# Molecular imaging in oncology drug development

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**Tremendous breakthroughs are being made in cancer drug discovery and development. However, such breakthroughs come at a high financial cost. At a time when there is increasing pressure on drug pricing, in part because of increased life expectancy, it is more important than ever to drive new therapeutics towards patients as efficiently as possible. In this review we discuss the applications of molecular imaging in oncology drug development, with a focus on its ability to enable better early decision making, to increase efficiency and thereby to lower costs.**

## Introduction

Despite advances in our understanding of cancer biology and drug discovery, mortality as a result of cancer remains high [1]. Meanwhile, there is continual pressure to reduce drug development costs [2,3], not least because treatments are likely to emerge that will lead to some cancers being considered chronic diseases, thereby changing the way drugs are developed, approved and valued. To capitalise on discoveries in cancer biology and reduce the development time for new drugs, we need to find ways to predict efficacious doses of mono and combination therapies earlier and with greater certainty [4], as well as ways to identify individual patients most likely to benefit from a particular therapy.

Two key goals of early-phase clinical trials are to provide evidence of a biological effect to support drug development and to predict the efficacious dose range. Establishing the maximum tolerable dose (MTD) is a logical starting point when toxicity and efficacy are closely linked; but this is often not the case with targeted 'noncytotoxics' where efficacious doses can be lower [5]. In addition, early studies typically include patients for whom standard therapy has failed or patients for whom the most appropriate therapy can be unclear. Such patients often have heterogeneous tumour types, different numbers of prior therapies, metastatic disease and impaired organ function, which can make it difficult to obtain evidence of a meaningful biological drug effect. Molecular markers of altered physiological processes can change before and in the absence of tumour morphology and could enable earlier determination of responders. In this review, we will discuss how molecular imaging techniques, such as positron emission tomography (PET), can help overcome some of the challenges.

## Imaging applications: operational definitions

For the purpose of this paper we will categorise the imaging methods and biomarkers according to their application or utility (Table 1). There will be cases where a given approach or method might fall into more than one category (e.g., a labelled biologic can be used to infer biodistribution of the drug as well as target expression)

## Biodistribution

In many cases discrete target expression means that distribution into the relevant compartment is necessary. Drug exposure can be limited by physiological barriers such as the blood–brain barrier [6] or efflux transporters such as P-glycoprotein (P-gp), breast-cancer-resistance protein (BCRP) and other multidrug-resistance proteins

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**TABLE 1**

**Biomarker definitions.**

| Type of biomarker or study | Utility |
|---|---|
| **Biodistribution** | Evaluate tissue distribution of a drug<br>Confirm that a drug reaches its target organ<br>Highlight potential safety risks<br>Potentially confirm target expression |
| **Pharmacodynamic** | Examine the effects of a drug's interaction with its target and investigate the consequences of that interaction |
| **Biomarkers of disease** | Monitor pathophysiological parameters related to disease progression, providing an indication of drug efficacy |
| **Patient selection biomarkers** | Prospectively identify patients most likely to respond to treatment |

(MRPs) [7]. In addition, therapeutic effects can be short lived owing to rapid redistribution away from the intended site of action or pathological changes such as altered renal function. In such cases a detailed evaluation of drug biodistribution might be warranted [8]. For example, it may be useful to demonstrate tumour uptake of a biologic therapy in the brain tumour setting (Fig. 1).

PET and single-photon emission computed tomography (SPECT) biodistribution studies, in which a drug is radiolabelled, provide information on the total drug concentration in different organs. However, one should remain aware that the distribution (and associated kinetics) of a tracer and a pharmacological dose of the same drug can differ, for instance as a result of drug effects on blood flow, nonlinearity of metabolic or transport processes or saturable clearance mechanisms. To test the assumption that the disposition of the tracer reflects that of a therapeutic dose, one can perform scans following administration of a tracer dose, and a tracer co-administered with a therapeutic dose. In addition, any differences in the route and administration scheme should be considered. Different radionuclides are available for labelling and the selection should be based on the desired imaging time points. As a general rule the biological half-life of the drug and the radioactive half-life of the radioisotope should be in the same range.

