MOLECULAR IMAGING OF CANCER WITH POSITRON EMISSION TOMOGRAPHY

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The imaging of specific molecular targets that are associated with cancer should allow earlier diagnosis and better management of oncology patients. Positron emission tomography (PET) is a highly sensitive non-invasive technology that is ideally suited for pre-clinical and clinical imaging of cancer biology, in contrast to anatomical approaches. By using radiolabelled tracers, which are injected in non-pharmacological doses, three-dimensional images can be reconstructed by a computer to show the concentration and location(s) of the tracer of interest. PET should become increasingly important in cancer imaging in the next decade.

TRACER
Also known as molecular probe or reporter probe. This molecule has a radioisotope attached to it and is injected in non-pharmacological amounts to provide imaging signal related to target(s) of interest. For PET tracers, the radioisotope is a positron emitter (e.g. ^{18}F).

POSITRON
A particle that has the same mass as an electron, but that carries a positive charge.

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The fields of cancer biology, pharmacology and clinical oncology have grown rapidly over the past few years, owing to the increase in technologies that allow the study of gene and protein expression from cell extracts and intact cells in culture. The time is now ripe for technologies that can go to the next level: the study of cancer cells in their normal environment within intact living subjects.

Much of biological and medical imaging has previously been driven by anatomy-based imaging, such as computed tomography (CT). The field of nuclear medicine, by contrast, has focused on studying molecular events in living subjects through the use of technologies that can localize TRACERS. Various tracers have been discovered, by chance, to be useful, and some have been specifically designed to target molecular events. With the recent advances in molecular/cell biology that have led to target discovery, it is now possible to design specific tracers to image events non-invasively in small animals and humans with positron emission tomography (PET)[1]. PET is ideally suited for monitoring cell/molecular events early in the course of a disease, as well as during pharmacological or radiation therapy. Furthermore, it can be used for prognostic information and to image for disease recurrence.

Principles of PET

PET can be thought of as a camera that can take pictures of a subject of interest and requires an exposure time of a few seconds to several minutes. The camera does not image visible light, but images high-energy gamma-rays that are emitted from inside the subject (FIG. 1). Natural biological molecules can be labelled with an isotope that is capable of producing two gamma-rays by emitting a POSITRON from its nucleus. The positron eventually collides with a nearby electron and they annihilate each other to produce energy in the form of two 511,000 eV gamma-rays, which are emitted in directions ~180 degrees apart. Frequently used positron-emitting isotopes include ^{15}O, ^{13}N, ^{11}C and ^{18}F; the latter is often used as a substitute for hydrogen in the molecule of interest. Other less commonly used positron emitters include ^{14}O, ^{64}Cu, ^{62}Cu, ^{124}I, ^{76}Br, ^{82}Rb (rubidium) and ^{68}Ga (gallium). Most of these isotopes are produced in a CYCLOTRON[2], but some can be produced with a GENERATOR (for example, ^{68}Ga, ^{82}Rb). Labelled tracers can be introduced into the subject and then PET imaging can follow their distribution and concentration (FIG. 1). Many of the positron-emitting isotopes that are used have relatively short half-lives — the half life of ^{18}F is 110 minutes — so the chemistry leading to incorporation of the isotope into the parent molecule and its subsequent introduction into the subject must take place relatively quickly. PET radiopharmacies exist throughout the world and are capable of providing commonly used PET tracers on a daily basis.

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Summary

- **Positron emission tomography (PET) is a method by which cellular and molecular events can be followed. Injected radiolabelled molecular probes (tracers) are used to map out the underlying biochemistry.**
- **Both small-animal and clinical PET are being used to study cancer in living subjects.**
- **2-^{18}F-fluoro-2-deoxy-D-glucose (FDG) is actively taken up and accumulates in cancer cells. It is useful for diagnosis, staging and monitoring the recurrence of various cancers, including lung, colorectal, melanoma, lymphoma, head and neck, as well as other malignancies.**
- **Many tracers already exist for PET that measure cell proliferation, bone remodelling, perfusion, oxygen metabolism, tumour-receptor density and reporter-gene expression. A new generation of tracers is being developed that should help to form libraries of molecular probes for 'customized' imaging approaches.**
- **Clinical PET/CT (computed tomography) scanners are now rapidly being installed, and form the basis for merging anatomical information (CT) with functional molecular information (PET) to further advance cancer management with FDG and, eventually, new-generation tracers.**
- **Drug and tracer research and development are rapidly evolving and should help to accelerate both the pharmaceutical and imaging industries.**

Isotopes that are beta-emitters — ^{3}H, ^{14}C — are not useful for non-invasive imaging of living subjects because beta-particles (electrons) do not travel significant distances in tissue and do not lead to annhilation events, as do positrons. Gamma-emitting isotopes — ^{99m}Tc (technetium), ^{111}In (indium), ^{123}I — can also be used with tracers for imaging living subjects, but require different types of cameras ('gamma-cameras') that do not require the production of two coincident gamma-rays. Gamma-cameras are rotated around the subject in a process known as single-photon-emission computed tomography (SPECT), to produce tomographic images[3]. PET is at least tenfold more sensitive than SPECT, and positron-emitting isotopes can readily be substituted for naturally occurring atoms, producing less perturbation to the biochemical behaviour of the radiolabelled parent molecule. SPECT systems can be developed with improved spatial resolution, but not without a further loss in sensitivity. For these reasons, PET is a more robust technique for imaging most molecular events.

The spatial resolution of most clinical PET scanners is ~$(6–8)^3$ mm^3, but higher-resolution clinical brain scanners have been developed that approach resolutions of ~3^3 mm^3. The sensitivity of PET is relatively high — in the range of 10^{-11}–10^{-12} moles/litre — and is independent of the location depth of the tracer of interest. Typically, several hundred million cells in relatively close proximity must accumulate the tracer for a PET scanner to visualize them against the background. The exact number of cells that can be imaged depends on numerous factors, including the level of tracer uptake in the surrounding tissues ('background'). It is important to note that all isotopes used produce two gamma-rays of the same energy, so if two molecular probes — each with a separate isotope — are injected simultaneously, there is no way for the PET camera to distinguish them. Therefore, to perform studies that involve multiple molecular events, molecular probes are usually injected separately, which allows for the decay of the isotope. SPECT can be used with several distinctly radiolabelled tracers because of its ability to distinguish gamma-rays of different energies. The images from a PET camera, although often shown in colour, reflect gamma-ray events with the same energy — the colour scale usually reflects the concentration of isotope.

