyl-[1,2-^{2}H$_4$]-choline (D4-[^{18}F]FCH), which exhibits greater *in vivo* stability, and sensitivity particularly for late tumour imaging. Moreover, the authors report that D4-[^{18}F]FCH was a biomarker of response in mice treated with the mitogenic inhibitor of the extracellular signal-regulated kinase, PD0325901 (Leyton *et al.*, 2009).

Apoptosis

Apoptosis, or programmed cell death, is the likely mechanism behind the tumourcidal effects of both standard chemotherapeutic agents and many novel agents (Hanahan and Weinberg, 2000). An early event in apoptosis involves phosphatidylserine, which is normally confined to the cytoplasmic leaflet of the cell translocating to the external leaflet of the cell membrane. This has been exploited for imaging by labelling annexin V, an endogenous protein that has a high affinity for membrane bound phosphatidylderine, for imaging of apoptosis *in vivo* (Blankenberg *et al.*, 1998). Annexin V was initially labelled with technetium-99 m and utilized as a SPECT radionuclide, to detect cardiac thrombosis in patients with atrial fibrillation (Blankenberg *et al.*, 1999; Hofstra *et al.*, 2000). SPECT imaging differs from PET imaging in that it involves the administration of a gamma-emitting tracer. An improved labelling methodology resulted in the

development of [^{99m}Tc]-HYNIC-annexin V, which has been used in the assessment of lung cancer, where a patient responding to therapy had an increased uptake of the radiotracer (Blankenberg, 2008). Other annexin V-based radioisotopes with longer half-lives have been developed including iodine-124 with the specific advantage that its long half-life allows the characterization of longer-term biologicalal processes, and [^{18}F]-annexin V (Glaser *et al.*, 2003; Yagle *et al.*, 2005).

There is still considerable interest in imaging apoptosis, as this is the predominant outcome from many cytotoxic and mew mechanism-based anticancer agents. [^{18}F]-labelled 5-fluoropentyl-2-methyl-malonic acid ([^{18}F]-ML10) is a ^{18}F radiotracer which is a member of the ApoSense family of small-molecule apoptosis probes that utilize the γ-carboxyglutamic-acid (Gla)-domain protein as a potential critical structural moiety, enabling binding to cells with anionic phospholipid surfaces (Cohen *et al.*, 2009). Early studies reported that induction of apoptosis is associated with cellular uptake of the probe, which is also caspase-dependent, and is blocked by caspase inhibition. Uptake of the tracer occurs in parallel with binding of Annexin-V and disruption of mitochondrial membrane potential. Uptake and accumulation of ([^{18}F]-ML10 is lost upon membrane disruption and therefore may be utilized to differentiate apoptosis versus necrosis (Cohen *et al.*, 2009). Whilst there are currently no published studies of the utility of [^{18}F]-ML10 in the imaging of malignancies, a recent study has validated the use of an analogue of ML-10, ML-9. In this study [^{3}H]-ML-9 was used to detect apoptosis induced by doxorubicin in *in vivo* models of malignancy (Grimberg *et al.*, 2009). The authors report marked uptake of [^{3}H]-ML-9 following treatment which correlated with both caspase activation and DNA fragmentation. Clinical validation of [^{18}F]-ML10 as a marker of apoptosis is underway.

The process of cell death involves the family of caspases that execute cell death. Given the role of these enzymes in cell death there is considerable interest in imaging caspases. Isatin sulphonamides are novel agents in development that bind selectively to the active site of caspase-3 (and -7). Work by our group illustrates the utility of a specific [^{18}F]isatin sulfonamide, [^{18}F]ICMT-11, which binds to a range of drug-induced apoptotic cancer cells *in vitro* and to murine lymphoma xenografts (Smith *et al.*, 2008; Nguyen *et al.*, 2009). Furthermore, the increased signal intensity in tumours after drug treatment was associated with increased apoptosis. This tracer is currently undergoing further validation.

Angiogenesis

Angiogenesis is an essential process that supports tumour growth and metastasis through neovascularization that is driven by growth factors including vascular endothelial growth factor (VEGF) (Shibuya, 2008). There are two main strategies for the targeting of angiogenesis for therapeutic benefit: direct inhibition of angiogenesis growth factors and their receptors, for example, the anti-VEGF inhibitor bevacizumab, and indirect inhibition of blood flow through targeting of established vasculature, for example, the tubulin binding drug combretastatin A4-phosphate. Despite the introduction of these agents into clinical practice, there are few validated imaging biomarkers that can currently be used to predict or assess response to these therapies. Naturally there is considerable interest in this area

There have been a number of PET methodologies developed for imaging angiogenesis. Direct imaging of VEGF and its receptor has been studied by a number of investigators with IgG radiolabelled with long-lived isotopes but these are yet to be used routinely in the clinic. In a study by Collingridge and colleagues, the free and membrane bound VEGF in tumours was imaged directly in mice through utilization of an anti-VEGF antibody labelled with iodine-124 ([^{124}I]-SHPP-VG76e) (Collingridge *et al.*, 2002). This study highlighted the need to evaluate antibody diagnostics following labelling to assure that they retain immunoreactivity and the use of control antibodies to assess blood pooling. Specific imaging of VEGF at 24 h after injection of the radiotracer was demonstrated in tumours expressing different levels of VEGF, as well as following presaturation of VEGF binding sites by unlabelled VG76e. In a phase I study, HuMV833, a humanized anti-VEGF monoclonal antibody, was labelled with iodine-124 and the distribution of the antibody, as well as its clearance, was assessed (Jayson *et al.*, 2002). The results indicated heterogenous uptake both within individual tumours and between individual patients. Recently, bevacizumab was labelled with zirconium-89 and assessed in an ovarian cancer model *in vivo* (Nagengast *et al.*, 2007). The study reported higher uptake of radiolabeled bevacizumab compared with control radiolabelled IgG in the human xenografts. However, compared with other studies investigating radiolabelled bevacizumab the tumour uptake was lower. This may have been as there is no clear evidence that VEGF is up-regulated in this tumour model, which again underscores the importance of appropriate tumour models in proof of concept studies. With respect to the targeting of the VEGF receptor, Cai and colleagues recently published an *in vivo* study of [^{64}Cu]-1,4,7,10-tetraazacyclododecane-*N,N′,N″,N‴*-tetraacetic acid (DOTA)-$VEGF_{121}$ (Cai *et al.*, 2006). The report suggests rapid and specific uptake of [^{64}Cu]-DOTA-$VEGF_{121}$ in tumours that express high levels of VEGFR-2. Furthermore, the authors have developed a VEGFR-2-specific PET tracer based on mutated $VEGF_{121}$ that has lower binding affinity for VEGFR-1 without compromising VEGFR-2 binding affinity (Wang *et al.*, 2007). Given the angiogenesis inducing properties of VEGF isoforms, it will be interesting to see how VEGFR-targeting probes are transferred into clinical use.