In the case of small organic molecules, isotopic substitution of $^{12}C$ or $^{12}F$ by $^{11}C$ and $^{18}F$ is often possible, when this is not the case further chemical modification (e.g., bioconjugation of a monoclonal antibody (mAb) with a metal chelating group) can be performed to enable labelling. In such cases further testing should be performed to ensure labelling has not changed important properties of the drug such as the immunoreactivity of mAbs. Metal isotopes with a chelating group or an iodine isotope are commonly used to label antibodies and other proteins because of their relatively long half-lives (e.g., $^{89}Zr$ $t_{1/2}$ = 78.41 h). $^{89}Zr$ and $^{111}In$ in their chelated forms are residualising (intracellularly retained), whereas the isotopes of iodine, including $^{131}I$

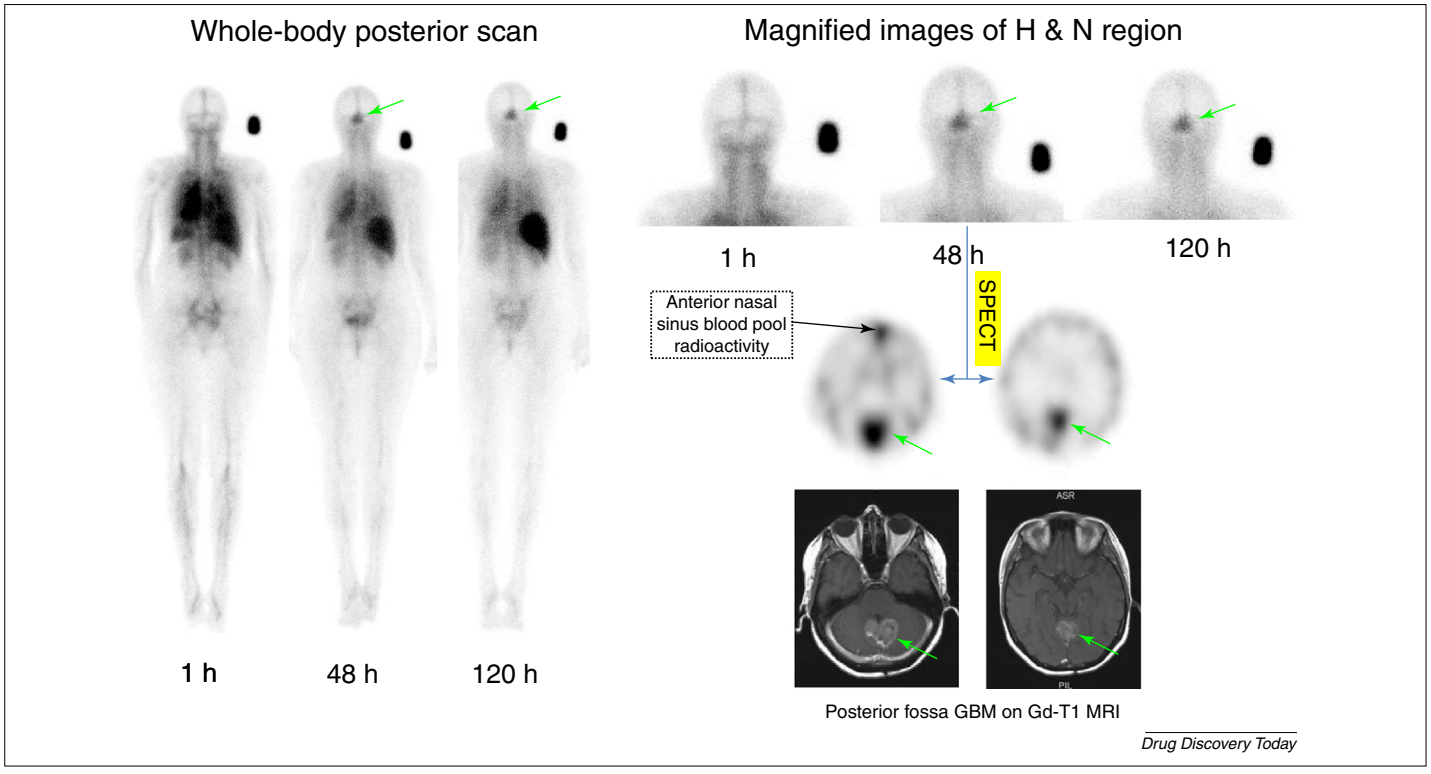


**FIGURE 1**

Single-photon emission computed tomography (SPECT) images of ABT-806i, an $^{111}In$-labelled anti-epidermal growth factor receptor (EGFR) antibody, in a brain tumour patient. Tumours are indicated by a green arrow.

and $^{124}$I, are non-residualising and rapidly exported from the interior of the cell after internalisation. Hence in the former case there is an accumulation of radioactivity in proportion to the area under the curve (AUC) of the exposure, whereas in the latter case there is a significant loss of radioactivity, which tends to accumulate in the thyroid and stomach.