Small-animal imaging with PET

In recent years, small-animal PET cameras have been designed, which facilitate the development of molecular-imaging assays that can be used in small rodents and primates before they are tested in humans. These approaches allow the rapid testing of human cell targets that are implanted into mice, and the optimization of the imaging signal and PHARMACOKINETICS of the tracer. These systems typically have a spatial resolution of ~2^3 mm^3 (REF. 4), but newer-generation systems that are undergoing completion will have a resolution of ~1^3 mm^3 (REF. 5). Small-animal imaging requires tracers with a higher specific radioactivity, owing to the limited sensitivity of the small-animal scanners and the need to inject a smaller mass of tracer.

The development of molecular imaging assays with PET is made much easier by the ability to validate the assay in cell culture and small-animal models, and then to use the same tracer in established clinical PET sites around the world. The ability to translate from cell culture to pre-clinical models to clinical applications is an important and powerful feature of PET technology[4,6].

Molecular and cellular targets

For tracers to be successful as imaging probes for PET, they must fulfil several criteria: the positron-emitting isotope that is chemically linked to the molecule of interest must not easily dissociate. If this occurs, it is the isotope that is followed with PET, rather than the tracer. It is important that the label does not significantly alter the biological properties of the parent molecule (for example, transport, elimination or affinity of interaction with target). The tracer must also clear rapidly from sites where there is no target molecule, and from the blood, so that a high contrast can be obtained between the tumour and surrounding tissues. This is one of the

CYCLOTRON
A device that is used to accelerate charged particles to create a collision between the charged particle and a target, so that a radioactive isotope can be produced for further incorporation into a molecule of interest.

GENERATOR
A device that is used to separate and extract a radioisotope through the use of a 'parent' isotope that constantly leads to a 'daughter' isotope.

PHARMACOKINETICS
The study of the time course of absorption, distribution, metabolism and excretion of drugs and their metabolites in body tissues and fluids.

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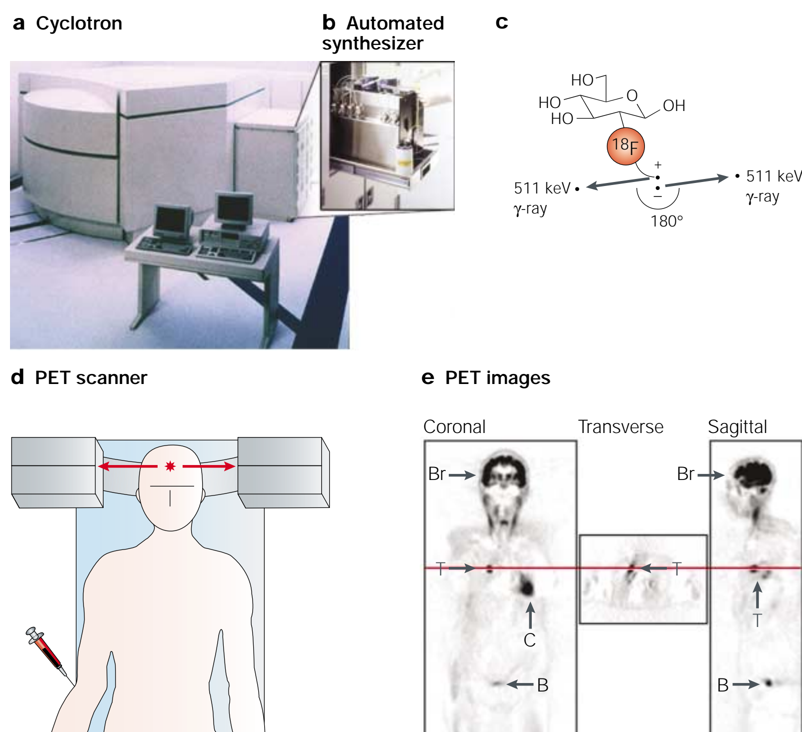

Figure 1 | **Principles of positron emission tomography (PET). a** | A cyclotron is used to accelerate charged particles to create relatively short-lived positron-emitting isotopes (for example, ^{18}F, the half-life of which is 110 minutes). **b** | Automated synthesizers can then couple the isotope to a molecule of interest to produce the molecular probe (tracer). **c** | The molecule 2-[^{18}F]fluoro-2-deoxy-D-glucose (FDG) can be synthesized and intravenously injected in non-pharmacological doses into a subject of interest. The positron emitter decays by emitting a positron from its nucleus. The positron loses energy and eventually annihilates with a nearby electron to produce two gamma-rays that are almost 180 degrees apart. **d** | The PET scanner can detect the coincident gamma-rays, and images can be reconstructed showing the location(s) and concentration of the tracer of interest. **e** | Cross-sectional FDG PET images are shown, of a patient 40 minutes after injection with FDG. Normal uptake in brain (Br) and myocardium (C), and renal excretion into the urinary bladder (B) are visible. Also seen is a tumour (T) in the lungs that takes up more FDG than the surrounding tissues.

unique features of a PET tracer compared with many existing pharmaceuticals. Without the efflux of the tracer from non-target sites, it would be impossible to image sites of specific targeting. In contrast to activatable probes or 'SMART PROBES' for optical and MRI (magnetic resonance imaging) approaches[7], which produce signal only when they interact with a target, a radiolabelled tracer constantly produces signal by decaying. This leads to areas of signal that are not related to specific targeting. For example, at early time-points, signal can often be seen in the renal and hepatobiliary systems, depending on the routes of tracer clearance. These non-specific sites of signal can sometimes cause difficulties in image interpretation, but by looking at the PET images as a function of time, or by waiting long enough — half-life of isotope permitting — to let the background signal clear by urinary and/or faecal elimination, specific and non-specific signals can usually be separated. Tracer kinetic modelling with time–activity data obtained from PET studies is a powerful way of mathematically modelling the underlying biochemical and physiological processes that govern tracer movement and metabolism within the body. Tracer metabolites must be limited in cases in which tracer kinetic modelling is used to quantify specific biochemical processes (for example, rate of phosphorylation)[8]. The importance of quantification cannot be over-emphasized, as it is crucial to the initial validation of a particular tracer, even if quantification is not usually performed in a routine clinical application.

SMART PROBES
Probes that are used in optical and magnetic resonance imaging that can be kept relatively silent until they interact with the target. After interaction, they become activated and produce a detectable signal.

MRI
(Magnetic resonance imaging). A technique to image subjects through the use of a magnetic field that aligns endogenous (for example, proton) or exogenous (for example, gadolinium) magnetic moments. Provides both anatomical imaging and functional imaging.

Many approaches exist, and are being developed, that allow target cancer cells to be imaged with PET. These are reviewed in detail next.

Glucose utilization. 2-Deoxy-D-[^{14}C]glucose (DG) — formed by replacing the OH in the 2-position of D-glucose with a hydrogen — was developed in the early 1950s as a drug to block accelerated rates of glycolysis in cancer, and hence tumour growth[9]. However, it also blocked glycolysis in the brain, so it could not be used as a drug. Over 20 years later, in 1977, Sokoloff *et al.*[10] developed a new use for DG in imaging glycolysis, by labelling DG with carbon-14 and using autoradiography, which requires the animal to be killed. This use was extended when 2-[^{18}F]fluoro-2-deoxy-D-glucose (FDG) was synthesized[11] to image living subjects specifically and non-invasively with PET.