There is increasing interest in imaging integrin $\alpha_v\beta_3$. Integrins are a family of cell-adhesion receptors that allow the interaction of tumour cells and tumour endothelial cells with the extracellular matrix (ECM). The integrin $\alpha_v\beta_3$ is highly expressed on activated endothelial cells during angiogenesis. The tripeptide amino acid sequence, RGD, is present in a number of ECM proteins including vitronectin, fibronectin, and thrombospondin. Cyclic peptide ligands possessing the RGD sequence have high affinity for these integrins and, when labelled appropriately with positron- or γ-emitting radioisotopes, can be used for imaging $\alpha_v\beta_3$ receptor by PET. Haubner and colleagues synthesized [^{18}F]Galacto-RGD for evaluation of tumour angiogenesis in a number of tumour types (Haubner *et al.*, 2001). In these studies it was noted that tracer uptake corresponded to expression of $\alpha_v\beta_3$ and tracer uptake could be blocked by excess unlabelled compound. [^{18}F]Galacto-RGD has been evaluated clinically in a number

of tumour types illustrating good biodistribution, low background uptake and heterogeneous uptake within tumours that correlated with expression of $\alpha_v\beta_3$ within tumour samples. More recently a new pegylated RGD-based radioligand for $\alpha_v\beta_3$ ([^{18}F]AH111585/[^{18}F]fluciclatide) was developed with high tumour retention. In a recent phase I study, Kenny and colleagues reported that [^{18}F]fluciclatide is retained in tumour tissues and detects primary and metastatic breast cancer by PET (Kenny *et al*., 2008 #125). A number of other clinical studies are currently underway utilizing this tracer as a response marker and results are awaited.

Visualization of blood flow offers another strategy for evaluating the vascular phenotype. Clinically, [^{15}O]H_2O has been extensively used to measure tissue perfusion in response to anti-vascular therapy. [^{15}O]H_2O is freely diffusible into and out of tissue water and is biologically inert, having the desired qualities for a tracer of perfusion. In a seminal study in phase I patients, [^{15}O]H_2O-PET was performed in patients receiving the anti-vascular agent combrestatin A4. The authors reported a 30–60% reduction in tumour flow at higher dose levels. This trial was important in delineating the role of PET imaging in phase I drug development. In particular, results from the PET study confirmed the mechanism of action of combrestatin A4 and allowed for the selection of dosing schedule to be taken forward into phase II (Collins, 2003).

Conclusion

In summary, the development of a novel radiotracer is a complicated process involving an experienced multidisciplinary research team. Described here are the initial steps in identifying and developing a potential radioligand for clinical use. As described, this involves extensive *in vitro* and *in vivo* characterization. A number of oncology drugs have been radiolabelled for PET imaging of PK and various drugs have been evaluated for target modulation and response using PET. Imaging of the tumours and normal tissues preclude extrapolation of plasma PK-PD to tissue PK-PD and the inherent assumptions associated with that as these are measured directly by the imaging method. As oncology continues to move towards the concept of personalized medicine, the role of PET imaging in achieving this aim will develop further. PET imaging allows a non-invasive, functional assessment of pathophysiological processes occurring within a patient, and is becoming an essential tool in the assessment of disease diagnosis, progression and response to therapy. Furthermore, as the cost of drug development increases, PET may be useful in demonstrating proof of concept for the proposed molecular and biological mechanisms of action for a drug, as well as being a useful biomarker. This is an area where reduced regulatory burden on investigators will boost innovation and rapid transition of research technology into patient tools.

Acknowledgements

We thank Kaiyumars Contractor, Lynn Maslen and Graham Smith for their help with this MS and Laura Kenny and Julius Leyton for allowing us to use unpublished images.

This work was supported by Cancer Research UK/Engineering and Physical Sciences Research Council/Medical Research Council/National Institute for Health Research Grant C2536/A10337.

Conflicts of interest

The authors have no conflict of interest to declare.

References

Ahmed FY, Johnston SJ, Cassidy J, O'Kelly T, Binnie N, Murray GI *et al*. (1999). Eniluracil treatment completely inactivates dihydropyrimidine dehydrogenase in colorectal tumors. J Clin Oncol 17: 2439–2445.

Apisarnthanarax S, Alauddin MM, Mourtada F, Ariga H, Raju U, Mawlawi O *et al*. (2006). Early detection of chemoradioresponse in esophageal carcinoma by 3′-deoxy-3′-3H-fluorothymidine using preclinical tumor models. Clin Cancer Res 12: 4590–4597.

Bading JR, Shields AF (2008). Imaging of cell proliferation: status and prospects. J Nucl Med 49 (Suppl. 2): 64S–80S.

Barthel H, Cleij MC, Collingridge DR, Hutchinson OC, Osman S, He Q *et al*. (2003). 3′-deoxy-3′-[18F]fluorothymidine as a new marker for monitoring tumor response to antiproliferative therapy in vivo with positron emission tomography. Cancer Res 63: 3791–3798.

Barthel H, Perumal M, Latigo J, He Q, Brady F, Luthra SK *et al*. (2005). The uptake of 3′-deoxy-3′-[18F]fluorothymidine into L5178Y tumours in vivo is dependent on thymidine kinase 1 protein levels. Eur J Nucl Med Mol Imaging 32: 257–263.