As well as decay properties suitable for the design of mAb-based radiotracers, using $^{89}$Zr to label biomolecules is convenient from an operational standpoint because the longer half-life means that tracers can be transported from the site of production removing the need for all scanning sites to have a cyclotron. However, the use of $^{89}$Zr is not without its challenges, in particular the high energy and long half-life of $^{89}$Zr results in a relatively high radiation exposure that could limit the number of doses administered and/or the populations that can be studied [9] – 37 MBq can be administered to patients and provides sufficient image quality [10].

Novel methods are being developed to circumvent the limitations of half-life, dosimetry and low specific activity. For example, a pre-targeted PET imaging strategy leverages the advantages of mAb selectivity and the rapid pharmacokinetic properties of small molecules designed to radiolabel the mAb after it has cleared the blood and reached the targeted tissue [11,12]. Another possible solution could be the use of radiolabelled antibody fragments. While displaying good affinity for their biological target, fragments also show faster blood clearance potentially resulting in better tissue:blood ratios at earlier time points [13–15].

In addition to shedding light on the efficacy potential of a drug, understanding the biodistribution of a drug can provide insight into potential safety risks. For example, accumulation of an antibody–drug conjugate (ADC) in a non-target organ might warrant closer inspection of potential safety measures associated with that organ's function [16]. However, heightened accumulation in an organ does not necessarily confirm toxicity, because treatment duration and organ sensitivity are also important factors.

Biodistribution studies can also provide proof-of-targeting [17]. For example the biodistribution of a labelled bispecific antibody could be compared to its two parent antibodies (the therapeutic and targeting components) to confirm that the distribution is restricted to the targeted tissue. Another example is $^{89}$Zr-trastuzumab for PET imaging of HER2-positive lesions in patients with metastatic breast cancer [10].

To conclude our discussion of biodistribution with a cautionary note, although it is appealing to estimate target availability as a function of labelled mAb concentration over time, uptake can be influenced by a number of factors including tracer delivery, internalisation, receptor recycling, downregulation and occupancy by endogenous and exogenous ligand. Furthermore, regional uptake does not account for various compartments of the signal [i.e., specific (tracer bound to the molecular target of the drug), non-specific (tracer bound to other macromolecular components) and free (tracer that is not bound at all)]. Although a number of groups are currently developing compartmental models [18,19], in contrast to neuroreceptor imaging [20], we are not currently aware of any fully validated models to quantify the specific binding of biomolecules with PET imaging. In addition, there remain a number of challenges to interpreting studies performed involving pre-dosing or co-dosing labelled and unlabelled antibodies with respect to blocking, occupancy of the target or changes in target expression. The long biological half-life of mAbs and complex cellular biology makes interpreting such homologous competition results difficult. For example, decreases in mAb uptake could be the result of changes in target expression, occupancy of the target, tumour cell death or changes in blood flow to the tumour. Clinical imaging studies have been done to investigate the impact of mass dose on tumour uptake [10,21]. Once models are established for determining occupancy, methods could be extended to quantify target density.

## Pharmacodynamics

Pharmacodynamic biomarkers are used to monitor downstream effects after the drug has engaged its target. If mechanistically relevant (i.e., key to the proposed mode-of-action of a drug), such markers can be used to confirm the mechanism-of-action in humans.

### *Metabolism*

$^{18}$F-Fluoro deoxy glucose (FDG) is a radiolabelled glucose analogue routinely used in the clinic for diagnosis and assessment of treatment response. The underlying principle is the increased glycolysis of malignant cells. FDG is taken up by cells through glucose transporters (GLUT), particularly GLUT-1 and GLUT-3, and phosphorylated by hexokinase. Because the oxygen at the C-2 position has been replaced with $^{18}$F it cannot be further metabolised and is trapped in the cell.

Studies in a variety of settings have demonstrated the value of FDG in predicting metabolic responses before changes on anatomical imaging and in differentiating residual active disease from fibrotic changes [22–27]. However, treatment-induced changes in FDG uptake are likely to vary depending on the nature of individual tumours and their degree or speed of response to different treatments. In addition, other technical and physiologic factors might affect FDG uptake and should therefore also be considered in the design and analysis of such studies [28–30]. Several studies have shown tumour FDG uptake to correlate with GLUT-1 upregulation whereas correlations with other markers including hexokinase, GLUT-3, hypoxia-inducible factor (HIF)-1α, cellularity, proliferation markers and others have been reported less consistently [31].

