In vivo pharmacokinetics and pharmacodynamics in drug development using positron-emission tomography

Eric O. Aboagye, Patricia M. Price and Terry Jones

Positron-emission tomography (PET) is a sensitive technique that can be used to measure drug pharmacokinetics and pharmacodynamics non-invasively in target tissues of patients. Here we focus on the application of this technology to address some of the bottlenecks in drug development, including: elucidation of pathophysiology, evaluation of pharmacokinetics, proof of principle of mechanism, and assessment of efficacy and/or response to therapy.

***Eric O. Aboagye, Patricia M. Price and Terry Jones**
PET Oncology Group
Department of Cancer Medicine
Imperial College School of Medicine
MRC Cyclotron Unit
Hammersmith Hospital
Du Cane Road
London, UK W12 0NN
*tel: +44 (0)20 8383 3759
fax: +44 (0)20 8383 2029
e-mail: eric.aboagye@ic.ac.uk

▼ The process of drug discovery and development has reached a turning point, and the empirical screening of drugs is being abandoned for more mechanism-based approaches. A rational approach to the testing of new compounds is therefore required so that optimized leads can progress rapidly to the clinic. Non-invasive technologies such as positron-emission tomography (PET) could be used to address some of the bottlenecks in the early clinical testing of new drugs.

What is PET?

PET is a sensitive and specific non-invasive technique that uses external detectors to measure the three-dimensional (3D) distribution and kinetics of injected compounds that have been labelled with short-lived positron-emitting radioisotopes. Drugs and molecular probes can be radiolabelled with positron emitters such as carbon-11 (physical half-life, $t_{1/2}$ = 20.4 min), nitrogen-13 ($t_{1/2}$ = 10 min), oxygen-15 ($t_{1/2}$ = 2.03 min), fluorine-18 ($t_{1/2}$ = 109.8 min) and iodine-124 ($t_{1/2}$ = 4.2 days). The short half-life of most positron-emitting radionuclides limits the time available for radiosynthesis and formulation, and hence the need for an on-site cyclotron. Current commercially available clinical scanners can achieve in-plane spatial resolution of the order of 4–8 mm, whereas animal scanners can achieve 0.5–2 mm resolution. Considering the steps involved in the application of PET to clinical research (Fig. 1), early interaction and shared risks in technology development between academia and industry can provide rapid results and can reduce cost.

Bottlenecks in drug development

There are bottlenecks in drug development, and PET can make an impact on these. The hurdles that present in early drug discovery and development include: (1) elucidation of pathophysiology, (2) target selection, (3) target validation, (4) lead generation, (5) lead optimization and (6) preclinical development. In oncology, this phase is aimed at discovering and developing compounds that target specific oncogenic alterations (e.g. overexpressed Ras protein), which are active *in vivo*, have suitable physicochemical and pharmacokinetic properties and can be formulated for administration. A late phase of drug development, which occurs in the clinic, is aimed at providing data on toxicity, pharmacokinetics and efficacy. Rational drug discovery requires an early appraisal of all these issues. In a recent article, Workman[1] summarized the added value that PET can provide for drug development by asking the following questions:

(1) Is the drug getting to the site of action in the required concentration?

1359-6446/01/$ – see front matter PII: S1359-6446(01)01684-1

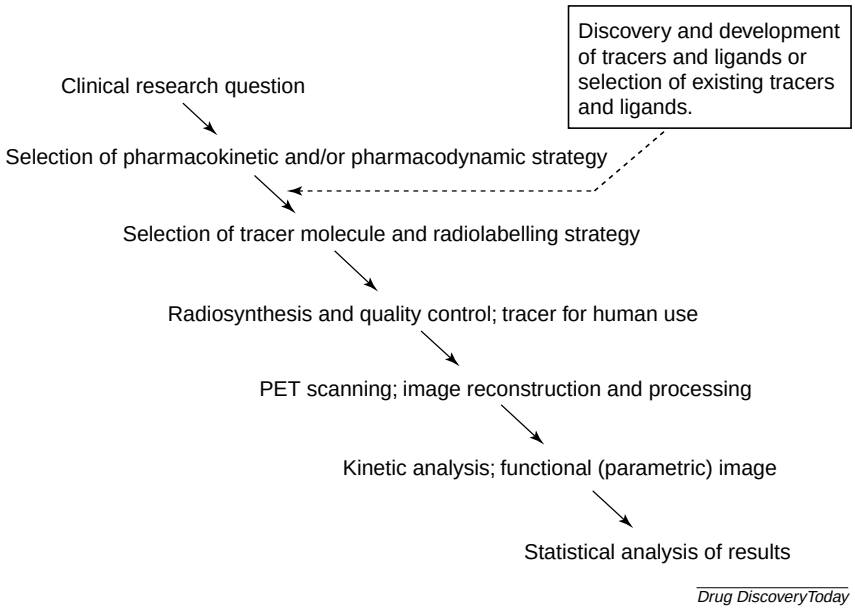

Figure 1. The imaging science of positron-emission tomography.

(2) What is the spatial heterogeneity?
(3) What is the clearance rate?
(4) Is the drug having the desired effect?
(5) How do these parameters relate to the biochemistry and physiology of the disease? Owing to the complex nature of the technology and cost considerations, the application of PET to drug development must be justified. The following questions should be asked:

- Is new information provided?
- Is the information in the 'crucial' or 'nice' category?
- Will the information speed up development?
- Are the logistics feasible?
- Is it cost effective[1]?
- Will the information provide 'scientific feedback' on mechanism of action and efficacy?
- Does imaging data provide an early indicator that the drug will be ineffective?

To make such decisions, the UK Cancer Research Campaign (CRC), for instance, has set up a Pharmacokinetics/Pharmacodynamics Technologies Advisory Committee to advise developers of anticancer agents on the use of PET and other technologies at various phases of development for CRC-funded clinical trials.

Elucidation of pathophysiology

To date, a major application of PET has been the elucidation of pathophysiology. This is because radiochemists can label various ligands and tracers, and can apply them to studies in animal models of disease in addition to subjects. The imaging of cardiac or brain function or of tumour properties enables scientists to understand physiology or pathophysiology in living beings and to evaluate the effects of clinical interventions. Table 1 summarizes some of the tracers and ligands used to elucidate pathophysiology *in situ* and their stage of development. Most of the probes have been validated and are being used clinically. Several others are in preclinical validation. Preclinical imaging of experimental animal models has, until recently, been difficult because of low sensitivity, resolution and the effect of partial volume artefacts. Nevertheless, the 'Ratpet' animal scanner has been used to evaluate new tracers in animal models of disease[2].