FDG was first used to study tumours in the 1980s by Di Chiro and others, who showed that the degree of malignancy of cerebral tumours was correlated with their FDG uptake[12,13]. Tumours have a higher rate of glucose use; FDG accumulation therefore also increases. In the early 1990s, FDG PET started to be used in conjunction with whole-body imaging protocols. The first applications of FDG PET outside neuro-oncology were primarily in the detection of lung cancer[14–16].

After FDG has been injected into the bloodstream, it is transported from the vascular space into the interstitial space. From here, specific glucose transporters recognize and transport it into cells. FDG is phosphorylated by hexokinase to form FDG-6-phosphate[17]. Unlike glucose, FDG lacks a hydroxyl group in the 2-position (FIG. 2a) and its first metabolite, FDG-6-phosphate, cannot act as a substrate for further glycolysis. Importantly, FDG is also eliminated via the renal system and, unlike glucose, is not reabsorbed well in the renal tubules, which leads to low levels of FDG in the blood. It is fortunate that the pharmacokinetics of glucose and FDG uptake, trapping and blood clearance are in the 30–60-minute time frame because this allows PET imaging with ^{18}F. The molecular targets of FDG are therefore glucose transporters and hexokinase[17,18]. The relative importance of each of these targets continues to be a subject of debate[19,20], and it is likely that in a given cell type, one might predominate.

Because all cells metabolize glucose, FDG is not specific for malignant transformation. This lack of specificity can be a potential problem in some cases, but it also serves to provide anatomical features in the image — by creating a low background signal in all tissues — that would otherwise not be present and would make image interpretation more difficult. Malignant transformation is often associated with increasing energy demands, a decrease in glucose-6-phosphate and the

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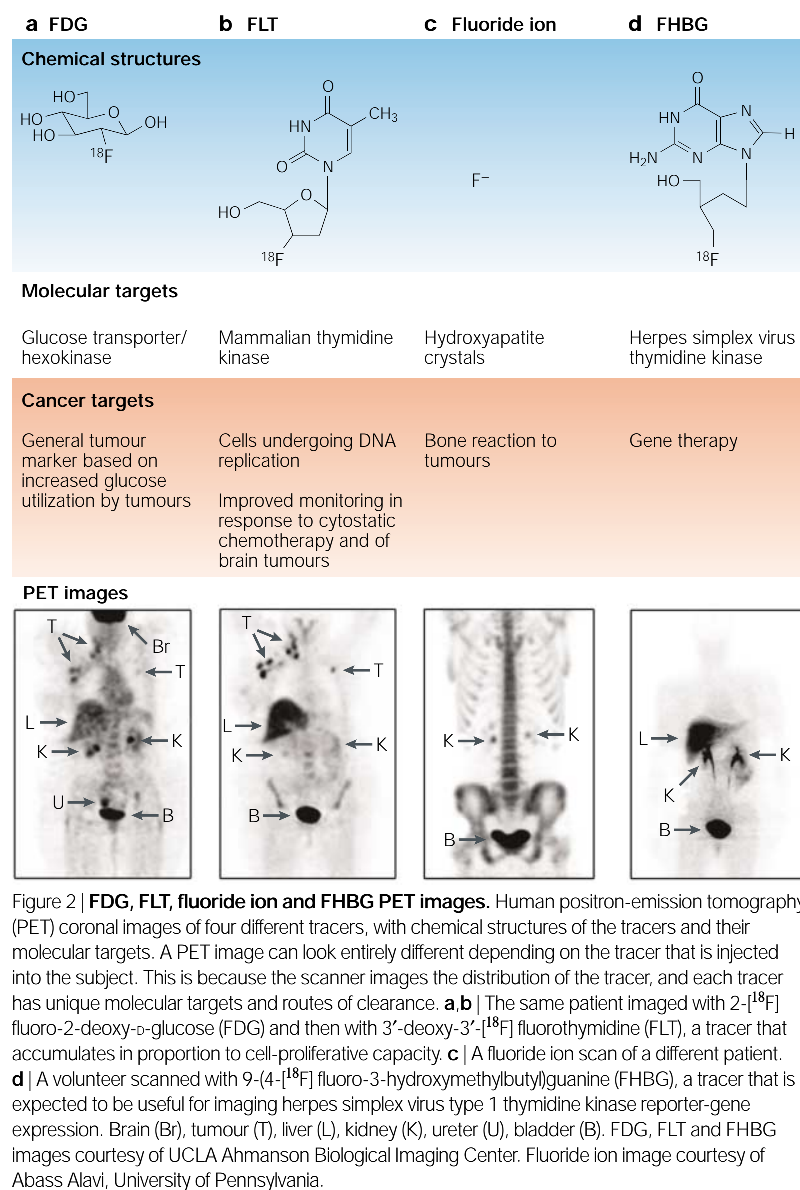

Figure 2 | **FDG, FLT, fluoride ion and FHBG PET images.** Human positron-emission tomography (PET) coronal images of four different tracers, with chemical structures of the tracers and their molecular targets. A PET image can look entirely different depending on the tracer that is injected into the subject. This is because the scanner images the distribution of the tracer, and each tracer has unique molecular targets and routes of clearance. **a**,**b** | The same patient imaged with 2-[^{18}F] fluoro-2-deoxy-D-glucose (FDG) and then with 3′-deoxy-3′-[^{18}F] fluorothymidine (FLT), a tracer that accumulates in proportion to cell-proliferative capacity. **c** | A fluoride ion scan of a different patient. **d** | A volunteer scanned with 9-(4-[^{18}F] fluoro-3-hydroxymethylbutyl)guanine (FHBG), a tracer that is expected to be useful for imaging herpes simplex virus type 1 thymidine kinase reporter-gene expression. Brain (Br), tumour (T), liver (L), kidney (K), ureter (U), bladder (B). FDG, FLT and FHBG images courtesy of UCLA Ahmanson Biological Imaging Center. Fluoride ion image courtesy of Abass Alavi, University of Pennsylvania.

upregulation of glucose transporters (especially GLUT-1) and hexokinase. It is also important to note that glucose competes with FDG at each step of uptake and trapping. In clinical practice, this is dealt with by fasting the patient for 4–6 hours before FDG injection, to minimize competition.