In studies of the tyrosine kinase inhibitor imatinib in gastrointestinal stromal tumours, early reduction of FDG uptake was attributed to a direct mechanistic inhibition of glucose metabolism by treatment [32]. Although in other cases changes in uptake can reflect reduced cellularity owing to cell death rather than decreased metabolism [33,34], it is noteworthy that, despite the success of FDG as a biomarker of response to cytotoxics, there are limited clinical data available to support its value in the assessment of response to more-targeted therapies [35]. In addition, inflammatory changes can be associated with increased FDG uptake which can confound response assessments [36].

### *Proliferation*

Many therapeutics target tumour proliferation by modulating DNA synthesis [37]. Proliferation [38] can be imaged by monitoring the incorporation of nucleosides into DNA. At present [$^{11}$C]

thymidine is considered to be the best characterised proliferation radiotracer. However, the short half-life of $^{11}C$ and complex modelling required to account for its rapid catabolism have led to the development of fluorinated analogues, such as $^{18}F$-3′-fluoro-3′-deoxy-L-thymidine (FLT). FLT is taken up by cells via a nucleoside transporter and phosphorylated by thymidine kinase (TK)1. It cannot be incorporated into DNA, although studies have shown a good correlation between kinase activity (measured as FLT cell uptake) and proliferation [39,40]. However, one must remain aware that therapies affecting the *de novo* and salvage pathways of DNA synthesis could confound analysis of imaging data from thymidine analogue imaging agents [41,42].

### *Apoptosis*

The ability of tumour cells to avoid apoptosis is another hallmark of cancer that is of therapeutic interest [37]. In common with other imaging targets, key challenges for apoptosis imaging have been proven to be the identification and validation of targets