Barwick T, Bencherif B, Mountz JM, Avril N (2009). Molecular PET and PET/CT imaging of tumour cell proliferation using F-18 fluoro-L-thymidine: a comprehensive evaluation. Nucl Med Commun 30: 908–917.

Bates RC, Edwards NS, Yates JD (2000). Spheroids and cell survival. Crit Rev Oncol Hematol 36: 61–74.

Been LB, Hoekstra HJ, Suurmeijer AJ, Jager PL, van der Laan BF, Elsinga PH (2009). [18F]FLT-PET and [18F]FDG-Pet in the evaluation of radiotherapy for laryngeal cancer. Oral Oncol 45: e211–e215.

Bergstrom M, Grahnen A, Langstrom B (2003). Positron emission tomography microdosing: a new concept with application in tracer and early clinical drug development. Eur J Clin Pharmacol 59: 357–366.

Blankenberg FG (2008). *In vivo* detection of apoptosis. J Nucl Med 49 (Suppl. 2): 81S–95S.

Blankenberg FG, Katsikis PD, Tait JF, Davis RE, Naumovski L, Ohtsuki K *et al*. (1998). In vivo detection and imaging of phosphatidylserine expression during programmed cell death. Proc Natl Acad Sci U S A 95: 6349–6354.

Blankenberg FG, Katsikis PD, Tait JF, Davis RE, Naumovski L, Ohtsuki K *et al*. (1999). Imaging of apoptosis (programmed cell death) with 99mTc annexin V. J Nucl Med 40: 184–191.

Brepoels L, Stroobants S, Verhoef G, De Groot T, Mortelmans L, De Wolf-Peeters C (2009). (18)F-FDG and (18)F-FLT uptake early after cyclophosphamide and mTOR inhibition in an experimental lymphoma model. J Nucl Med 50: 1102–1109.

Buck AK, Kratochwil C, Glatting G, Juweid M, Bommer M, Tepsic D *et al.* (2007). Early assessment of therapy response in malignant lymphoma with the thymidine analogue [18F]FLT. Eur J Nucl Med Mol Imaging 34: 1775–1782.

Burton C, Ell P, Linch D (2004). The role of PET imaging in lymphoma. Br J Haematol 126: 772–784.

Cai W, Wu Y, Chen K, Cao Q, Tice DA, Chen X (2006). In vitro and in vivo characterization of 64Cu-labeled Abegrin, a humanized monoclonal antibody against integrin alpha v beta 3. Cancer Res 66: 9673–9681.

Chen W, Delaloye S, Silverman DH, Geist C, Czernin J, Sayre J *et al.* (2007). Predicting treatment response of malignant gliomas to bevacizumab and irinotecan by imaging proliferation with [18F] fluorothymidine positron emission tomography: a pilot study. J Clin Oncol 25: 4714–4721.

CHMP (C.f.M.P.f.H.U.) (2004). Position Paper on Non-Clinical Safety Studies to Support Clinical Trials with A Single Microdose. Agency EM (ed.) European Medicines Agency: London.

Cohen A, Shirvan A, Levin G, Grimberg H, Reshef A, Ziv I (2009). From the Gla domain to a novel small-molecule detector of apoptosis. Cell Res 19: 625–637.

Collingridge DR, Carroll VA, Glaser M, Aboagye EO, Osman S, Hutchinson OC *et al.* (2002). The development of [(124)I]iodinated-VG76e: a novel tracer for imaging vascular endothelial growth factor in vivo using positron emission tomography. Cancer Res 62: 5912–5919.

Collins JM (2003). Functional imaging in phase I studies: decorations or decision making? J Clin Oncol 21: 2807–2809.

Contractor KB, Aboagye EO (2009). Monitoring predominantly cytostatic treatment response with 18F-FDG PET. J Nucl Med 50 (Suppl. 1): 97S–105S.

Di Fabio F, Pinto C, Rojas Llimpe FL, Fanti S, Castellucci P, Longobardi C *et al.* (2007). The predictive value of 18F-FDG-PET early evaluation in patients with metastatic gastric adenocarcinoma treated with chemotherapy plus cetuximab. Gastric Cancer 10: 221–227.

Dimasi JA (2001). Risks in new drug development: approval success rates for investigational drugs. Clin Pharmacol Ther 69: 297–307.

Dimitrakopoulou-Strauss A, Strauss LG, Schlag P, Hohenberger P, Irngartinger G, Oberdorfer F *et al.* (1998). Intravenous and intra-arterial oxygen-15-labeled water and fluorine-18-labeled fluorouracil in patients with liver metastases from colorectal carcinoma. J Nucl Med 39: 465–473.

Dittmann H, Dohmen BM, Kehlbach R, Bartusek G, Pritzkow M, Sarbia M *et al.* (2002). Early changes in [18F]FLT uptake after chemotherapy: an experimental study. Eur J Nucl Med Mol Imaging 29: 1462–1469.

Dubessy C, Merlin JM, Marchal C, Guillemin F (2000). Spheroids in radiobiology and photodynamic therapy. Crit Rev Oncol Hematol 36: 179–192.

EANM Radiopharmacy Committee (2007). *Guidelines on Current Good Radiopharmacy Practice (cGRPP) in the Preparation of Radiopharmaceuticals*.

Ebenhan T, Honer M, Ametamey SM, Schubiger PA, Becquet M, Ferretti S *et al.* (2009). Comparison of [18F]-tracers in various experimental tumor models by PET imaging and identification of an early response biomarker for the novel microtubule stabilizer patupilone. Mol Imaging Biol 11: 308–321.

EMEA (2009). Questions & Answers on the CHMP Guideline on the Limits of Genotoxic Impurities (EMA/CHMP/SWP/431994/2007/ Revision 2), Agency TEME (ed.) London, http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002907.pdf.

Everitt S, Hicks RJ, Ball D, Kron T, Schneider-Kolsky M, Walter T *et al.* (2009). Imaging cellular proliferation during chemo-radiotherapy: a pilot study of serial 18F-FLT positron emission tomography/computed tomography imaging for non-small-cell lung cancer. Int J Radiat Oncol Biol Phys 75: 1098–1104.