New cameras (Micropet, Concorde Microsystems, Knoxville, TN, USA; HIDAC, Oxford Positron Systems, Oxford, UK) are being installed in several institutions for animal imaging, and this should help to bridge the gap between *in vitro* science and *in vivo* human studies with PET. The imaging paradigm could be set up and refined in animals before implementation in humans and the image data related to *ex vivo* assays. High-resolution images can be obtained with such animal scanners (Fig. 2). The PET scans show high uptake of [^{18}F]fluorodeoxyglucose ([^{18}F]FDG) in the myocardium, gastrointestinal tract, brain and tumour; this was confirmed after mice were sacrificed. This has significant implications for post-genome drug development and organ-regeneration research, wherein the effects of genetic manipulations or drug effects on gene expression and tissue function will need to be demonstrated in small animals.

It is apparent that some of the probes have multiple utilities, for example, [^{18}F]FDG follows the same route as glucose into cells, where it is phosphorylated by hexokinase to [^{18}F]FDG-6-phosphate[3,4]. Unlike glucose, there is little further metabolism and [^{18}F]FDG-6-phosphate remains essentially trapped within cells. [^{18}F]FDG-6-phosphate has low membrane permeability and although dephosphorylation does occur, it is slow in brain, heart and tumour, which have low levels of glucose-6-phosphatase. Based on these facts, it has been extensively used in cardiology to access myocardial viability and revascularization[5], and in neurology to study and delineate areas of cerebrovascular disease, epileptic foci, dementia and movement disorders[6,7]. In oncology, [^{18}F]FDG is used to determine tumour

Table 1. Ligands and tracers used to study pathophysiology in oncology, cardiology and neurology

Radiotracer	Process studied	Utility
Oncology		
6-[^{18}F]fluorodeoxyglucose	Tumour glucose utilization	Clinical
2-[^{11}C]thymidine	Cell proliferation	Clinical
3′-[^{18}F]fluorothymidine	Cell proliferation	Clinical
[^{11}C]methionine	Protein synthesis	Clinical
[^{15}O]H_2O	Tumour blood flow	Clinical
[^{15}O]CO	Tumour blood volume	Clinical
[^{11}C]HCO_3	pH (intra and extracellular)	Clinical
[^{18}F]fluoromisonidazole	Tumour hypoxia	Clinical
16α-[^{18}F]fluoro-17β-oestradiol	Oestrogen receptor status	Clinical
5-[^{124}I]iodo-2′-fluoro-1β-D-arabinofuranosyluracil	Gene expression (HSV1tk reporter)	Preclinical
9-[(1-[^{18}F]fluoro-3-hydroxy-2-propoxy)methylguanine	Gene expression (HSV1tk reporter)	Preclinical
[^{11}C]daunorubicin	Multidrug resistance phenotype	Preclinical
[^{124}I]Annexin-V	Apoptosis	Preclinical
[^{124}I]VG76e	VEGF levels	Preclinical
Cardiology		
6-[^{18}F]fluorodeoxyglucose	Cardiac glucose utilization	Clinical
[^{15}O]H_2O	Myocardial blood flow	Clinical
[^{11}C]acetate	Tricarboxylic acid cycle rate	Clinical
(*S*)-[^{11}C]CPG-12177	β-adrenoceptor status	Clinical
[^{11}C]methyl quinuclidinyl benzylate	Cardiac muscarinic receptors	Clinical
[^{11}C]*meta*-hydroxyephedrine	Presynaptic catecholamine reuptake	Clinical
[^{18}F]fluoromisonidazole	Myocardial ischaemia	Preclinical
[^{11}C]GB67G	α_1-adrenoceptor status	Preclinical
[^{124}I]Annexin-V	Apoptosis during transplant rejection	Preclinical
Neurology and psychiatry		
6-[^{18}F]fluorodeoxyglucose	Cerebral glucose utilization	Clinical
[^{15}O]H_2O	Cerebral blood flow	Clinical
[^{15}O]CO	Cerebral blood volume	Clinical
[^{11}C]raclopride	Dopamine ($D_{2/3}$) receptor density	Clinical
[^{11}C]SCH 23390	Dopamine (D_1) receptor density	Clinical
[^{11}C]RTI 121	Dopamine reuptake status	Clinical
[^{18}F]fluorodopa	Dopaminergic neurone density	Clinical
[^{11}C]flumazenil	Benzodiazepine receptor density	Clinical
[^{11}C]WAY100635	5-HT_{1A} receptor density	Clinical
[^{11}C]methylspiperone	5-HT_2 receptor density	Clinical
[^{11}C]diprenorphine	Opioid receptor density	Clinical
[^{11}C]deprenyl	Monoamine oxidase-B activity	Clinical
(*R*)-[^{11}C]PK11195	Microglial activation	Clinical

Abbreviations: HSV1tk reporter, herpes simplex virus-1 thymidine kinase; VEGF, vascular endothelial growth factor.

extent, stage and treatment response[8]. In addition, PET studies with some of these ligands and tracers (Table 1) have enabled the biological basis of diseases to be established. For example, focal pathophysiology in early stroke, Parkinson's disease, Huntington's disease, epilepsy, schizophrenia, anxiety disorders, memory loss, depression and addiction can now be classified according to PET-receptor occupancy, glucose metabolism and blood flow. The next step is to use this information to develop better drugs.