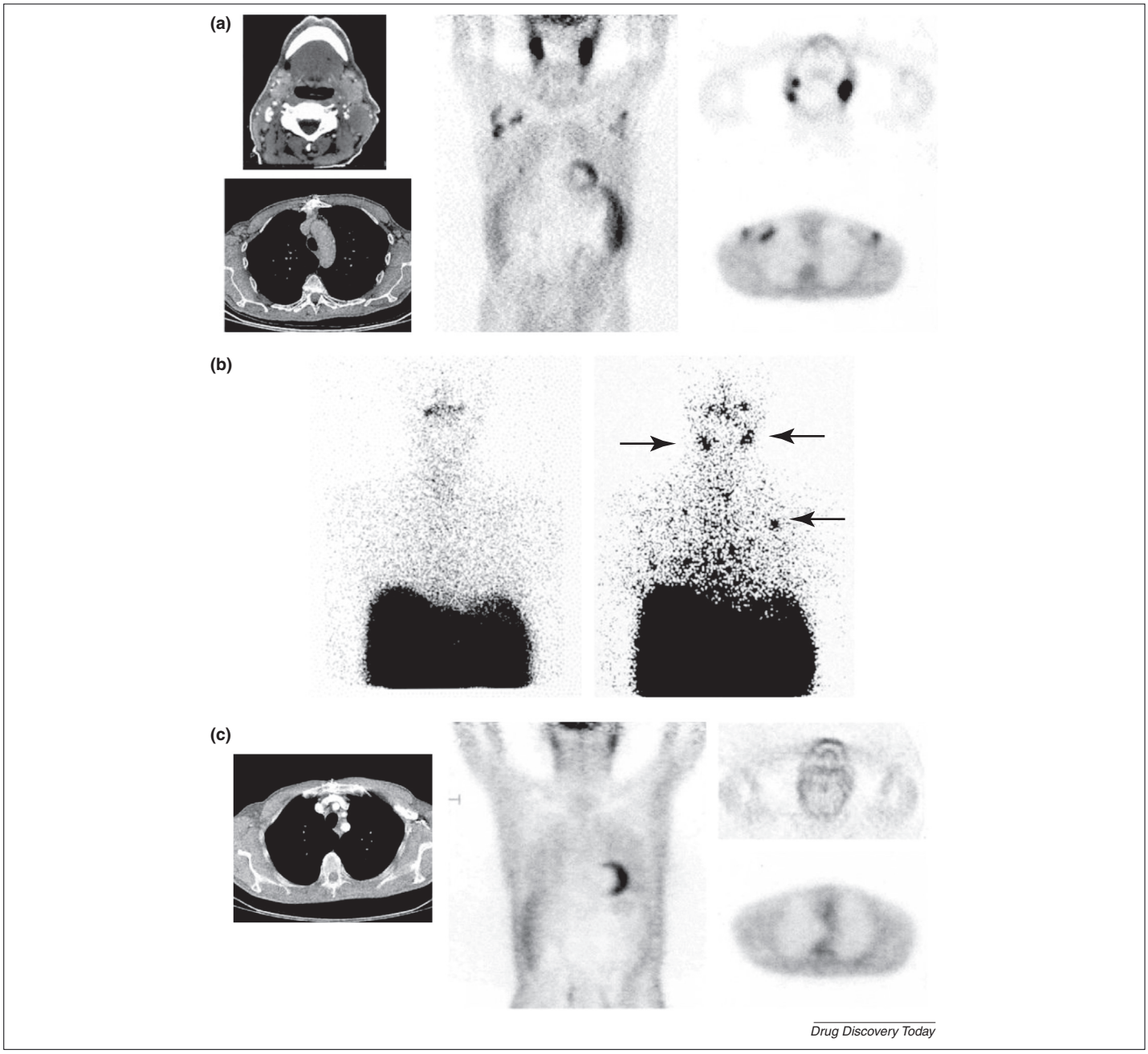


**FIGURE 2**

A case of non-Hodgkin lymphoma (NHL) stage IV treated by cyclophosphamide-doxorubicine-vincristine-prednisone protocol with a positive 99mTc-Annexin V study. **(a)** The computerised tomography (CT) scans of the neck and of the thorax (left) and the 18FDG positron emission tomography (PET) scan (middle and right) performed before treatment showed a lymph node dissemination at the cervical and axillary levels. **(b)** The Annexin V imaging performed immediately before (left) and 48 h after (right) chemotherapy demonstrated an increased uptake of the apoptosis agent at the tumour sites (arrows). **(c)** The post-treatment evaluation by the CTs and PETs showed complete disappearance of disease.
*Source*: Adapted, with permission, from Ref. [44].

for imaging and timing of assessments. In addition the half-life of apoptotic cells is short [43] and the apoptotic signal will eventually fade as a result of cell death and the timing might vary depending on treatment.

Several apoptosis tracers have been reported. Annexin V is an endogenous protein with high affinity for membrane-bound lipid phosphatidylserine (PS), which is externalised during the apoptotic process. Belhocine *et al.* used $^{99m}$Tc-Annexin-V SPECT to assess apoptosis induced by chemotherapy in patients with multiple tumour types. In all seven patients with increased Annexin-V tumour uptake, a subsequent objective response was observed. An example of a positive Annexin-V image can be seen in Fig. 2 [44]. However, studies have shown that necrotic cells [45] also externalise PS. Recently, imaging of apoptosis through targeting of intracellular activated caspase-3 has been demonstrated preclinically [46]. ML-10 is another newer tracer for imaging apoptosis, the accumulation of which is thought to be the result of membrane depolarisation and acidification during early apoptosis [47–49].

### *Angiogenesis*

Angiogenesis, the formation of new vasculature, is required for tumour progression by enabling a nutrient supply [37] and as such is another target for therapy and imaging. Imaging the integrin $\alpha_v\beta_3$ receptor can be used to monitor angiogenesis. $\alpha_v\beta_3$ expression is low on epithelial cells and mature endothelial cells but higher on activated endothelial cells in the neovasculature of many tumours, and is involved in growth, invasion and metastasis. Binding of the amino acid sequence Arg-Gly-Asp (RGD) to $\alpha_v\beta_3$ led to the development of multiple peptidic analogues for imaging angiogenesis. $^{18}$F-Galacto-RGD has been shown to exhibit favourable tumour uptake, kinetics and biodistribution in patients with multiple tumour types [50]. $^{68}$Ga-Labelled RGD peptides have also been studied clinically and might enable imaging without the necessity of a cyclotron [51,52].

### *Immunotherapy*

Immunotherapy has emerged as a promising option to treat patients with cancer. Immune checkpoint inhibitors designed to restore a patient's antitumour immune response, which can be suppressed during tumour development, have been approved for the treatment of advanced melanoma and metastatic non-small-cell lung cancer (NSCLC). Increased uptake of FLT in the spleen of patients treated with tremelimumab has provided evidence of the pharmacodynamic effect on lymphoid cell activation [53]. A new class of drugs that block programmed cell death protein (PD)-1, an immune checkpoint that prevents the activation of T cells, or its ligand PDL-1 have also shown success in treating certain types of cancer. Immunotherapy of solid tumours can delay response to treatment assessed by lesion size. As a result the utilisation of Response Evaluation Criteria in Solid Tumours (RECIST) can lead to erroneous classifications because progressive disease and molecular imaging tools can provide very useful insights on the response to treatments.