FDA (2004). Innovation or stagnation: challenge and opportunity on the critical path to new medical products.

FDA (2010). Critical Path 2010 Update. U.S. Department of Health & Human Services.

Fowler JS, MacGregor RR, Wolf AP, Farrell AA, Karlstrom KI, Ruth TJ (1981). A shielded synthesis system for production of 2-deoxy-2-[18F]fluoro-D-glucose. J Nucl Med 22: 376–380.

Fracasso G, Colombatti M (2000). Effect of therapeutic macromolecules in spheroids. Crit Rev Oncol Hematol 36: 159–178.

Galldiks N, Kracht LW, Burghaus L, Ullrich RT, Backes H, Brunn A *et al.* (2010). Patient-tailored, imaging-guided, long-term temozolomide chemotherapy in patients with glioblastoma. Mol Imaging 9: 40–46.

Gambhir SS, Czernin J, Schwimmer J, Silverman DH, Coleman RE, Phelps ME (2001). A tabulated summary of the FDG PET literature. J Nucl Med 42: 1S–93S.

Ginos JZ, Cooper AJ, Dhawan V, Lai JC, Strother SC, Alcock N *et al.* (1987). [13N]cisplatin PET to assess pharmacokinetics of intra-arterial versus intravenous chemotherapy for malignant brain tumors. J Nucl Med 28: 1844–1852.

Glaser M, Collingridge DR, Aboagye EO, Bouchier-Hayes L, Hutchinson OC, Martin SJ *et al.* (2003). Iodine-124 labelled annexin-V as a potential radiotracer to study apoptosis using positron emission tomography. Appl Radiat Isot 58: 55–62.

Glunde K, Jie C, Bhujwalla ZM (2004). Molecular causes of the aberrant choline phospholipid metabolism in breast cancer. Cancer Res 64: 4270–4276.

Glunde K, Raman V, Mori N, Bhujwalla ZM (2005). RNA interference-mediated choline kinase suppression in breast cancer cells induces differentiation and reduces proliferation. Cancer Res 65: 11034–11043.

Graf N, Herrmann K, den Hollander J, Fend F, Schuster T, Wester HJ *et al.* (2008). Imaging proliferation to monitor early response of lymphoma to cytotoxic treatment. Mol Imaging Biol 10: 349–355.

Gray KR, Contractor KB, Kenny LM, Al-Nahhas A, Shousha S, Stebbing J *et al.* (2010). Kinetic filtering of [(18)F]Fluorothymidine in positron emission tomography studies. Phys Med Biol 55: 695–709.

Grimberg H, Levin G, Shirvan A, Cohen A, Yogev-Falach M, Reshef A *et al.* (2009). Monitoring of tumour response to chemotherapy in vivo by a novel small-molecule detector of apoptosis. Apoptosis 14: 257–267.

Haioun C, Itti E, Rahmouni A, Brice P, Rain JD, Belhadj K *et al.* (2005). [18F]fluoro-2-deoxy-D-glucose positron emission tomography (FDG-PET) in aggressive lymphoma: an early prognostic tool for predicting patient outcome. Blood 106: 1376–1381.

Hamacher K, Coenen HH, Stocklin G (1986). Efficient stereospecific synthesis of no-carrier-added 2-[18F]-fluoro-2-deoxy-D-glucose using aminopolyether supported nucleophilic substitution. J Nucl Med 27: 235–238.

Hanahan D, Weinberg RA (2000). The hallmarks of cancer. Cell 100: 57–70.

Hara T, Kosaka N, Kishi H (1998). PET imaging of prostate cancer using carbon-11-choline. J Nucl Med 39: 990–995.

Harte RJ, Matthews JC, O'Reilly SM, Price PM (1998). Sources of error in tissue and tumor measurements of 5-[18F]fluorouracil. J Nucl Med 39: 1370–1376.

Haubner R, Wester HJ, Weber WA, Mang C, Ziegler SI, Goodman SL *et al*. (2001). Noninvasive imaging of alpha(v)beta3 integrin expression using 18F-labeled RGD-containing glycopeptide and positron emission tomography. Cancer Res 61: 1781–1785.

Herrmann K, Wieder HA, Buck AK, Schoffel M, Krause BJ, Fend F *et al*. (2007). Early response assessment using 3′-deoxy-3′-[18F]fluorothymidine-positron emission tomography in high-grade non-Hodgkin's lymphoma. Clin Cancer Res 13: 3552–3558.

Hofstra L, Liem IH, Dumont EA, Boersma HH, van Heerde WL, Doevendans PA *et al*. (2000). Visualisation of cell death in vivo in patients with acute myocardial infarction. Lancet 356: 209–212.

Holdsworth CH, Badawi RD, Manola JB, Kijewski MF, Israel DA, Demetri GD *et al*. (2007). CT and PET: early prognostic indicators of response to imatinib mesylate in patients with gastrointestinal stromal tumor. AJR Am J Roentgenol 189: W324–W330.

Iwata R, Ido T, Takahashi T, Monma M (1984). Automated synthesis system for productino of 2-deoxy-[18F]fluoro-D-glucose with computer control. Appl Radiat Isot 35: 445–454.

Jayson GC, Zweit J, Jackson A, Mulatero C, Julyan P, Ranson M *et al*. (2002). Molecular imaging and biological evaluation of HuMV833 anti-VEGF antibody: implications for trial design of antiangiogenic antibodies. J Natl Cancer Inst 94: 1484–1493.

de Jong IJ, Pruim J, Elsinga PH, Vaalburg W, Mensink HJ (2003). 11C-choline positron emission tomography for the evaluation after treatment of localized prostate cancer. Eur Urol 44: 32–38; discussion 38–39.

Kawada K, Murakami K, Sato T, Kojima Y, Ebi H, Mukai H *et al*. (2007). Prospective study of positron emission tomography for evaluation of the activity of lapatinib, a dual inhibitor of the ErbB1 and ErbB2 tyrosine kinases, in patients with advanced tumors. Jpn J Clin Oncol 37: 44–48.