The current clinical applications of FDG in cancer diagnosis and management are very diverse. Because most types of cancer cell accumulate FDG, and most non-cancer cells accumulate much less, FDG PET is a very general approach to cancer imaging[21]. The overall mean sensitivity and specificity across various applications are ~85%. The primary established clinical roles for FDG PET are in the diagnosis and management of various malignancies. The assessment of solitary pulmonary nodules[22] and pre-operative staging of non-small-cell lung cancer to determine sites of metastases not seen on CT[23] are improving the management of patients. Staging in colorectal cancer, melanoma and lymphoma are all relatively well-established applications[24–27]. Imaging of brain metastases is limited because of the relatively high uptake of FDG by grey matter. Emerging roles include monitoring for recurrence and during therapy[28,29] (FIG. 3). By providing early information on the metabolic response of a tumour for a given therapy, FDG PET can aid in continuing or changing therapy to avoid potential side effects of a given therapeutic regimen. FDG PET has not been particularly effective in managing patients with prostate[30] or ovarian cancer[31], and screening high-risk patients remains to be validated. At present, the vast majority (~95%) of all clinical PET studies use FDG.

FDG is a great example of a failed drug that has become quite successful as an imaging agent. Many other such agents could be present in the databases of pharmaceutical and biotechnology companies, waiting to be exploited.

Cell proliferation. Cell proliferation is increased with malignant transformation, which consequently increases the number of cells undergoing DNA replication. The use of thymidine analogues provides a useful way of targeting DNA replication[32]. Upregulation in thymidine transport and mammalian thymidine kinases provides molecular targets for imaging. These are upregulated by cancer cells because thymidine is needed for DNA synthesis. Several agents, including [^{11}C]thymidine, have been studied, but the most promising agent so far is 3′-deoxy-3′-[^{18}F]fluorothymidine (FLT)[33,34] (FIG. 2b).

Imaging in dogs and clinical patients[34] shows the potential superiority of FLT over FDG. FLT PET imaging during therapy has the potential to be more useful than FDG for three reasons. First, there would potentially be less uptake of FLT than FDG after an inflammatory response. Second, cytostatic chemotherapeutics, which often have a greater impact on cell division than on glucose metabolism, might be better monitored with FLT. Third, brain tumours might also be better imaged with FLT: it accumulates at lower levels in most regions of the brain, because of a lack of significant neuronal cell division.

Bone remodelling. Metastasis to bone is common for several cancers, including those of prostate, breast and lung. The most common clinical procedure used to evaluate bone metastasis is [^{99m}Tc]methylenediphosphonate (MDP), which uses the gamma-camera and SPECT imaging[35]. The positron-emitting [^{18}F]fluoride ion was first described as an excellent bone-imaging tracer 40 years ago, and its use for PET imaging is still being clinically validated[35,36] (FIG. 2c).

Skeletal uptake of bone-seeking radiopharmaceuticals, such as MDP and [^{18}F]fluoride ion, is influenced by bone blood flow, the molecular size and net electric charge of the molecule, capillary surface, capillary permeability, local pH and, most importantly, metabolic activity of the bone tissue[37]. In contrast to anionic complexes, such as ^{99m}Tc-labelled diphosphonates,

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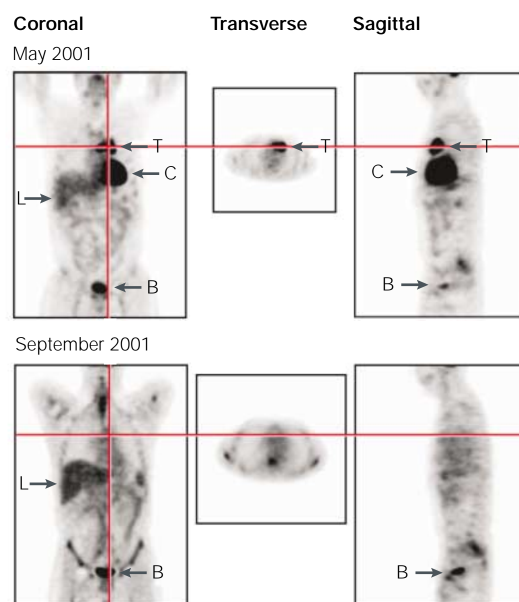

Figure 3 | **FDG PET imaging for monitoring therapy.** Shown are 2-[^{18}F]fluoro-2-deoxy-D-glucose (FDG) positron-emission tomography (PET) cross-sectional images of the same patient before and after chemotherapy for lymphoma. The tumour seen on the top image set is not well visualized in the images taken four months later. Cardiac activity is also not well visualized in the post-therapy images, and is probably related to dietary fasting differences. FDG PET is being actively investigated as an agent to monitor therapy and might show changes long before anatomical imaging, such as computed tomography, shows any response to therapy. Tumour (T), cardiac (C), bladder (B), liver (L). Images courtesy of J. Czernin, UCLA Ahmanson Biological Imaging Center.

[^{18}F]fluoride is small and is naturally incorporated into the bone matrix. After diffusion through capillaries into bone extracellular fluid, [^{18}F]fluoride exchanges slowly with hydroxyl groups in the hydroxyapatite crystal of bone to form fluoroapatite, and the activity is deposited preferentially at the surface of bone, where remodelling and turnover are greatest[38]. The relationship between bone formation and resorption is believed to determine the amount of CHEMISORPTION and incorporation of [^{18}F]fluoride into the bone matrix. The greater sensitivity and resolution of PET, as compared with SPECT, should lead to the more effective characterization of bony metastases[35], and it is likely that this technique will continue to gain more widespread acceptance as higher-throughput PET scanner technology is developed.

Perfusion. Tumours are in constant need of nutrients from the blood, and tumour neovascularization provides a crucial lifeline for rapidly dividing tumour cells[39]. Tracers that are extracted from the blood can therefore be used to assess tumour blood perfusion. The diffusion into tissues is proportional to delivery, and so is a measure of perfusion. Although a relatively non-specific assay, blood-perfusion imaging uses simple tracers and has the potential to be useful. ^{15}O-labelled water is one of the most extensively studied perfusion tracers[40] and there is a relatively good correlation between perfusion and tracer accumulation, but several other tracers for blood-perfusion assessment exist[8]. Quantitative measurements of perfusion require determination of the blood time–activity curve (input function), which can be obtained by arterial sampling or images of the left ventricle. Good studies for the validation of various tracers for specific use with tumours are still lacking. Attempts to image tumour angiogenesis specifically have also been preliminarily validated[41], and it is likely that these techniques can be extended for use with anti-angiogenesis pharmaceuticals.