### *Considerations for PD biomarker implementation*

One crucial issue with the use of PD biomarkers is the timing of imaging relative to therapy. For example, some efficacious therapies can cause an increase in tumour FDG uptake immediately after therapy instead of a decrease. It is known that an increase in tumour FDG uptake, or flare, after tamoxifen therapy in oestrogen-receptor-positive breast cancer is indicative of responsiveness [54]. The potential of flares should be investigated before routine use of a therapy–biomarker combination. A more common example is illustrated by FLT where it is expected that the drug effect has a

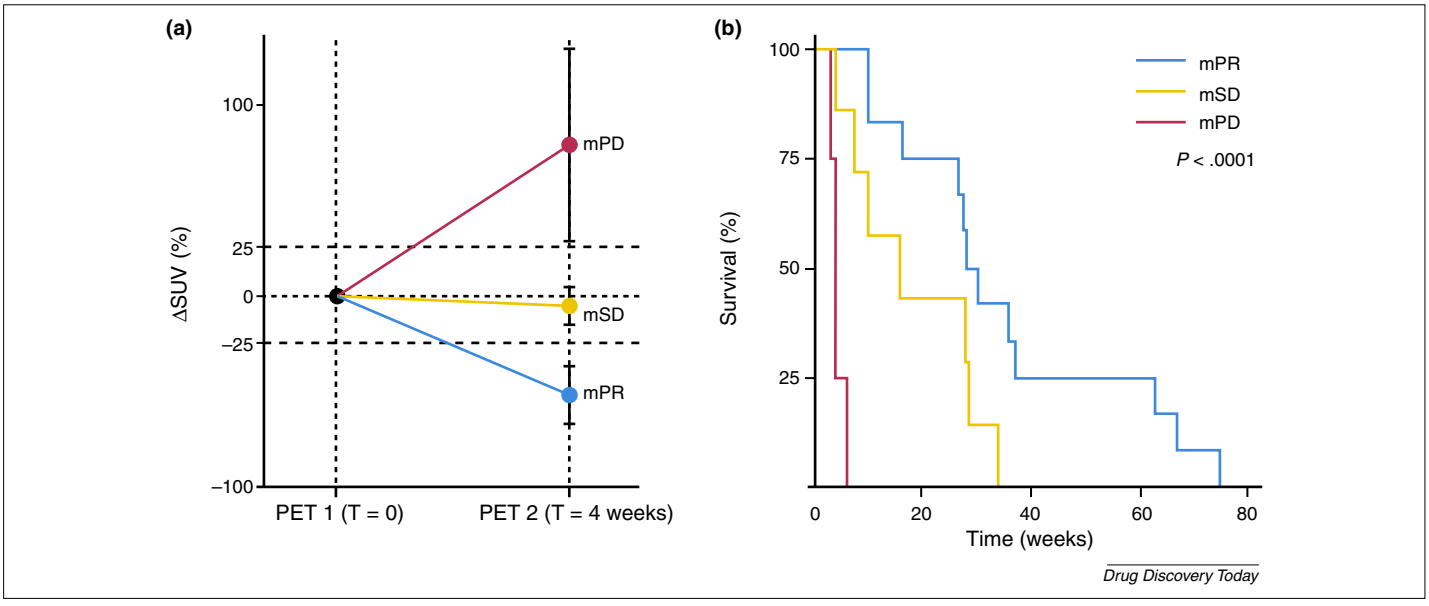


**FIGURE 3**

**(a)** Changes in standardised uptake value (ΔSUV; mean ± standard deviation) between baseline [positron emission tomography (PET) 1] and after 4 weeks of sunitinib (PET 2) are grouped according to metabolic response: metabolically progressive disease (mPD, red); metabolically stable disease (mSD, yellow); and metabolic partial response (mPR, blue). **(b)** Corresponding Kaplan–Meier estimates of progression-free survival.
*Source*: Adapted, with permission, from Ref. [56].

time course of induction of effect on the biomarker, followed by a period of normalisation [55]. The time course of the biomarker effect can have a complicated relation to the plasma pharmacokinetics, often not directly reflecting it, and a temporal course that is dose-dependent.