Evaluation of drug pharmacokinetics

Studies of the kinetics of drug absorption, distribution, metabolism and excretion (ADME and pharmacokinetics) form an important part of any drug development process,

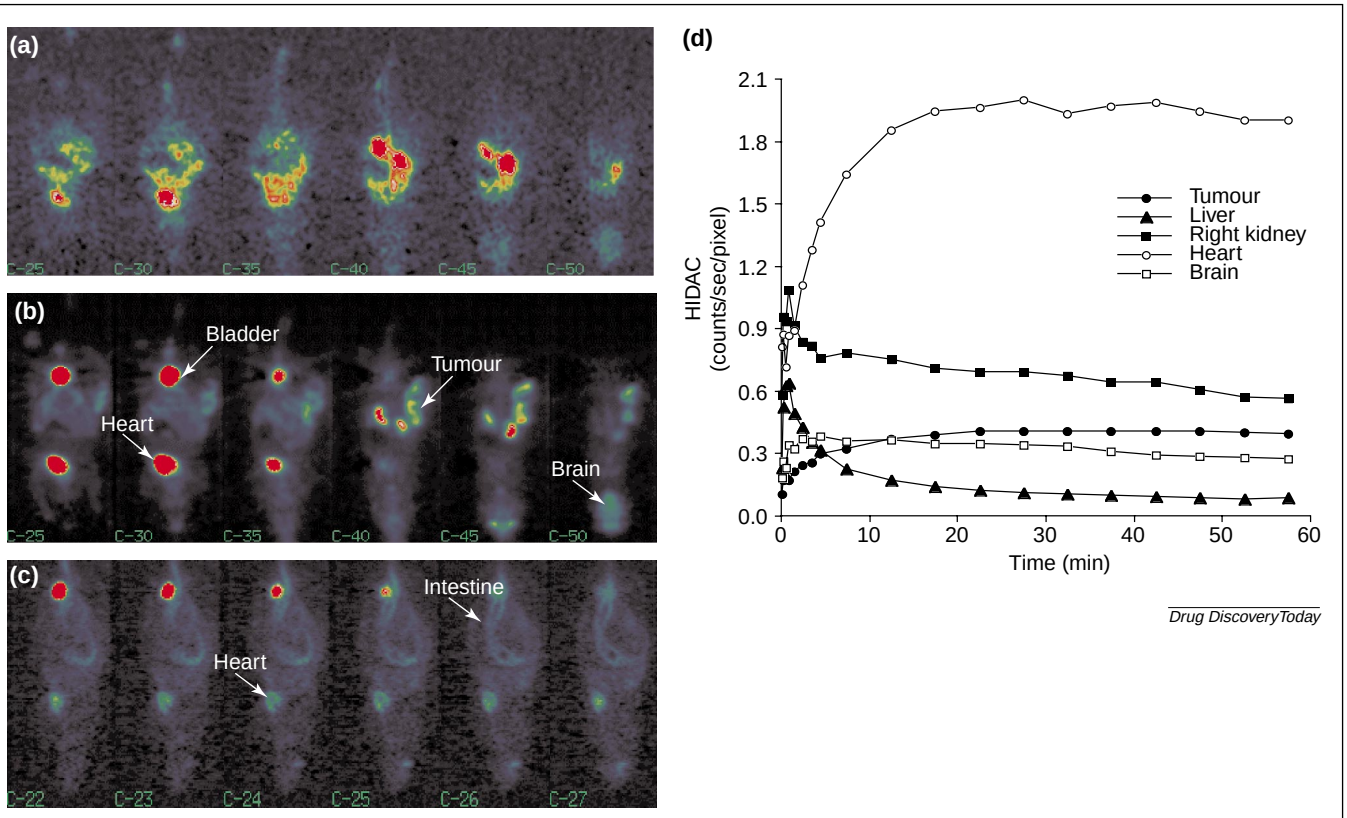

Figure 2. (a) Typical whole-body coronal slices (pixel size, 0.5 × 0.5 × 0.5 mm) along the length of a RIF-1 tumour-bearing mouse 0–5 min after an intravenous injection of [^{18}F]fluorodeoxyglucose. **(b)** A 30–60 min image of the same mouse in (a). **(c)** A 30–60 min image of a non-tumour bearing mouse showing good definition of the heart. For (a), (b), and (c), time frames have been added to provide composite images; selected coronal slice numbers are indicated at the bottom left of each image. **(d)** Typical time versus fluorine-18-radioactivity curves obtained from user-defined regions of interest placed on tumour, liver, kidney, heart and brain of the mouse in (a) and (b). Each time-point represents mean data from at least five adjacent transaxial planes. Aboagye, E.O *et al.*, unpublished.

and poor pharmacokinetics is a major cause of drug 'failure'. PET studies of drug pharmacokinetics can be performed early in patients, even before conventional Phase I studies[9] ('pre-Phase I'; at tracer doses). The ability to measure drug pharmacokinetics in tissues with PET needs to be underpinned by strong radiochemistry input. In general, radiopharmaceutical synthesis should be fast enough to allow the target drug to be isolated, purified and formulated as a sterile, pyrogen-free, isotonic solution and assessed for quality within 2–3 half-lives of the radionuclide. PET can provide information on the kinetics, dosimetry and distribution of drugs in diseased and normal tissue within the field of view of the scanner, in addition to hepatobiliary and renal clearance.

General pharmacokinetic parameters can be quantified and therefore can be calculated relatively easily. These parameters include: peak radioactivity (C_{max}), time to reach peak radioactivity (t_{max}), area under the radioactivity–time curve (AUC), uptake [standardized uptake value (SUV)] and proportion of drug in various tissues relative to that in blood. The mathematical modelling of tissue data enables other important kinetic parameters relating to the uptake, distribution and washout to be derived, for example, clearance from plasma to tissue (K_1), clearance from tissue to plasma (K_2), selective binding (K_3), permeability–product surface area (PS product), net unidirectional influx constant from plasma to tissue (K_I), mean residence time, binding potential and tissue volume of distribution (partitioning between blood and tissue).

In most PET pharmacokinetic studies, tracer quantities of drugs are administered. For drugs that exhibit non-linear pharmacokinetics, when the dose of the stable drug associated with the radiotracer is much lower than the pharmacological dose range, the main information obtained relates to drug localization and partitioning in the absence of saturation effects (these are optimum conditions). When pharmacokinetic information at pharmacological doses is sought, it is appropriate to carry out the studies with a formulation containing the appropriate pharmacological dose combined with the radiotracer. The latter

studies are mainly limited to the intravenous route of administration.

For several compounds, however, the oral route of administration is preferred, which limits the application of PET. For carbon-11-labelled compounds, the rate of absorption from an oral dose could be too slow in comparison with the physical half-life of the isotope, and this might lead to sensitivity limitations for most current clinical PET scanners. Furthermore, there could be concerns for large radiation doses being delivered to the upper gastrointestinal tract. It is still possible to administer the radiotracer intravenously at a specific time (e.g. steady state) after an oral dose has been administered. The plasma–tissue exchange rate constants calculated from such studies then apply only to the dose of drug administered orally, the schedule and the time-point used. Knowing the plasma concentrations of the drug, the tissue flux can be calculated. Other routes of administration such as the nasal route (inhalation) are more suited to PET studies. Another major consideration is drug metabolism because PET is incapable of distinguishing between chemical species, that is, PET measures total tissue radioactivity. Drug metabolism needs to be assessed, for example, by radioHPLC, on plasma samples from patients and supported with plasma and tissue data from animal studies.