Oxygen metabolism. Tumour oxygenation is a crucial factor in the successful treatment of tumours by various approaches. Hypoxia seems to be a significant prognostic variable, and it is likely that the use of tracers to monitor hypoxia before, during and after treatment will continue to increase. [^{18}F] Fluoromisonidazole (FMISO), an analogue of 2-nitroimidazole, has been used to image hypoxia in tumours[42]. FMISO diffuses into all cells and is reduced and reoxidized in normal cells; however, it continues to be reduced and binds to cell components in hypoxic cells. The use of FMISO is not optimal because of its relatively poor cellular uptake and slow clearance from normal tissues[43]. Copper bis(thiosemicarbazones), such as Cu(II)-diacetyl-bis(N^4-methylthiosemicarabaone) (Cu-ATSM), has been shown to wash out of normal tissues quickly and yet be retained in hypoxic tissues owing to its reduction by oxygen-depleted mitochondria[44]. It is probable that increasing research into the development of new hypoxia tracers, as well as the testing of existing ones, will increase over the next few years.

Tumour receptors and antigens. Although tracers for metabolism, proliferation, perfusion and hypoxia provide useful imaging of neoplasms, they are relatively non-specific and are usually less useful for imaging tumours that have very low growth rates. The development of tracers that target specific tumour antigens is therefore essential for the development and usefulness of clinical PET.

Many intracellular and cell-surface receptors are upregulated in cancer cells. To target potential receptors, it would be optimal to use tracers that have a very high affinity for the target receptor and a minimal background accumulation; this could be achieved by lowering LIPOPHILICITY. Relatively small ligands, as well as larger proteins such as antibodies, have been labelled and used as tracers to target specific receptors. Examples of small ligands include ^{11}C-labelled *N*-methylspiperone and ^{18}F-labelled spiperone for targeting the dopamine receptors on pituitary adenomas[45,46], and ^{64}Cu-labelled octreotide[47] and ^{68}Ga-labelled octreotide analogues[48] for targeting somatostatin-receptor-positive tumours. Sigma receptors are also found on many tumours, and small PET ligands to image these receptors are under investigation[49,50].

CHEMISORPTION
A chemical adsorption process in which weak chemical bonds are formed between gas or liquid molecules and a solid surface.

LIPOPHILICITY
The degree of affinity for fat.

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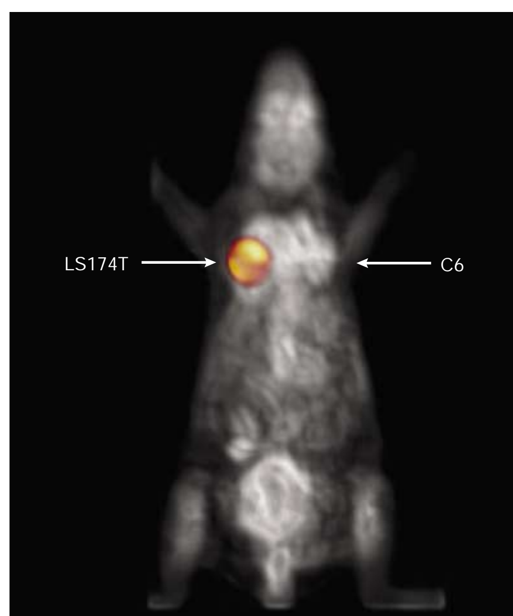

Figure 4 | **Small-animal FDG/^{124}I minibody imaging.** MicroPET (positron-emission tomography) imaging of a mouse 1 hour after tail-vein injection of 2-[^{18}F]fluoro-2-deoxy-D-glucose (FDG) (grey scale) superimposed on microPET imaging of the same mouse imaged 18 hours after injection of an ^{124}I (~4-day half-life)-labelled minibody (colour scale) targeted against carcinoembryonic antigen (CEA). The mouse carries two tumour xenografts: a C6 rat glioma as a negative control and an LS174T line that expresses CEA. The minibody signal is seen almost exclusively from the LS174T tumour only, because most background activity has already cleared 18 hours after tracer injection. Images courtesy of Anna Wu and the Crump Institute for Molecular Imaging.

MINIBODIES
An engineered antibody construct that consists of the variable-heavy- and variable-light-chain domains of a native antibody that is fused to the hinge region and to the CH3 domain of the immunogloblin molecule. Minibodies are small versions of whole antibodies, encoded in a single protein chain, that retain the antigen-binding region, the CH3 domain (to allow assembly into a bivalent molecule), and the antibody hinge (to accommodate dimerization by disulphide linkages).

DIABODIES
Engineered antibody fragments that are bivalent or bispecific molecules generated by dimerization of two variable-heavy–variable-light fragments. These molecules clear much more rapidly from the blood than do full antibodies.

Antibodies are also a potential tracer for targeting cell-surface receptors. Although primarily explored for imaging with gamma-cameras and SPECT, newer small-animal studies and clinical trials are starting with positron-labelled antibody fragments. Monoclonal antibodies developed against a specific antigen target are problematic, because their relatively slow clearance from blood leads to images with very high background signals, even at up to one week after injection of the antibody. Efforts have been made in the systematic construction of engineered antibody fragments, such as MINIBODIES and DIABODIES[51,52], against carcinoembryonic antigen (CEA). These agents show much more rapid blood clearance (owing to their smaller size) — at the expense of some affinity for CEA — in comparison with intact antibodies. Humanized versions of these engineered antibody fragments have been labelled with ^{64}Cu and ^{124}I, and mouse tumour xenograft imaging has been performed with microPET[52] (FIG. 4). Clinical PET trials with these agents are now starting. The engineered antibody fragments have the ability to be adapted for targeting other tumour-cell-surface targets (for example, ERBB2 (also known as HER2/neu)) and it remains to be seen what advantages these tracers have in the clinical setting over existing tracers such as FDG. Further reviews of antibodies and engineered antibody fragments for imaging are provided elsewhere[53].

Relatively small radioligands have been used to target intracellular receptors. One of the most extensively studied systems is radiolabelled steroids for targeting hormone-receptor-positive tumours[54]. As there are only a few thousand steroid receptors per cancer cell, there is a limited imaging signal potential from each cell. 16α[^{18}F]Fluoro-17β-oestradiol (FES), an oestrogen analogue, has good imaging characteristics in human studies and has been preliminarily studied to monitor the effectiveness of hormonal therapy with tamoxifen in breast cancer[55–57]. Tamoxifen competes with FES, so the PET signal can decrease after treatment and this can be used to gauge treatment efficacy. Androgen receptors have also been targeted by PET tracers for imaging prostate cancer. ^{18}F has been used to label several hormone analogues, including 16β-^{18}F-substituted testosterone, 5α-dihydrotestosterone and mibolerone, 16α- and 16β-[^{18}F]7α-methyl-19-nortesotsteorne, 20-[^{18}F]fluoro-R1881 (metribolone) and 20-[^{18}F]fluoromiboloerone. Some animal studies have been performed with these agents, but more detailed studies are still needed[58,59]. These tracers might eventually be used to improve the staging and monitoring of prostate cancer. More studies are still needed to determine the utility of these tracers for imaging human prostate cancer.