### *Biomarkers of disease*

Disease biomarkers are most often markers of pathophysiological changes linked to the disease. They can be the same biomarker used for assessment of PD response but applied with a different question in mind. Whereas PD biomarkers measure acute responses, disease biomarkers assess sustained effects often judged to be indicative of clinical treatment response. Ideally, a disease biomarker will differentiate treatment response versus nonresponse before changes in tumour volume.

FDG-PET was determined to be predictive of treatment response for sunitinib, a multitargeted tyrosine kinase inhibitor, in patients with gastrointestinal stromal tumours (GIST). Patients were imaged before therapy and 4 weeks after beginning therapy while still on treatment. A response by FDG-PET at 4 weeks correlated with progression-free survival as seen in Fig. 3 [56]. Regulatory agencies do not currently recognise functional imaging measures as surrogate endpoints because of a lack of data. However, these imaging methods can be used to great effect in early clinical trials to support internal decision making around progression of drug candidates.

## Patient selection biomarkers

The final type of biomarker discussed here is the patient selection biomarker, used to identify patients prospectively who are likely to respond to treatment (either to enrich clinical trials or as a companion diagnostic). The presence of a protein or receptor can be necessary for many targeted therapies to have an effect. $^{99m}$Tc-Etafolatide has been used to identify folate-receptor-positive patients. Positivity by $^{99m}$Tc-etafolatide-SPECT correlated with overall response to vintafolide in a Phase II study [57]. In March 2014, Merck and Endocyte announced that the Committee for Medicinal Products for Human Use of the European Medicines Agency issued positive opinions for the conditional marketing of vintafolide and its companion imaging agent in platinum-resistant folate-positive ovarian cancer [58]. Meng *et al.* have also demonstrated that the maximum standardised uptake value (SUVmax) of $^{11}$C-PD153035, an imaging agent for epidermal growth factor receptor (EGFR), had a positive correlation with overall survival and progression-free survival in NSCLC patients treated with erlotinib (Fig. 4). Increasingly, this concept is also being applied to labelled mAbs and combination therapies [59,60].

Important considerations for patient selection methods are cost and technical feasibility. This is especially relevant if the target population is relatively small. To increase the chance of a positive result in early clinical trials, it might be cost-effective to include imaging as a component in the screening. However, the rationale and use need to be carefully considered. An additional motivation can be that the imaging component can aid in the qualification and understanding of other, more readily applied, biomarkers. Having a patient selection strategy is important for increasing the probability of clinical success as treatments are becoming more targeted. As such, options for patient selection strategies should be evaluated for robustness, availability and cost for screening patients.