Kelloff GJ, Hoffman JM, Johnson B, Scher HI, Siegel BA, Cheng EY *et al*. (2005). Progress and promise of FDG-PET imaging for cancer patient management and oncologic drug development. Clin Cancer Res 11: 2785–2808.

Kenny LM, Vigushin DM, Al-Nahhas A, Osman S, Luthra SK, Shousha S *et al*. (2005). Quantification of cellular proliferation in tumor and normal tissues of patients with breast cancer by [18F]fluorothymidine-positron emission tomography imaging: evaluation of analytical methods. Cancer Res 65: 10104–10112.

Kenny L, Coombes RC, Vigushin DM, Al-Nahhas A, Shousha S, Aboagye EO (2007). Imaging early changes in proliferation at 1 week post chemotherapy: a pilot study in breast cancer patients with 3′-deoxy-3′-[18F]fluorothymidine positron emission tomography. Eur J Nucl Med Mol Imaging 34: 1339–1347.

Kenny LM, Coombes RC, Oulie I, Contractor KB, Miller M, Spinks TJ *et al*. (2008). Phase I trial of the positron-emitting Arg-Gly-Asp (RGD) peptide radioligand 18F-AH111585 in breast cancer patients. J Nucl Med 49: 879–886.

Kenny LM, Contractor KB, Stebbing J, Al-Nahhas A, Palmieri C, Shousha S *et al*. (2009). Altered tissue 3′-deoxy-3′-[18F]fluorothymidine pharmacokinetics in human breast cancer following capecitabine treatment detected by positron emission tomography. Clin Cancer Res 15: 6649–6657.

Kenny LM, Contractor KB, Hinz R, Stebbing J, Palmieri C, Jiang J *et al*. (2010). Reproducibility of [11C]choline-positron emission tomography and effect of trastuzumab. Clin Cancer Res 16: 4236–4245.

Kissel J, Brix G, Bellemann ME, Strauss LG, Dimitrakopoulou-Strauss A, Port R *et al*. (1997). Pharmacokinetic analysis of 5-[18F]fluorouracil tissue concentrations measured with positron emission tomography in patients with liver metastases from colorectal adenocarcinoma. Cancer Res 57: 3415–3423.

Krause BJ, Souvatzoglou M, Herrmann K, Weber AW, Schuster T, Buck AK *et al*. (2010). [11C]Choline as pharmacodynamic marker for therapy response assessment in a prostate cancer xenograft model. Eur J Nucl Med Mol Imaging 37: 1861–1868.

Kunz-Schughart LA, Freyer JP, Hofstaedter F, Ebner R (2004). The use of 3-D cultures for high-throughput screening: the multicellular spheroid model. J Biomol Screen 9: 273–285.

Kurdziel KA, Kiesewetter DO, Carson RE, Eckelman WC, Herscovitch P (2003). Biodistribution, radiation dose estimates, and in vivo Pgp modulation studies of 18F-paclitaxel in nonhuman primates. J Nucl Med 44: 1330–1339.

Lee JH, Rosen EL, Mankoff DA (2009). The role of radiotracer imaging in the diagnosis and management of patients with breast cancer: part 1 – overview, detection, and staging. J Nucl Med 50: 569–581.

Leyton J, Latigo JR, Perumal M, Dhaliwal H, He Q, Aboagye EO (2005). Early detection of tumor response to chemotherapy by 3′-deoxy-3′-[18F]fluorothymidine positron emission tomography: the effect of cisplatin on a fibrosarcoma tumor model *in vivo*. Cancer Res 65: 4202–4210.

Leyton J, Alao JP, Da Costa M, Stavropoulou AV, Latigo JR, Perumal M *et al*. (2006a). In vivo biological activity of the histone deacetylase inhibitor LAQ824 is detectable with 3′-deoxy-3′-[18F]fluorothymidine positron emission tomography. Cancer Res 66: 7621–7629.

Leyton J, Lockley M, Aerts JL, Baird SK, Aboagye EO, Lemoine NR *et al*. (2006b). Quantiflying the activity of adenoviral E1A CR2 deletion mutants using renilla luciferase bioluminescence and 3′-deoxy-3′-[18F]fluorothymidine positron emission tomography imaging. Cancer Res 66: 9178–9185.

Leyton J, Smith G, Lees M, Perumal M, Nguyen QD, Aigbirhio FI *et al*. (2008). Noninvasive imaging of cell proliferation following mitogenic extracellular kinase inhibition by PD0325901. Mol Cancer Ther 7: 3112–3121.

Leyton J, Smith G, Zhao Y, Perumal M, Nguyen QD, Robins E *et al*. (2009). [18F]fluoromethyl-[1,2-2H4]-choline: a novel radiotracer for imaging choline metabolism in tumors by positron emission tomography. Cancer Res 69: 7721–7728.

Marchetti S, Schellens JH (2007). The impact of FDA and EMEA guidelines on drug development in relation to Phase 0 trials. Br J Cancer 97: 577–581.

MHRA (2007). *Rules and Guidance for Pharmaceutical Manufacturers and Distributors 2007*.

MHRA (2010). Applying to Conduct A Clinical Trial: Additional Information, Vol. 2010. Medicines and Healthcare products Regulatory Agency (MHRA) http://www.mhra.gov.uk/

Howweregulate/Medicines/Licensingofmedicines/Clinicaltrials/Applyingforaclinicaltrialauthorisation/Additionalinformation/index.htm.

Moehler M, Dimitrakopoulou-Strauss A, Gutzler F, Raeth U, Strauss LG, Stremmel W (1998). 18F-labeled fluorouracil positron emission tomography and the prognoses of colorectal carcinoma patients with metastases to the liver treated with 5-fluorouracil. Cancer 83: 245–253.

Molthoff CF, Klabbers BM, Berkhof J, Felten JT, van Gelder M, Windhorst AD *et al.* (2007). Monitoring response to radiotherapy in human squamous cell cancer bearing nude mice: comparison of 2′-deoxy-2′-[18F]fluoro-D-glucose (FDG) and 3′-[18F]fluoro-3′-deoxythymidine (FLT). Mol Imaging Biol 9: 340–347.