N-[2-(dimethylamino)ethyl]acridine-4-carboxamide (DACA; XR5000; NSC 601316) is a DNA-intercalating acridine derivative that stimulates DNA breakage via the formation of cleavable complexes between DNA and either topoisomerase I or II (Refs 10,11). Several important translational research questions were posed before Phase I clinical trials, including whether (1) the metabolite profile of DACA was altered in humans compared with preclinical models, (2) the drug is distributed adequately to human tumours (predictive of activity), and (3) the degree of uptake into normal tissues, for example, the brain (predictive of neurotoxicity). To address these issues, carbon-11-labelled DACA ([^{11}C]DACA) was synthesized and used in a pre-Phase I radiotracer study to evaluate the tissue pharmacokinetics and plasma metabolite profile of DACA[9]. Pre-Phase I studies were performed at a radiotracer dose equivalent to 0.001% of the Phase I starting dose[9]. Analysis of plasma samples in this and other studies have shown that DACA is extensively metabolized and therefore the kinetics of parent drug alone could not be derived[12]. Total radioactivity was shown to localize in the following order: vertebra< brain<tumour<kidney<lung<myocardium<spleen<liver. The low absolute peak concentrations and overall distribution of [^{11}C]DACA-derived radioactivity attained in vertebra and brain suggested that myelotoxicity and neurotoxicity were less likely to be dose-limiting. Tumour uptake of [^{11}C]DACA was found to be variable and was moderately correlated with blood flow[9].

Temozolomide is an imidazotetrazine derivative, which unlike dacarbazine, does not require metabolic activation to alkylate DNA[13]. The drug undergoes pH-dependent activation to reactive species. Alkylation of DNA guanine in the O^6 position appears to be the primary cytotoxic event. It was envisaged that knowledge regarding the tumour versus normal tissue biodistribution and mechanism of action of temozolomide could be important for patient selection and for optimizing therapy, for example, through biochemical modulation or scheduling. Temozolomide has been labelled with carbon-11 in either the 3-*N*-methyl or the 4-carbonyl position[14]. PET pharmacokinetic studies with [^{11}C-methyl]temozolomide in patients with high-grade glioma showed that temozolomide is distributed to brain tumours to a higher extent compared with the contralateral normal brain[15]. Within the scan time (60–90 min), the differences in radiotracer kinetics could be explained by differences in influx rather than in washout. The exposure of tumours to [^{11}C-methyl]temozolomide-derived radioactivity correlated with patient response duration but not survival[15]. Ongoing work in our group involves clinical evaluation and confirmation of the proposed mode of action of temozolomide in humans. It was proposed that radiolabelling in the 3-*N*-methyl position will result in the incorporation of the radiolabel in DNA, whereas radiolabelling in the 4-carbonyl position will result in the loss of label as [^{11}C]CO_2 in expired air before its incorporation into DNA. Paired clinical studies are currently underway to confirm this. These studies have demonstrated higher levels of plasma and exhaled [^{11}C]CO_2 for [^{11}C-methyl]temozolomide compared with [^{11}C-carbonyl]temozolomide (Saleem, A. *et al.*, unpublished).

Fluconazole is an anti-fungal agent that acts by inhibiting fungal cytochrome P450. This results in the depletion of normal fungal sterols and the accumulation of 14-methyl sterols, which affects the function of the fungal cell membrane[16]. Fluconazole undergoes minimal metabolism *in vivo* and therefore tissue radioactivity measurements reflect accurate concentrations of the intact drug. The kinetics of [^{18}F]fluconazole have been monitored in human subjects by PET scanning[17]. [^{18}F]fluconazole was given during the last 15 min of a 2 h infusion of a pharmacological dose of fluconazole (5 mg kg^{-1}). The drug was extensively distributed, from a high concentration of 23 μg g^{-1} in the spleen to a low concentration of 1.2 μg g^{-1} in the bone. Because levels of fluconazole of >6 μg g^{-1} are needed to treat infections with most strains of *Candida* spp. and levels of >10 μg g^{-1} are needed for *Cryptococcus neoformans*, *Coccidioides immitis* and *Histoplasma capsulatum*, it was felt

that the standard dose of 400 mg day^{-1} should be more than adequate in the treatment of urinary tract and hepatosplenic candidiasis but problematic in the treatment of candidal osteomyelitis. It was also proposed that higher doses should be considered in patients infected with *C. neoformans*, *C. immitis* and *H. capsulatum*, which involve the CNS and musculoskeletal systems.

Proof of principle of mechanism of action *in vivo*

There is an increasing need to obtain information on the mechanism of action of drugs in patients to ensure that a new drug behaves in a predictable manner. These studies could be based on alterations in metabolism, perfusion, retention or receptor occupancy. Examples of such studies are described below.

The synthetic pyrimidine, 5-fluorouracil (5-FU), is the most commonly used anticancer agent for the treatment of gastrointestinal malignancies. Up to 80% of systemically administered 5-FU is degraded by catabolism to α-fluoro-β-alanine (FBAL)[18], decreasing the amount of drug available for the formation of cytotoxic anabolites. One aspect of current research with 5-FU has been to provide proof of principle of the mechanism of action of eniluracil, an inactivator of the proximal and rate-limiting catabolic enzyme, dihydropyrimidine dehydrogenase. Using PET, it was demonstrated that in eniluracil-naïve patients, 5-[^{18}F]FU-derived radioactivity localized strongly (0.0234% of the injected activity per ml at 11 min) in normal liver because of the rapid formation and retention of [^{18}F]FBAL[19]. Furthermore, there was a distinct localization of radioactivity in the gall bladder consistent with hepatobiliary clearance of [^{18}F]FBAL[19]. In eniluracil-treated patients, there was a substantial reduction in radiotracer disposition in normal liver and kidneys. Other effects observed were the absence of hepatobiliary clearance and an increase in plasma uracil and unmetabolized 5-[^{18}F]FU. The half-life of 5-[^{18}F]FU-derived radioactivity in tumours increased from 2.3 h to >4 h following eniluracil administration. This is a large increase given that tumour radioactivity in eniluracil-naïve patients predominantly comprises [^{18}F]FBAL. These studies demonstrated proof of principle of mechanism of action of eniluracil.