Gene therapy with reporter genes/probes. Imaging gene expression is important for monitoring the location(s), magnitude and time-variation of gene expression from gene-therapy vectors, and can be important in measuring the efficacy of the therapy. Without imaging methods to monitor expression, the field of gene therapy will continue to face significant obstacles for routine use. For monitoring the expression of therapeutic genes during gene therapy, several approaches to 'linking' a therapeutic gene to a 'PET reporter gene' have been studied[60]. The PET reporter gene allows an indirect method of following the expression of the linked therapeutic gene of interest. It 'reports' back on the status of expression of the gene of interest when properly linked.

The linkage can be made in one of several ways. First, an internal ribosomal entry site (IRES) can be used in a bicistronic vector that encodes both transcribed genes in one mRNA, which is translated into two proteins[61,62]. Second, an inducible bi-directional vector can be used in which doxycycline initiates the transcription of both the therapeutic gene and the reporter gene[63] (FIGS 5,6). Third, the reporter gene and therapeutic gene can be delivered by using two separate vectors[64]. Finally, fusion approaches in which a fusion protein contains both the therapeutic and reporter protein can be used[65]. All of these approaches are dependent on having a useful PET reporter gene, as described next.

Several approaches have been validated to extend the reporter-gene techniques that are normally used in cell studies to develop PET reporter genes. Although optical reporters such as green fluorescent protein (GFP) and firefly/*Renilla* luciferase offer significant advantages in cell assays, and more recently have been

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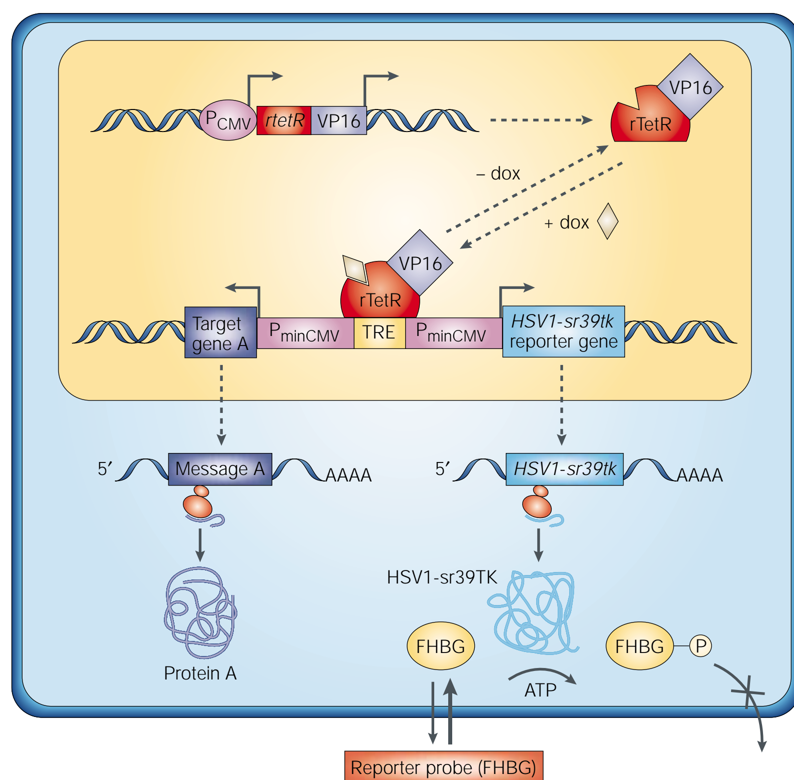

Figure 5 | **Bi-directional inducible therapeutic and reporter-gene expression.** Target (therapeutic) gene expression can be measured indirectly by imaging reporter-gene expression if expression of the two genes is 'linked'. Both genes can be simultaneously expressed from two minimal cytomegalovirus (CMV) promoters that are regulated by a single bi-directional tetracycline-responsive element (TRE). The rTetR–VP16 fusion protein is produced constitutively from a CMV promoter. When the rTetR–VP16 fusion protein binds doxycycline, the fusion protein plus doxycycline complex binds to the TRE regulatory sequence and substantially enhances expression from the two minimal CMV promoters. The target gene *A* in one coding region and a reporter gene (for example, a reporter kinase such as *HSV1-sr39tk*) in the alternative coding region are transcribed simultaneously into two mRNA molecules. Translation of the two mRNA molecules yields two distinct proteins in amounts that are directly correlated with each other. Quantitative imaging of the location(s) and magnitude of PET reporter-gene expression by trapping of a PET tracer inside the cell (for example, by phosphorylation of FHBG by the HSV1-sr39TK reporter protein) provides an indirect measure of target-gene expression.

Na/I SYMPORTER
A transporter that is found primarily in thyroid epithelial tissue that co-transports both iodide and sodium from extracellular fluid into cells.

SUICIDE GENE
A gene that can be introduced into target cells that will, under the appropriate conditions, lead to destruction of that cell. The herpes simplex virus type 1 thymidine kinase gene (*HSV1-tk*) is an example of a suicide gene. It encodes a protein that, in the presence of pro-drugs such as ganciclovir, leads to cell death. Suicide-gene-therapy approaches have been attempted as a way to destroy cancer cells.

used in small-animal models[7,60], they are not easily translated to human applications because of the limited penetration of visible light through tissues. PET reporter genes — including those that encode herpes simplex virus type 1 thymidine kinase (*HSV1-tk*)[66–68] and the dopamine type 2 receptor (*D2R*)[69] and their mutants[70,71] — have been validated in animals. Other reporter genes, including those that encode the somatostatin receptor and Na/I SYMPORTER, have also been preliminarily studied and are reviewed elsewhere[60]. The principle in using each of these PET reporter genes is to obtain sufficient PET signal primarily from those cells that express the reporter gene due to accumulation of the appropriately chosen tracer.

The *HSV1-tk* reporter gene, which encodes the HSV1-TK protein, can trap several positron-labelled tracers by phosphorylating a transported tracer. This tracer then becomes charged, so it cannot leave the cell (FIG. 5). Both acycloguanosines (for example, derivatives of ganciclovir and penciclovir) and derivatives of thymidine such as 2′-fluoro-2′-deoxy-1-β-D-arabinofuranosyl-5-iodo-uracil (FIAU) have been used as tracers for imaging *HSV1-tk* reporter-gene expression by adenovirus-mediated gene expression in the liver and stably transfected tumour cells[72]. We have also described imaging with a mutant *HSV1-sr39tk* reporter gene, which has a greater imaging sensitivity when used with acycloguanosines[70] and is less sensitive to changes in intracellular thymidine concentration.

The *D2R* reporter gene has been validated for the PET imaging of reporter-gene expression with [^{18}F]fluoroethylspiperone (FESP) as the tracer ligand[69], and, more recently, a mutant D2R has also been reported[71] that is uncoupled from signal transduction, while maintaining affinity for FESP. Although it might seem that a receptor approach might lead to less imaging sensitivity than an enzyme-based approach (for example, *HSV1-tk*), there are advantages, including no requirement for tracer transport into the cell for cell-surface receptors. Detailed comparisons of various approaches can be found elsewhere[72].