Monazzam A, Razifar P, Simonsson M, Qvarnstrom F, Josephsson R, Blomqvist C *et al.* (2006). Multicellular tumour spheroid as a model for evaluation of [18F]FDG as biomarker for breast cancer treatment monitoring. Cancer Cell Int 6: 6.

Monazzam A, Josephsson R, Blomqvist C, Carlsson J, Langstrom B, Bergstrom M (2007). Application of the multicellular tumour spheroid model to screen PET tracers for analysis of early response of chemotherapy in breast cancer. Breast Cancer Res 9: R45.

Monazzam A, Razifar P, Ide S, Rugaard Jensen M, Josephsson R, Blomqvist C *et al.* (2009). Evaluation of the Hsp90 inhibitor NVP-AUY922 in multicellular tumour spheroids with respect to effects on growth and PET tracer uptake. Nucl Med Biol 36: 335–342.

Mueller-Klieser W (2000). Tumor biology and experimental therapeutics. Crit Rev Oncol Hematol 36: 123–139.

Munch-Petersen B, Cloos L, Jensen HK, Tyrsted G (1995). Human thymidine kinase 1. Regulation in normal and malignant cells. Adv Enzyme Regul 35: 69–89.

Nagengast WB, de Vries EG, Hospers GA, Mulder NH, de Jong JR, Hollema H *et al.* (2007). In vivo VEGF imaging with radiolabeled bevacizumab in a human ovarian tumor xenograft. J Nucl Med 48: 1313–1319.

Nguyen QD, Smith G, Glaser M, Perumal M, Arstad E, Aboagye EO (2009). Positron emission tomography imaging of drug-induced tumor apoptosis with a caspase-3/7 specific [18F]-labeled isatin sulfonamide. Proc Natl Acad Sci U S A 106: 16375–16380.

van Oosterom AT, Judson I, Verweij J, Stroobants S, Donato di Paola E, Dimitrijevic S *et al.* (2001). Safety and efficacy of imatinib (STI571) in metastatic gastrointestinal stromal tumours: a phase I study. Lancet 358: 1421–1423.

Osman S, Rowlinson-Busza G, Luthra SK, Aboagye EO, Brown GD, Brady F *et al.* (2001). Comparative biodistribution and metabolism of carbon-11-labeled N-[2-(dimethylamino)ethyl]acridine-4-carboxamide and DNA-intercalating analogues. Cancer Res 61: 2935–2944.

Ott K, Weber WA, Lordick F, Becker K, Busch R, Herrmann K *et al.* (2006). Metabolic imaging predicts response, survival, and recurrence in adenocarcinomas of the esophagogastric junction. J Clin Oncol 24: 4692–4698.

Oyama N, Akino H, Suzuki Y, Kanamaru H, Ishida H, Tanase K *et al.* (2001). FDG PET for evaluating the change of glucose metabolism in prostate cancer after androgen ablation. Nuclear Med Commun 22: 963–969.

Pan MH, Huang SC, Liao YP, Schaue D, Wang CC, Stout DB *et al.* (2008). FLT-PET imaging of radiation responses in murine tumors. Mol Imaging Biol 10: 325–334

Pardo OE, Latigo J, Jeffery RE, Nye E, Poulsom R, Spencer-Dene B *et al.* (2009). The fibroblast growth factor receptor inhibitor PD173074 blocks small cell lung cancer growth in vitro and in vivo. Cancer Res 69: 8645–8651.

Perumal M, Pillai RG, Barthel H, Leyton J, Latigo JR, Forster M *et al.* (2006). Redistribution of nucleoside transporters to the cell membrane provides a novel approach for imaging thymidylate synthase inhibition by positron emission tomography. Cancer Res 66: 8558–8564.

Pfannenberg C, Aschoff P, Dittmann H, Mayer F, Reischl G, von Weyhern C *et al.* (2010). PET/CT with 18F-FLT: does it improve the therapeutic management of metastatic germ cell tumors? J Nucl Med 51: 845–853.

Pillai RG, Forster M, Perumal M, Mitchell F, Leyton J, Aibgirhio FI *et al.* (2008). Imaging pharmacodynamics of the alpha-folate receptor-targeted thymidylate synthase inhibitor BGC 945. Cancer Res 68: 3827–3834.

Pimlott SL (2005). Radiotracer development in psychiatry. Nucl Med Commun 26: 183–188.

Pio BS, Park CK, Pietras R, Hsueh WA, Satyamurthy N, Pegram MD *et al.* (2006). Usefulness of 3′-[F-18]fluoro-3′-deoxythymidine with positron emission tomography in predicting breast cancer response to therapy. Mol Imaging Biol 8: 36–42.

Ramirez de Molina A, Rodriguez-Gonzalez A, Gutierrez R, Martinez-Pineiro L, Sanchez J, Bonilla F *et al.* (2002). Overexpression of choline kinase is a frequent feature in human tumor-derived cell lines and in lung, prostate, and colorectal human cancers. Biochem Biophys Res Commun 296: 580–583.

Ramirez de Molina A, Sarmentero-Estrada J, Belda-Iniesta C, Taron M, Ramirez de Molina V, Cejas P *et al.* (2007). Expression of choline kinase alpha to predict outcome in patients with early-stage non-small-cell lung cancer: a retrospective study. Lancet Oncol 8: 889–897.

Reske SN, Blumstein NM, Neumaier B, Gottfried HW, Finsterbusch F, Kocot D *et al.* (2006). Imaging prostate cancer with 11C-choline PET/CT. J Nucl Med 47: 1249–1254.

Ribas A, Benz MR, Allen-Auerbach MS, Radu C, Chmielowski B, Seja E *et al.* (2010). Imaging of CTLA4 blockade-induced cell replication with (18)F-FLT PET in patients with advanced melanoma treated with tremelimumad. J Nucl Med 51: 340–346.

Romer W, Hanauske AR, Ziegler S, Thodtmann R, Weber W, Fuchs C *et al.* (1998). Positron emission tomography in non-Hodgkin's lymphoma: assessment of chemotherapy with fluorodeoxyglucose. Blood 91: 4464–4471.