Several new therapies are being developed for which proof of principle of mechanism of action is crucial to the development of the compounds. This is particularly the case for anti-angiogenic agents, cell transplantation, gene therapies and antibody-directed therapies. These therapies are either meant to be tumour-specific with minimal normal tissue toxicity, or should only target focal pathophysiology in the case of therapies used in neuropsychiatry and cardiology. Thus, classical Phase I dose-finding approaches (to define the maximum tolerated dose) might be inappropriate. Angiogenesis, the sprouting of capillaries from pre-existing vasculature, represents an important target and a challenge for drug development. Two main therapeutic approaches are being pursued including: (1) inhibition of processes that constitute the angiogenic phenotype such as high vascular endothelial growth factor (VEGF) and growth factor receptor activity (anti-angiogenic agents), and (2) inhibition of blood flow (anti-vascular agents). To assess the effects of the anti-vascular agent combretastatin A4 (Ref. 20) on tumour blood flow and blood volume, as part of a CRC Phase I trial, Anderson and colleagues[21] performed paired PET scans with $H_2{}^{15}O$ and $C^{15}O$, respectively. Blood flow and volume parameters were unchanged in untreated patients and at low doses of the drug. At higher doses, a 30–60% decrease in tumour blood flow was seen in four out of five patients at 30 min resolving in three out of four patients by 24 h after injection of combretastatin A4, demonstrating proof of mechanism of action of the drug[21]. Reduction in blood flow for tumours and spleen were similar to that seen in tumour-bearing animals[20].

One area in which PET imaging could be crucial clinically is in cell transplantation and genetic therapy. In Parkinson's disease, for instance, cell transplantation has been applied to correct impairment of striatal dopaminergic neurotransmission caused by degeneration of substantia nigral neurons[22]. The restoration of basal and drug-induced dopamine release (with [^{18}F]flurodopa and [^{11}C]raclopride) 10 years after a patient had received human embryonic mesencephalic tissue in the right putamen has been demonstrated[22] (Fig. 3). This demonstrated restoration of function (i.e. storage and amphetamine release of dopamine) after transplantation, which paralleled substantial symptomatic relief[22]. Several probes are being developed to monitor gene therapy[23,24]. These analogues of thymidine or uracil undergo phosphorylation (and trapping) when herpes simplex virus-1 thymidine kinase (HSV1tk) is expressed and can be used in monitoring HSV1tk gene therapy (location and levels over time) or as *in vivo* reporter probes when the gene of interest is linked to HSV1tk. Antibody-directed enzyme pro-drug therapy (ADEPT) is a cancer-cell selective approach that is being explored to increase efficacy[25]. ADEPT is based on the delivery to cancer cells of a non-toxic antibody–enzyme conjugate. After a suitable time to allow for clearance of unbound conjugate, a non-toxic pro-drug is administered, which is selectively converted to a toxic compound. By radiolabelling the pro-drug or conjugates with a positron emitter, it would be possible to quantitate distributions of the pro-drug or conjugate. This will provide information

on the selectivity between tumours and normal tissues and aid in the appropriate timing of therapy. A prodrug that is a substrate for carboxypeptidase G2 has been labelled with carbon-11 for monitoring the ADEPT strategy[25]. It is anticipated that by providing proof of principle, these types of pharmacodynamic assessment will cut down the time involved in early clinical trials of new agents.

Receptor occupancy studies have underpinned most of the PET studies aimed at providing proof of principle of mechanism of action. Several growth factors produce their therapeutic effects by interacting with specific receptors or binding to cell surface molecules. It is possible to study such drug–receptor interactions with ligands or antibodies radiolabelled with a positron emitter. Such studies can either be direct (radiolabelling the molecule of interest) or indirect (displacement of radioligand by the molecule of interest) and can measure the regional uptake of radioligands to enable the derivation of values for receptor number (B_{max}), affinity (K_d) and binding potential (B_{max}/K_d). Drug receptor studies are helpful in: (1) predicting response or for selecting patients likely to respond to specific therapies, and (2) predicting optimal dose.

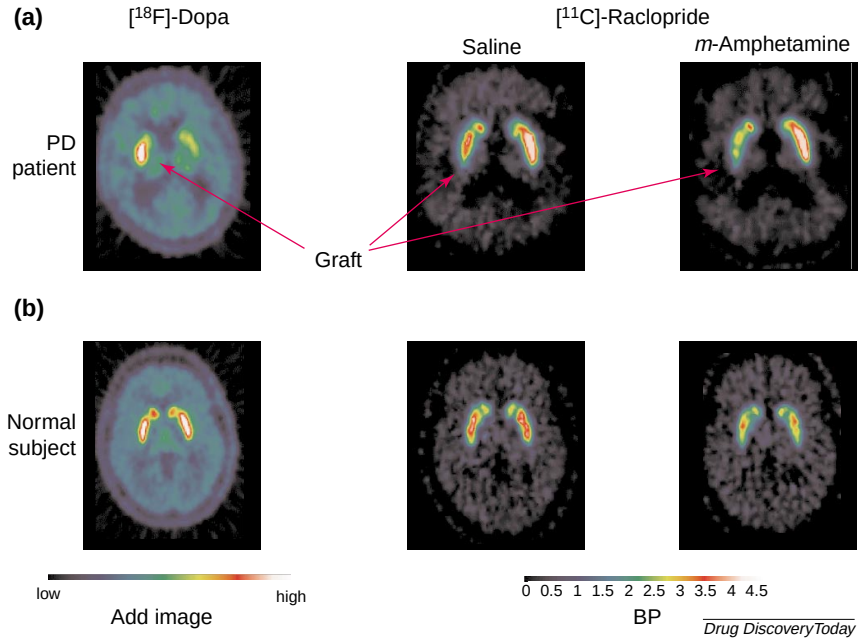

Figure 3. Nigral transplants re-innervate the putamen to a level sufficient to normalize both [18F]-dopa uptake (dopamine storage capacity of dopaminergic terminals in striatum) and D_2 receptor occupancy. Composite images of [18F]-dopa and parametric images of [11C]-raclopride-binding potential at the basal ganglia level for a patient, with Parkinson's disease (PD), 10 years after transplantation of embryonic mesencephalic tissue to the right putamen **(a)** and for a normal subject **(b)**. At baseline (saline), D_2 site availability was upregulated (43.7% higher [11C]-raclopride binding) in the non-grafted putamen, whereas the grafted putamen had normal receptor occupancy. Following amphetamine treatment (decreases receptor site availability via an increase in extracellular dopamine levels), there was a 26.6% reduction in [11C]-raclopride binding in the grafted putamen compared with 4.5% in the non-grafted putamen. (Reproduced, with permission, from Ref. 21.)