Studies in imaging reporter-gene expression in human subjects have only recently started. As might be expected, as *HSV1-tk* is both a reporter gene and a SUICIDE GENE[73], imaging its expression in humans would be a good initial proof of principle for the use of PET imaging in gene therapy. We have studied the kinetics, biodistribution, stability, dosimetry and safety of 9-(4-[^{18}F]fluoro-3-hydroxymethylbutyl)guanine (FHBG) in healthy human volunteers[74], for eventual use in human gene-therapy trials (FIG. 2d). Imaging *HSV1-tk* reporter gene expression near the gall bladder, kidneys and bladder with FHBG might be difficult owing to the background signal in these regions, which is caused by routes of tracer clearance. Furthermore, imaging within the brain would be difficult because FHBG does not significantly cross the blood–brain barrier. Clinical trials with FHBG in patients undergoing *HSV1-tk* gene therapy are now starting. In another preliminary study, Jacobs *et al.*[75] have recently shown that [^{124}I]FIAU PET imaging might be useful in patients with glioblastoma who are undergoing *HSV1-tk* suicide-gene therapy. The *HSV1-tk* gene was delivered by the intratumoral infusion of cationic liposomes. The disruption of the blood–brain barrier allows imaging in these patients. Although still in validation, it is likely that the PET reporter-gene approaches will aid various gene-therapy trials directly. Issues of aberrant gene expression in unintended sites will be particularly helpful in making safe the routine use of gene therapy.

Other applications. The use of ^{11}C- and ^{18}F-labelled choline has recently gained attention for applications in prostate cancer imaging[76–78]. This approach relies on the fact that these tracers are readily incorporated into cells through phosphorylcholine synthesis and integration in membrane phospholipids. The use of radiolabelled amino acids, including methionine and tyrosine, is also a growing area, and might prove to be useful in specific applications (for example, brain metastases);

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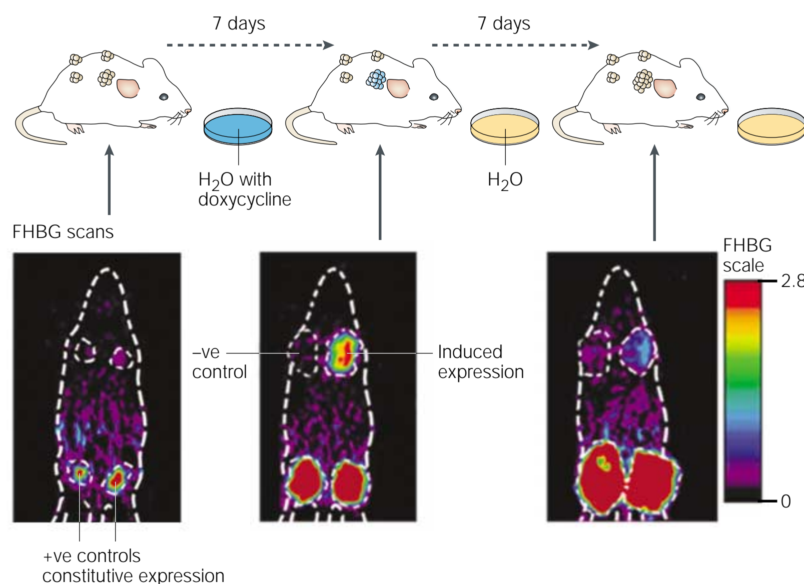

Figure 6 | **MicroPET imaging of bi-directional inducible therapeutic and reporter-gene expression.** Sequential microPET (positron-emission tomography) imaging studies of a nude mouse carrying four tumours. Four tumour cell lines — two positive controls (constitutive reporter-gene expression), one negative control and one inducible line (reporter-gene expression induced by doxycycline (see FIG.5 for expression system)) — were injected subcutaneously into four separate sites in a single mouse. When tumours reached a size of at least 5 mm, the mouse was imaged with 9-(4-[^{18}F] fluoro-3-hydroxymethylbutyl)guanine (FHBG). Doxycycline was then added to the water supply for 7 days. The mouse was then scanned again with FHBG. Doxycycline was removed from the water supply for the next 7 days, and the mouse was again scanned with FHBG. The locations of the four tumours and the mouse outline are shown by the dotted regions of interest. All images are 1–2 mm coronal sections through the four tumours. The %ID/g (% injected dose per gram tissue) scale for FHBG is shown on the right. The negative control tumours show no gene expression, and the positive control tumours show increased expression over the time course. The tumour on the top right, with inducible gene expression, initially does not accumulate FHBG, then at 7 days after addition of doxycycline, induction of reporter-gene expression traps FHBG. Seven days after withdrawal of doxycycline, there is decreased induction and minimal trapping of FHBG.The FHBG image signal correlates well with target-gene expression (not shown). Images reproduced with permission from REF. 63.© (2001) Nature Publishing Group.

this is reviewed elsewhere[79]. It remains to be determined under what circumstances radiolabelled choline and amino acids outperform FDG.

The use of antisense tracers for targeting endogenous mRNA levels would be very useful because of the ability to adapt these approaches to target the expression of almost any endogenous gene. By rearranging the base sequence of the tracer, while keeping the radiolabelling strategy the same, large libraries of such tracers could be generated. Antisense probes of high specific radioactivity have been developed, but it remains uncertain whether these approaches will be robust enough to image endogenous gene expression *in vivo*[80–82]. Problems of efficient delivery, non-specific interactions and sufficient efflux from cells that do not contain target mRNA will need to be resolved. Investigations to couple approaches with split reporter proteins[83] or trans-splicing approaches[84–86], in conjunction with HIV Tat-mediated delivery of DNA, RNA or protein[87], are being studied to image mRNA levels. These approaches might provide signal amplification by using mRNA hybridization to provide specificity for the target, and could simultaneously provide sensitivity through signal amplification from the reporter protein. Applications of PET in assessing multidrug resistance[88], and intra-operative beta-probes with FDG for guiding surgery[89], are also areas of active investigation. Other tracers for various cancer molecular targets are reviewed elsewhere[8,90,91].