Rosenbaum SJ, Lind T, Antoch G, Bockisch A (2006). False-positive FDG PET uptake – the role of PET/CT. Eur Radiol 16: 1054–1065.

Rosso L, Brock CS, Gallo JM, Saleem A, Price PM, Turkheimer FE *et al.* (2009). A new model for prediction of drug distribution in tumor and normal tissues: pharmacokinetics of temozolomide in glioma patients. Cancer Res 69: 120–127.

Rueger MA, Ameli M, Li H, Winkeler A, Rueckriem B, Vollmar S *et al.* (2010). [(18)F]FLT PET for non-invasive monitoring of early response to gene therapy in experimental gliomas. Mol Imaging Biol 13: 547–557.

Saleem A, Aboagye EO, Price PM (2000a). In vivo monitoring of drugs using radiotracer techniques. Adv Drug Deliv Rev 41: 21–39.

Saleem A, Yap J, Osman S, Brady F, Suttle B, Lucas SV *et al.* (2000b). Modulation of fluorouracil tissue pharmacokinetics by eniluracil: in-vivo imaging of drug action. Lancet 355: 2125–2131.

Saleem A, Harte RJ, Matthews JC, Osman S, Brady F, Luthra SK *et al.* (2001). Pharmacokinetic evaluation of N-[2-(dimethylamino)ethyl] acridine-4-carboxamide in patients by positron emission tomography. J Clin Oncol 19: 1421–1429.

Saleem A, Brown GD, Brady F, Aboagye EO, Osman S, Luthra SK *et al.* (2003). Metabolic activation of temozolomide measured *in vivo* using positron emission tomography. Cancer Res 63: 2409–2415.

Saleem A, Aboagye EO, Matthews JC, Price PM (2008). Plasma pharmacokinetic evaluation of cytotoxic agents radiolabelled with positron emitting radioisotopes. Cancer Chemother Pharmacol 61: 865–873.

Saleem A, Ranson M, Callies S, Lahn M, Prenant C, Brown GD *et al.* (2009). Microdosing imaging pharmacokinetic (PK) study of the antisense oligonucleotide (ASO) to survivin (LY2181308) using positron emission tomography (PET): a novel paradigm in clinical drug development. J Clin Oncol 27: 15s (suppl; abstr 3578).

Schelling M, Avril N, Nahrig J, Kuhn W, Romer W, Sattler D *et al.* (2000). Positron emission tomography using [(18)F]Fluorodeoxyglucose for monitoring primary chemotherapy in breast cancer. J Clin Oncol 18: 1689–1695.

Scher B, Seitz M, Albinger W, Tiling R, Scherr M, Becker HC *et al.* (2007). Value of 11C-choline PET and PET/CT in patients with suspected prostate cancer. Eur J Nucl Med Mol Imaging 34: 45–53.

Seitz U, Wagner M, Neumaier B, Wawra E, Glatting G, Leder G *et al.* (2002). Evaluation of pyrimidine metabolising enzymes and in vitro uptake of 3′-[(18)F]fluoro-3′-deoxythymidine ([(18)F]FLT) in pancreatic cancer cell lines. Eur J Nucl Med Mol Imaging 29: 1174–1181.

Shah C, Miller TW, Wyatt SK, McKinley ET, Olivares MG, Sanchez V *et al.* (2009). Imaging biomarkers predict response to anti-HER2 (ErbB2) therapy in preclinical models of breast cancer. Clin Cancer Res 15: 4712–4721.

Shankar LK, Hoffman JM, Bacharach S, Graham MM, Karp J, Lammertsma AA *et al.* (2006). Consensus recommendations for the use of 18F-FDG PET as an indicator of therapeutic response in patients in National Cancer Institute Trials. J Nucl Med 47: 1059–1066.

Sherley JL, Kelly TJ (1988). Regulation of human thymidine kinase during the cell cycle. J Biol Chem 263: 8350–8358.

Shibuya M (2008). Vascular endothelial growth factor-dependent and -independent regulation of angiogenesis. BMB Rep 41: 278–286.

Shields AF, Graham MM, Kozawa SM, Kozell LB, Link JM, Swenson ER *et al.* (1992). Contribution of labeled carbon dioxide to PET imaging of carbon-11-labeled compounds. J Nucl Med 33: 581–584.

Shields AF, Grierson JR, Dohmen BM, Machulla HJ, Stayanoff JC, Lawhorn-Crews JM *et al.* (1998). Imaging proliferation in vivo with [F-18]FLT and positron emission tomography. Nat Med 4: 1334–1336.

Shreve PD, Anzai Y, Wahl RL (1999). Pitfalls in oncologic diagnosis with FDG PET imaging: physiologic and benign variants. Radiographics 19: 61–77; quiz 150–151.

Smith IC, Welch AE, Hutcheon AW, Miller ID, Payne S, Chilcott F *et al.* (2000). Positron emission tomography using [(18)F]-fluorodeoxy-D-glucose to predict the pathologic response of breast cancer to primary chemotherapy. J Clin Oncol 18: 1676–1688.

Smith G, Glaser M, Perumal M, Nguyen QD, Shan B, Arstad E *et al.* (2008). Design, synthesis, and biological characterization of a caspase 3/7 selective isatin labeled with 2-[18F]fluoroethylazide. J Med Chem 51: 8057–8067.

Sohn HJ, Yang YJ, Ryu JS, Oh SJ, Im KC, Moon DH *et al.* (2008). [18F]Fluorothymidine positron emission tomography before and 7 days after gefitinib treatment predicts response in patients with advanced adenocarcinoma of the lung. Clin Cancer Res 14: 7423–7429.

Solit DB, Santos E, Pratilas CA, Lobo J, Moroz M, Cai S *et al.* (2007). 3′-deoxy-3′-[18F]fluorothymidine positron emission tomography is a sensitive method for imaging the response of BRAF-dependent tumors to MEK inhibition. Cancer Res 67: 11463–11469.