Predicting response from assessment of receptor occupancy

Receptor occupancy studies involving the oestrogen receptor (ER) have been used to predict response. The majority of breast cancers are hormone dependent as indicated by an increase in ER and progesterone receptor content of breast tumours[26]. The clinical course of disease in patients with ER-positive tumours is less aggressive, characterized by a longer disease-free interval and greater overall survival. Moreover, ER-positive tumours are likely to respond to hormonal manipulation. 16α-[18F]fluoro-17β-oestradiol (FES), a radioligand for oestrogen receptors, has been used to assess the oestrogen receptor status of breast tumours. There is a decrease in the uptake of FES in metastatic breast cancers after the administration of tamoxifen, a partial agonist used in the prevention and treatment of breast cancer[27]. This demonstrated the presence of functional oestrogen receptors in the tumours. The decrease in the uptake of FES after tamoxifen was significantly greater in patients who responded to hormonal therapy[28]. Thus, the oestrogen receptor occupancy status can be used to predict response to hormonal therapy.

Predicting optimum dose from assessment of receptor occupancy

In general, neuroleptic drug action is thought to be largely mediated by the blockade of dopamine receptors[29,30]. For classical anti-psychotics, PET studies have suggested that a threshold D_2 occupancy of 70% is needed for anti-psychotic effect and 80% for extrapyramidal side effects[31,32]. This knowledge can be used to compare the receptor occupancy of novel anti-psychotic agents and to determine the doses needed for the optimum receptor occupancy. Bench and coworkers[33] used [11C]-raclopride to measure the occupancy of central dopamine D_2 receptors by the neuroleptic, CP-88,059-1 (Ziprasidone). Volunteers received 2–60 mg of CP-88,059-1 five hours before PET scanning; one subject received placebo only. It was found that the binding of

[^{11}C]-raclopride decreased in a dose-dependent manner and 85% dopamine D_2 receptor occupancy was achieved with the highest dose of CP-88,059-1. From these findings it was suggested that an effective anti-psychotic dose should be between 20 mg and 40 mg. Further studies showed that binding potential of the ligand was restored to the normal range after 18 h (Ref. 34). Similar studies have been reported for lazabemide (Ro196327), a reversible monoamine oxidase B (MAO B) inhibitor used to treat early-stage Parkinson's disease[35]. In this case, [^{11}C]L-deprenyl was used to demonstrate that a dose of 0.4 mg^{-1} or greater administered every 12 h is the minimum necessary to provide >90% inhibition of brain MAO B in patients with early Parkinson's disease. Brain MAO B activity returned to baseline values by 36 h after drug discontinuation. Thus, the minimum effective dose was defined rationally on the basis of enzyme inhibition.

Assessment of efficacy and/or response to treatment

After drugs have been shown to act in a predictable fashion, the next step in drug development is to show that they are efficacious. Several probes have been developed to assess efficacy, particularly in the field of oncology wherein alterations in processes downstream of receptor occupancy (e.g. phosphorylation of response elements), for example, could invalidate the relationship between receptor occupancy and efficacy. Currently, PET methods are being used predominantly as observational end-points because the level of validation required to use them in making 'go–no-go' decisions (i.e. pivotal decisions whether or not to take the drug to the next phase of development) in the course of drug development has not been achieved for most probes. PET methodology is available for measuring thymidine incorporation into DNA and thus for providing an index of proliferation rate[36,37], which can be superior to current methods for monitoring tumour response including tumour shrinkage and time to progression.

PET methods might be superior because response assessment with anatomical images can be confounded by inflammatory or fibrotic masses. Furthermore, metabolic changes can precede changes in size, an important consideration for monitoring anticancer agents that are cytostatic. The optimal probe for measuring proliferation is, however, an issue of current investigation. 2-[^{11}C]Thymidine is the PET 'gold standard' for measuring proliferation. The main limitation of using 2-[^{11}C]thymidine-PET is its rapid catabolism ultimately to $^{11}CO_2$. A dual-scan approach, involving initial scanning with $^{11}HCO_3$ to enable correction of $^{11}CO_2$ contribution to subsequent 2-[^{11}C]thymidine scans, has been developed and used to determine fractional retention and incorporation rate constants for 2-[^{11}C]thymidine[38,39]. This technique has been validated by the demonstration that fractional uptake of 2-[^{11}C]thymidine-derived radioactivity correlates with a well-known index of cell proliferation, the MIB-1 index[40]. Despite its limitations, 2-[^{11}C]thymidine has been used in several pilot studies to measure response to therapy. For example, in patients with metastatic small-cell lung cancer and abdominal sarcoma, the 2-[^{11}C]thymidine flux constant (measured at 1 week after chemotherapy) declined by 100% in complete responders and 35% in a partial responder compared with a much smaller decline (15%) in a patient showing progressive disease[41]. Another strategy for overcoming the rapid catabolism of 2-[^{11}C]thymidine is the use of less readily metabolized analogues. One of the more promising thymidine analogues for measuring proliferation is 3′-deoxy-3′-[^{18}F]fluorothymidine (FLT)[42]. This radiotracer is metabolized to a lesser extent than 2-[^{11}C]thymidine. The retention of FLT is determined not only by the degree of proliferation but also by levels of cell cycle-regulated thymidine kinase-1. Studies are ongoing to evaluate the role of this tracer in clinical imaging of proliferation.