Multimodality imaging

PET imaging provides unique information on tumour biochemistry and physiology, but without much anatomical information — that obtained is generally due to the non-specificity of the tracer. For example, with FDG PET, the muscles throughout the body are usually detected by their use of glucose. As more specific tracers with rapid clearance from non-specific tissues are developed, correlative anatomical imaging is crucially needed. Although software tools can be used to register data from scans obtained separately from CT and PET, this is difficult to do outside the brain as a result of many variables, including patient motion. Clinical PET/CT systems have been developed and are undergoing rapid refinement[92–94]. It is likely that most clinical PET systems around the world will be replaced with PET/CT systems during the next 5–10 years. The ability to obtain data from both modalities is already significantly improving clinical decision-making[95] (FIG. 7). Small-animal studies should also benefit from obtaining anatomical and functional images. MicroCT studies[96,97] and microPET studies can be performed separately[98], but the availability of a small-animal microPET/CT scanner will be useful, and several such instruments are in development. In addition to CT, the use of MRI[99] and even optical approaches, when coupled with PET, might prove useful in small-animal and perhaps even clinical imaging.

Drug development and PET

One of the most under-used applications of microPET and clinical PET is in the area of drug development[100,101]. For the most part, the imaging community and the pharmaceutical/biotechnology industries have remained isolated with minimal overlap, but this has started to change over the past few years, and many pharmaceutical companies are investing in research imaging programs that use microPET and PET.

Drugs that are ready to proceed to testing *in vivo* can have their pharmacokinetics analysed in small animals, as well as humans, with PET. This first requires labelling the pharmaceutical with a positron-emitting isotope, but is potentially highly cost-effective. This is because the costs of the time invested in the labelling technique(s) can be offset by a more rapid translation to human testing. Although trace levels of the drug can be introduced into humans, mass levels might need to be used to mimic the biodistribution of the drug of interest accurately. In addition, existing PET tracers can be used to monitor a new pharmaceutical. For example, a new drug that is a ligand for a receptor can be studied with PET by imaging displacement of an existing PET tracer before and after drug administration. The total radioactivity

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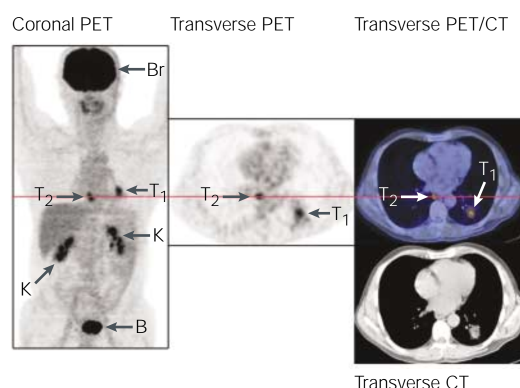

Figure 7 | **FDG PET/CT imaging for improved patient management.** Shown are images from a new generation of hybrid positron-emission tomography (PET) and computed tomography (CT) dual-modality scanner. This scanner can take both PET and CT images in the same patient, allowing registration of functional (PET) and anatomical (CT) information. The patient is a 42-year-old smoker with known non-small-cell lung cancer and referred for a PET/CT scan for staging his disease before potential surgery. 2-[^{18}F]fluoro-2-deoxy-D-glucose (FDG) PET coronal image shows the lung tumour in the left lower lobe (T1), but a second area of increased uptake is seen in the mediastinum (T2). On the FDG PET transverse images, this second lesion localizes to the middle mediastinum. FDG PET alone in this setting would indicate that this is probably a lymph node of the known lung carcinoma. However, the transverse section of the PET/CT shows that this second area is localized in the oesophageal wall and is not a distinct lymph node. This patient had an additional biopsy that proved oesophageal cancer. This case illustrates the importance of having anatomical definition for the PET study. As PET tracers further improve and localize only to the site of a tumour, it will be vital to have information on the exact anatomical location(s) of tracer uptake. Brain (Br), tumours (T1,T2), kidneys (K), bladder (B). Images provided courtesy of Gustav K. von Schulthess, University Hospital, Zurich, Switzerland.

measured by PET can contain significant amounts of radiolabelled metabolites, so that tracer kinetic modelling and blood sampling of metabolites are often needed to quantify the levels of drug.

It is also possible to start the process of merging the development of a PET tracer and a pharmaceutical from a very early stage. This can be done by developing libraries of molecules that can be conveniently radiolabelled and can also be used for screening for therapeutic efficacy against one or more targets. The advantage of being able to study a radiopharmaceutical in an animal model and then quickly move into human studies is an important area that is likely to be used for many drugs. Clinical trials might be accelerated through earlier human studies to rule out drugs that have unfavourable biodistribution and/or pharmacokinetics. This ability to translate easily from animal to human studies should help to increase the use of PET for drug development. Additionally, the development of new PET tracers should be enhanced by greater partnerships between the pharmaceutical and imaging communities[101–105].

Prospects for the future

Clinical and small-animal PET should expand significantly over the next decade, as more research groups and clinical centres acquire these technologies. In addition, small radiopharmacies with cyclotrons should continue to grow, providing for the routine availability of FDG and other existing tracers. The existing tracers could eventually give way to a newer generation of molecular probes that are more sensitive and specific. Advances in detector technology and image reconstruction techniques should help to produce a new generation of scanners that have better spatial resolution, sensitivity and significantly improved throughput time (less than 10 minutes for the entire body of a patient). PET will probably be replaced by PET/CT systems to provide anatomical and functional image information, especially as tracers become more specific and lead to minimal background signal. The use of PET/CT imaging in the diagnosis and management of cancer patients will be expanded with the development of new tracers. The use of PET/CT systems by radiation oncologists to perform more detailed therapy planning should also increase significantly.

It is likely that PET will be important in the molecular imaging of cancer; however, it will probably be part of a multimodality imaging approach. There should be continued roles for SPECT, CT, MRI, ultrasound and new optical imaging approaches (for example, optical breast-cancer imaging). PET will have an advantage in terms of its greater sensitivity at all depths, as well as the ability to use biological molecules that retain most, if not all, their properties after being radiolabelled. These advantages for PET will be coupled with significant challenges of developing relatively large libraries of useful tracers. PET assays will need to move towards generalized tracers in which the radioisotope labelling chemistry remains largely unchanged, but the underlying molecular structure can be easily modified to image a new molecular target. Alternatively, pre-targeting approaches, in which a non-radioactive molecule is administered first and followed by a tracer, might allow signal amplification and generalization to many targets.

The next decade should see applications in individualized or 'customized' imaging. Scenarios in which an individual's tumour is characterized in detail by gene and protein profiling should allow the use of tracers that are specific for the individual's tumour. This could allow better imaging during therapy and enhanced monitoring for recurrence by using tracers that are optimal for a given individual. Finally, the continued use of small-animal PET in developing new pharmaceuticals, as well as PET tracers for newly identified cancer targets, should help in significantly enhancing the fields of tumour biology, and cancer therapeutics and management. It will be important to use PET with a specific tracer in clearly defined populations of patients in which its efficacy has proved to be cost-effective. Finally, all practitioners of molecular imaging will need to work closely with engineering and biological scientists, pharmaceutical companies and clinical oncologists to build the next generation of useful tracers for PET and technologies that will some day significantly expand the capabilities of PET use in oncology.

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