Stroobants S, Goeminne J, Seegers M, Dimitrijevic S, Dupont P, Nuyts J *et al.* (2003). 18FDG-Positron emission tomography for the early prediction of response in advanced soft tissue sarcoma treated with imatinib mesylate (Glivec). Eur J Cancer 39: 2012–2020.

Stuart-Harris R, Caldas C, Pinder SE, Pharoah P (2008). Proliferation markers and survival in early breast cancer: a systematic review and meta-analysis of 85 studies in 32,825 patients. Breast 17: 323–334.

Subhas N, Patel PV, Pannu HK, Jacene HA, Fishman EK, Wahl RL (2005). Imaging of pelvic malignancies with in-line FDG PET-CT: case examples and common pitfalls of FDG PET. Radiographics 25: 1031–1043.

Sugiyama M, Sakahara H, Sato K, Harada N, Fukumoto D, Kakiuchi T *et al.* (2004). Evaluation of 3′-deoxy-3′-18F-fluorothymidine for monitoring tumor response to radiotherapy and photodynamic therapy in mice. J Nucl Med 45: 1754–1758.

Sunaga N, Oriuchi N, Kaira K, Yanagitani N, Tomizawa Y, Hisada T *et al.* (2008). Usefulness of FDG-PET for early prediction of the response to gefitinib in non-small cell lung cancer. Lung Cancer 59: 203–210.

Troost EG, Bussink J, Hoffmann AL, Boerman OC, Oyen WJ, Kaanders JH (2010). 18F-FLT PET/CT for early response monitoring and dose escalation in oropharyngeal tumors. J Nucl Med 51: 866–874.

Tyler JL, Yamamoto YL, Diksic M, Theron J, Villemure JG, Worthington C *et al.* (1986). Pharmacokinetics of superselective intra-arterial and intravenous [11C]BCNU evaluated by PET. J Nucl Med 27: 775–780.

Ullrich RT, Zander T, Neumaier B, Koker M, Shimamura T, Waerzeggers Y *et al.* (2008). Early detection of erlotinib treatment response in NSCLC by 3′-deoxy-3′-[F]-fluoro-L-thymidine ([F]FLT) positron emission tomography (PET). Plos One 3: e3908.

Vallabhajosula S (2007). (18)F-labeled positron emission tomographic radiopharmaceuticals in oncology: an overview of radiochemistry and mechanisms of tumor localization. Semin Nucl Med 37: 400–419.

VanBrocklin HF (2008). Radiopharmaceuticals for drug development: United States regulatory perspective. Current Radiopharmaceuticals 1: 2–6.

Verel I, Visser GW, Van Dongen GA (2005). The promise of immuno-PET in radioimmunotherapy. J Nucl Med 46 (Suppl. 1): 164S–171S.

Wahl RL, Jacene H, Kasamon Y, Lodge MA (2009). From RECIST to PERCIST: evolving considerations for PET response criteria in solid tumors. J Nucl Med 50 (Suppl. 1): 122S–150S.

Waldherr C, Mellinghoff IK, Tran C, Halpern BS, Rozengurt N, Safaei A *et al*. (2005). Monitoring antiproliferative responses to kinase inhibitor therapy in mice with 3′-deoxy-3′-18F-fluorothymidine PET. J Nucl Med 46: 114–120.

Wang H, Cai W, Chen K, Li ZB, Kashefi A, He L *et al*. (2007). A new PET tracer specific for vascular endothelial growth factor receptor 2. Eur J Nucl Med Mol Imaging 34: 2001–2010.

Wei LH, Su H, Hildebrandt IJ, Phelps ME, Czernin J, Weber WA (2008). Changes in tumor metabolism as readout for Mammalian target of rapamycin kinase inhibition by rapamycin in glioblastoma. Clin Cancer Res 14: 3416–3426.

Wieder HA, Geinitz H, Rosenberg R, Lordick F, Becker K, Stahl A *et al*. (2007). PET imaging with [18F]3′-deoxy-3′-fluorothymidine for prediction of response to neoadjuvant treatment in patients with rectal cancer. Eur J Nucl Med Mol Imaging 34: 878–883.

Workman P, Aboagye EO, Chung YL, Griffiths JR, Hart R, Leach MO *et al*. (2006). Minimally invasive pharmacokinetic and pharmacodynamic technologies in hypothesis-testing clinical trials of innovative therapies. J Natl Cancer Inst 98: 580–598.

Yagle KJ, Eary JF, Tait JF, Grierson JR, Link JM, Lewellen B *et al*. (2005). Evaluation of 18F-annexin V as a PET imaging agent in an animal model of apoptosis. J Nucl Med 46: 658–666.

Yang YJ, Ryu JS, Kim SY, Oh SJ, Im KC, Lee H *et al*. (2006). Use of 3′-deoxy-3′-[18F]fluorothymidine PET to monitor early responses to radiation therapy in murine SCCVII tumors. Eur J Nucl Med Mol Imaging 33: 412–419.

Yerushalmi R, Woods R, Ravdin PM, Hayes MM, Gelmon KA (2010). Ki67 in breast cancer: prognostic and predictive potential. Lancet Oncol 11: 174–183.

Young H, Baum R, Cremerius U, Herholz K, Hoekstra O, Lammertsma AA *et al*. (1999). Measurement of clinical and subclinical tumour response using [18F]-fluorodeoxyglucose and positron emission tomography: review and 1999 EORTC recommendations. European Organization for Research and Treatment of Cancer (EORTC) PET Study Group. Eur J Cancer 35: 1773–1782.

Zhang C, Yan Z, Painter CL, Zhang Q, Chen E, Arango ME *et al*. (2009). PF-00477736 mediates checkpoint kinase 1 signaling pathway and potentiates docetaxel-induced efficacy in xenografts. Clin Cancer Res 15: 4630–4640.

Zhu AX, Meyerhardt JA, Blaszkowsky LS, Kambadakone AR, Muzikansky A, Zheng H *et al*. (2010). Efficacy and safety of gemcitabine, oxaliplatin, and bevacizumab in advanced biliary-tract cancers and correlation of changes in 18-fluorodeoxyglucose PET with clinical outcome: a phase 2 study. Lancet Oncol 11: 48–54.