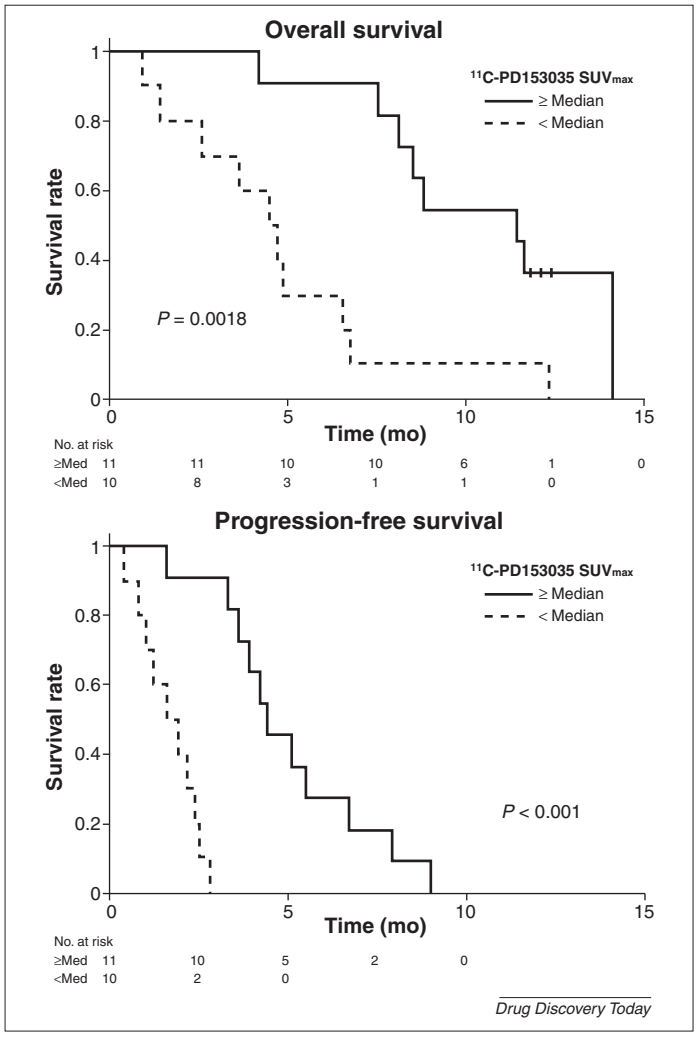


**FIGURE 4**

Kaplan–Meier overall survival (OS) and progression-free survival (PFS) curves stratified by baseline 11C-PD153035 maximum standardised uptake value (SUVmax) greater or less than median value of 2.92. Differences between survivals in high and low SUV groups were highly significant.
*Source*: Adapted, with permission, from Ref. [59].

## Practical considerations

### *Cost*

The inclusion of imaging increases the costs of the trial. These costs include good manufacturing practice (GMP)-qualified production of the radiotracer (if a GMP supply is not already available), generation of an investigational medicinal product dossier (IMPD), site management, scanning and image analysis. However, it remains the case that the costs associated directly with imaging generally make up a small part of the total. For example, if a task is to define a suitable dose range for a drug under development, the use of imaging can often be significantly cheaper and faster than searching for an optimal dose range using other methods that require larger patient cohorts. The dose-finding is especially complicated and expensive for antibody therapeutics where an MTD might be difficult to establish and the dose is limited not when

adverse effects are seen but when the mass of drug reaches a prohibitive amount.

### *Standardisation*

As mentioned above, there are guidelines with respect to standardisation and quality assurance of PET studies with FDG, but not necessarily for less common methodologies. Local technical expertise for relevant imaging subspecialties (e.g., radiochemistry, radiography, physicists, etc.) should be considered when choosing sites. For the more common types of imaging methodologies it might be reasonable to engage an imaging contract research organisation (CRO) for support, but more technically complex studies might be outside of the core competence of such companies. For these more-advanced studies, it might be rational to utilise only one site to avoid differences between sites in image acquisition and analysis and radiotracer production, but this should be balanced with the need for a sufficient recruitment rate.

### *Availability*

The more common types of imaging methodologies, such as FDG-PET, are sufficiently established that a clinical study could be planned based on site experience in a certain tumour type first, considering the imaging component second. However, more-advanced molecular imaging methods are usually confined to only a few sites and the studies need to be placed where the imaging is available and of sufficient quality.

### *Power*

Clinical trials utilising molecular imaging are often carried out as a subgroup of patients from a larger trial or as a small standalone experimental medicine study. The reasons for this include high costs, desire to limit imaging to selected sites and inclusion criteria that might limit patient recruitment rates. Standard statistical criteria for determination of power of the imaging cohort are usually not applied. Imaging can be quantitative with a high precision when applied as a comparison of drug treatment compared with baseline within each individual. Typical test–retest values show variability expressed as standard deviation of ~15% or less [61]. Thus, for these approaches technical variability is typically not the prominent factor if imaging acquisition and analysis is standardised. Instead, the statistical question as with most clinical trials is related to the variability of the disease and pharmacology, which is often unknown before the study and cannot therefore be accounted for in formal power calculations. For imaging trials performed in small populations, using a novel drug, and to support internal company decision-making rather than regulatory agency decision-making, it is often considered acceptable to specify the statistical description of data.

## Concluding remarks

The potential applications of translational imaging to oncology drug development are numerous. Biodistribution studies can be used to understand drug distribution, tissue kinetics and potential target exposure. Pharmacodynamic markers can be used to inform dosing regimens and confirm drug mechanism-of-action. In addition, as the way we treat cancer changes, imaging is likely to play an important part in applications such as demonstrating the delivery of biologically active macromolecules to the tumour(s) and distinguishing between potentially lethal cancers that require aggressive treatment, as well as nonlethal cancer which can instead be managed chronically.

## Conflicts of interest

Sarah Mudd, Robert Comley, Kyle Holen, Yanping Luo, Gerard Fox and Laurent Martarello are employees of AbbVie. John Beaver is an employee of Biogen. Sabin Carme is an employee of Merck. Mats Bergstrom is a paid consultant of AbbVie. AbbVie participated in the review and approval of the publication.

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