As a pharmacodynamic endpoint, [^{18}F]FDG has been used in several relatively small studies to monitor the response of tumours to treatment. These studies include: assessment of the response to temozolomide (in glioma; Fig. 4)[43], multidrug chemotherapy

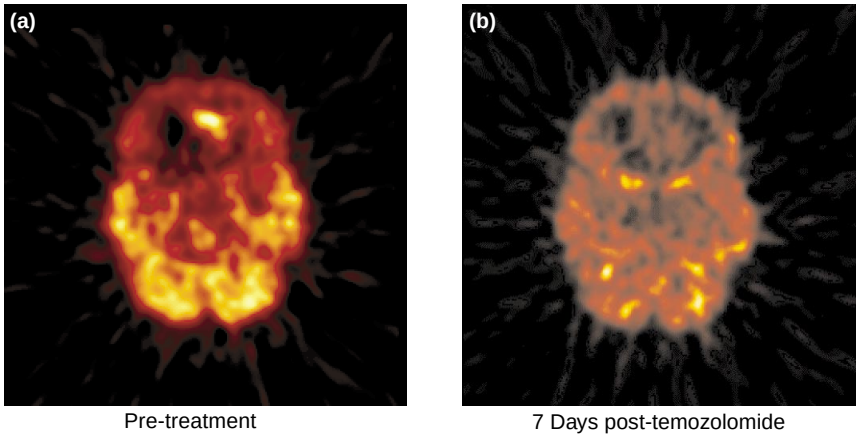

Figure 4. [^{18}F]fluorodeoxyglucose ([^{18}F]FDG) PET image of a glioma patient before **(a)** and 7 days after **(b)** chemotherapy with temozolomide. The hyperintense region in the top half of the pre-treatment image represents uptake into the tumour. There was a decrease in uptake after treatment.

(breast and glioma)[44,45], hormonal treatment[46] and protracted 5-FU with or without interferon[47] (colorectal liver metastases). Pharmacodynamic studies with [^{18}F]FDG are usually carried out at baseline and soon after the first or second cycle of therapy. The European Organization for Research and Treatment of Cancer-PET group (EORTC-PET group) has recently published guidelines for common measurement criteria and for reporting of alterations in FDG-PET studies to enable much needed comparison of smaller clinical studies and larger-scale multi-centre trials[48]. Tumour response assessment as defined by the group (progressive, stable, partial or complete metabolic response) is based on the observation that, on average, a 15–30% reduction in SUV or metabolic rate of glucose utilization can predict response and that this precedes tumour shrinkage and clinical response[48].

Summary

PET can be used to address important bottlenecks in drug discovery and development, including the evaluation of pathophysiology, pharmacokinetics and pharmacodynamics. The high sensitivity and specificity of PET assays enable the mechanism of action of drugs to be assessed in animal models of disease and in human tissues. There are, however, specific and difficult methodological challenges in ligand and tracer discovery, radiochemistry, data processing and modelling. To fully realize the potential of PET in drug discovery and development, there is a need to efficiently integrate scientists from different disciplines including molecular biologists, biochemists, physicists, mathematical modellers, pharmacologists and clinicians. The ability to mobilize such expertise focusing on molecular imaging requires long-term funding and close collaboration between PET centres and drug developers. The information gained from such an effort can speed up the drug discovery and development process and move drugs quickly from concept to bedside.

References

1 Workman, P. (1995) Bottlenecks in anticancer drug discovery and development: *in vivo* pharmacokinetic and pharmacodynamic issues and the potential role of PET. In *PET for Drug Development and Evaluation* (Comar, D., ed.), pp. 277–285, Kluwer Academic Publishers

2 Myers, R. *et al.* (1999) Radio-imaging in small animals. *J. Psychopharmacol.* 13, 352–357

3 Aloj, L. *et al.* (1999) Glut-1 and hexokinase expression: relationship with 2-fluoro-2-deoxy-D-glucose uptake in A431 and T47D cells in culture. *Cancer Res.* 59, 4709–4714

4 Waki, A. *et al.* (1998) The importance of glucose transport activity as the rate-limiting step of 2-deoxyglucose uptake in tumor cells *in vitro*. *Nucl. Med. Biol.* 25, 593–597

5 Bax, J.J. *et al.* (1997) Metabolic imaging using F18-fluorodeoxyglucose to assess myocardial viability. *Int. J. Card. Imaging* 13, 145–155

6 Kumar, A. *et al.* (1991) High-resolution PET studies in Alzheimer's disease. *Neuropsychopharmacology* 4, 35–46

7 Kushner, M. *et al.* (1987) Local cerebral metabolic changes in acute ischemic strokes. *Gerontology* 33, 265–267

8 Brock, C.S. *et al.* (1997) Does fluorine-18 flurodeoxyglucose metabolic imaging of tumours benefit oncology? *Eur. J. Nucl. Med.* 24, 691–705

9 Saleem, A. *et al.* Pharmacokinetic evaluation of *N*-[2-(Dimethylamino)ethyl]acridine-4–carboxamide (DACA; XR5000) in patients by positron emission tomography (PET). *J. Clin. Oncol.* (in press)

10 Atwell, G.J. *et al.* (1987) Potential antitumor agents. 50. *In vivo* solid-tumor activity of derivatives of *N*-[2-(dimethylamino)ethyl]acridine-4-carboxamide. *J. Med. Chem.* 30, 664–669

11 Finlay, G.J. *et al.* (1993) *In vitro* assessment of *N*-[2-(dimethylamino)ethyl]acridine-4-carboxamide, a DNA-intercalating antitumour drug with reduced sensitivity to multidrug resistance. *Cancer Chemother. Pharmacol.* 31, 401–406

12 Osman, S. *et al.* (1997) Studies on the metabolism of the novel antitumor agent [*N*-methyl-11C]*N*-[2-(dimethylamino)ethyl]acridine-4-carboxamide in rats and humans prior to phase I clinical trials. *Cancer Res.* 57, 2172–2180

13 Stevens, M.F. and Newlands, E.S. (1993) From triazines and triazenes to temozolomide. *Eur. J. Cancer* 7, 1045–1047

14 Brown, G.D. *et al.* (1994) Synthesis of [^{11}C-methyl]methylisocyanate and application of microwave heating to labelling the novel anticancer agent Temozolomide. *J. Label. Compd. Radiopharm.* 35, 100–103

15 Brock, C.S. *et al.* (1996) The kinetic behavior of temozolomide in man. *Proc. Am. Soc. Clin. Oncol.* 15, 475

16 Richardson, K. (1990) The discovery and profile of fluconazole. *J. Chemother.* 2, 1184–1187

17 Fischman, A.J. *et al.* (1993) Pharmacokinetics of ^{18}F-labelled fluconazole in healthy human subjects by positron emission tomography. *Antimicrob. Agents Chemother.* 37, 1270–1277

18 Pinedo, H.M. and Peters, G.F. (1988) Fluorouracil: biochemistry and pharmacology. *J. Clin. Oncol.* 6, 1653–1664

19 Saleem, A. *et al.* (2000) Modulation of fluorouracil tissue pharmacokinetics by eniluracil: *in vivo* imaging of drug action. *Lancet* 355, 2125–2131

20 Tozer, G.M. *et al.* (1999) Combretastatin A-4 phosphate as a tumor vascular-targeting agent: early effects in tumor and normal tissues. *Cancer Res.* 59, 1626–1634

21 Anderson, H. *et al.* (2000) Measurement of tumour and normal tissue perfusion (NT) by positron emission tomography (PET) in the evaluation of antivascular therapy: results in the phase I study of combretastatin A4 phosphate (CA4P). *Proc. Am. Soc. Clin. Oncol.* 19, 179a

22 Piccini, P. *et al.* (1999) Dopamine release from nigral transplants visualised *in vivo* in a Parkinsons's patient. *Nat. Neurosci.* 2, 1137–1140

23 Tjuvajev, J.G. *et al.* (1998) Imaging herpes virus thymidine kinase gene transfer and expression by positron emission tomography. *Cancer Res.* 58, 4333–4341

24 Gambhir, S.S. *et al.* (1999) Imaging adenoviral-directed reporter gene expression in living animals with positron emission tomography. *Proc. Natl. Acad. Sci. U. S. A.* 96, 2333–2338

25 Prenant, C. *et al.* (1997) An alternative synthesis of [^{11}C]ethylene and conversion to [^{11}C]chloroethyltosylate *in situ* via [^{11}C]1-chloro-2-iodoethane for radiolabelling ADEPT prodrugs. *J. Label. Compd. Radiopharm.* 40, 766–767

26 Collett, K. *et al.* (1996) Prognostic role of oestrogen and progesterone receptors in patients with breast cancer: relation to age and lymph node status. *J. Clin. Pathol.* 49, 920–925

27 McGuire, A.H. *et al.* (1991) Positron tomographic assessment of 16 alpha-[18F] fluoro-17 beta-estradiol uptake in metastatic breast carcinoma. *J. Nucl. Med.* 32, 1526–1531

28 Dehdashti, F. *et al.* (1999) Positron emission tomographic assessment of 'metabolic flare' to predict response of metastatic breast cancer to antiestrogen therapy. *Eur. J. Nucl. Med.* 26, 51–56

29 Creese, I. *et al.* (1976) Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. *Science* 192, 481–483

30 Seeman, P. *et al.* (1976) Antipsychotic drug doses and neuroleptic/dopamine receptors. *Nature* 261, 717–719

31 Farde, L. *et al.* (1989) D1- and D2-dopamine receptor occupancy during treatment with conventional and atypical neuroleptics. *Psychopharmacology* 99 (Suppl.), S28–31

32 Farde, L. *et al.* (1992) Positron emission tomographic analysis of central D1 and D2 dopamine receptor occupancy in patients treated with classical neuroleptics and clozapine. Relation to extrapyramidal side effects. *Arch. Gen. Psychiatry* 49, 538–544

33 Bench, C.J. *et al.* (1993) Dose dependent occupancy of central dopamine D2 receptors by the novel neuroleptic CP-88,059-01: a study using positron emission tomography and 11C-raclopride. *Psychopharmacology* 112, 308–314

34 Bench, C.J. *et al.* (1996) The time course of binding to striatal dopamine D_2 receptors by the neuroleptic ziprasidone (CP-88,059-01) determined by positron emission tomography. *Psychopharmacology* 124, 141–147

35 Fowler, J.S. *et al.* (1993) Monoamine oxidase B (MAO B) inhibitor therapy in Parkinson's disease: the degree and reversibility of human brain MAO B inhibition by RO 19 6327. *Neurology* 43, 1984–1992

36 Mankoff, D.A. *et al.* (1998) Kinetic analysis of 2-[carbon-11]thymidine PET imaging studies: compartmental model and mathematical analysis. *J. Nucl. Med.* 39, 1043–1055

37 Eary, J.F. *et al.* (1999) 2-[C-11]thymidine imaging of malignant brain tumors. *Cancer Res.* 59, 615–621

38 Gunn, R.N. *et al.* (2000) A general method to correct PET data for tissue metabolites using a dual-scan approach. *J. Nucl. Med.* 41, 706–711

39 Mankoff, D.A. *et al.* (1999) Kinetic analysis of 2-[^{11}C]thymidine PET imaging studies: validation studies. *J. Nucl. Med.* 40, 614–624

40 Wells, P. *et al.* (1997) Measurement of cell proliferation *in vivo* using [2-^{11}C] thymidine. *Proc. Am. Soc. Clin. Oncol.* 16, 548a

41 Shields, A.F. *et al.* (1998) Carbon-11-thymidine and FDG to measure therapy response. *J. Nucl. Med.* 39, 1757–1762

42 Shields, A.F. *et al.* (1998) Imaging proliferation *in vivo* with [F-18]FLT and positron emission tomography. *Nat. Med.* 4, 1334–1336

43 Brock, C.S. *et al.* (2000) Early evaluation of tumour metabolic response using [18F]fluorodeoxyglucose and positron emission tomography: a pilot study following the phase II chemotherapy schedule for temozolomide in recurrent high-grade gliomas. *Br. J. Cancer* 82, 608–615

44 Rozental, J.M. *et al.* (1989) Glucose uptake by gliomas after treatment. *Arch. Neurol.* 46, 1302–1307

45 Jansson, T. *et al.* (1995) Positron emission tomography studies in patients with locally advanced and/or metastatic breast cancer: a method for early therapy evaluation. *J. Clin. Oncol.* 13, 1470–1477

46 Wahl, R.L. *et al.* (1993) Metabolic monitoring of breast cancer chemohormonotherapy using positron emission tomography: initial evaluation. *J. Clin. Oncol.* 11, 2101–2111

47 Findlay, M. *et al.* (1996) Non-invasive monitoring of tumor metabolism using fluorodeoxyglucose and positron emission tomography in colorectal cancer liver metastases: correlation with tumor response to fluorouracil. *J. Clin. Oncol.* 14, 700–708

48 Young, H. *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