TeaserThe key to applying targeted imaging to personalized medicine is the choice of a radiolabeled probe for a disease control point.

Targeted imaging: an important biomarker for understanding disease progression in the era of personalized medicine

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The key to applying targeted imaging to personalized medicine is the choice of the right radiolabeled probe for the right target for the right disease following the lead of pharmaceutical development. The imaging approach differs depending on whether the target is a single disease control point (e.g. a specific receptor or transport protein linked to the mechanistic activity of a drug) or a general disease control point applicable to a number of treatment paradigms (e.g. proliferation, angiogenesis, inflammation). But in either case, the number of control points must be small given the time constraints on molecular imaging procedures in the clinic. Regardless of the choice, the radiotracer must be validated as binding to the target with the appropriate pharmacokinetics and pharmacodynamics for effective external imaging. Such an imaging agent developed in concert with drug development has a built in synergy that will accelerate the drug development process, targeted imaging and personalized medicine as well.

Introduction

Several publications in *Drug Discovery Today* have described the use of biomarkers including molecular imaging in personalized medicine [1,2]. The term 'personalized medicine' emerged in the late 1990s on the basis of progress in the Human Genome Project. Other terms for the same concept, such as integrated medicine, theranostics, pharmacodiagnostics and diagnostic/therapeutic (Dx/Rx) partnering, are similar in that they address the use of detailed information about a patient's genotype or level of gene expression and a patient's clinical data to select medication, therapy or preventative measures that are particularly suited to that patient at the time of treatment administration [3]. The benefits of this approach are in its accuracy, efficacy and safety. There are numerous biomarker approaches other than molecular imaging such that a case needs to be made for the time and place that imaging is most effective. Nunn [4] has recently reviewed the FDA's role in personalized medicine and their involvement to date.

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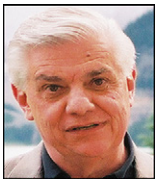

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Dr William C. Eckelman is presently an Adjunct Professor at UCSD, Chair of the Scientific Advisory Board at Molecular Insight Pharmaceuticals, Inc. and the CSO of Molecular Tracer LLC, which was formed in mid-2004. He began his research in ^{99m}Tc radiopharmaceuticals with the development of 'instant kits'. In receptor-binding radiotracers, Drs Eckelman, Reba and colleagues performed the first neuroreceptor image in humans in 1983. As the Vice President at the Squibb Institute, Dr Eckelman led the group that developed the Sr-82/Rb-82 generator, [^{99m}Tc]teboroxime and Prohance. Translational efforts include ligands for the estrogen, opioid, dopamine, muscarinic and serotonin receptors. In addition, Dr Eckelman has been the Editor-in-Chief of Nucl. Med. Biol. since 1985 and has published over 400 research papers and book chapters.

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GLOSSARY

Molecular imaging Molecular imaging is the visualization, characterization and measurement of biological processes at the molecular and cellular levels in humans and other living systems. Molecular imaging typically includes two- or three-dimensional imaging as well as quantification over time. The techniques used include radiotracer imaging/nuclear medicine, magnetic resonance imaging (MRI), magnetic resonance spectroscopy (MRS), optical imaging, ultrasound and others.

Targeted imaging External imaging of ligands targeted to specific protein expression products such as receptors, enzymes and antigens.

Nuclear medicine A subspecialty of medicine based primarily on the use of radioactive substances in medical diagnosis, treatment and research. In a typical examination a radioactive tracer is given, usually by injection intravenously, and the distribution of the radioactive substance within the body or a part of the body is portrayed in a series of nuclear images. The imaging is based on the emission of gamma rays by the tracer substance that passes out of the body and is recorded by a scintillation camera.

Nuclear medicine imaging External imaging using the techniques of nuclear medicine. The information is calibrated to provide concentration in units of radioactivity per volume. The volume is often referred to as a voxel (volumetric and pixel) representing a value on a regular grid in three-dimensional space. This is analogous to a pixel, which represents two-dimensional (2D) image data. While positron emission tomography (PET) imaging is the gold standard for quantitative imaging, single photon emission computed tomography (SPECT) imaging may yield reasonably accurate concentration estimates with further refinements in reconstruction algorithm design and measurement technology.

Radiotracer A radioactive substance, present in subpharmacologic doses, to monitor a biochemical pathway by measuring the emitted radioactivity. This term was originally introduced as the use of radioelements as indicators.

Radionuclide A radioactive nuclide – an unstable form of a chemical element that decays by emitting particles, gamma radiation or X-radiation.

Radioisotope An unstable isotope of an element that decays by emitting particles, gamma radiation or X-radiation. The term, 'radioisotopes', is limited to unstable atoms of the same element.

What impact can targeted imaging have on personalized medicine?

Although noninvasive targeted imaging is not explicitly addressed in the definition of personalized medicine, it is proposed as being capable of characterizing specific phenotypes on the basis of its capability to track molecular events, the key to the disease process. For example, targeted imaging has the advantage of defining biochemistry and physiology noninvasively in humans in situations where:

- Phase O (exploratory) target occupancy studies can optimize the drug dose and timing
- Whole body surveys are needed (disease staging)
- Repeat studies using the subject as its own control are beneficial (monitoring therapy).
- An additional advantage is that nuclear imaging can determine pharmacokinetics and pharmacodynamics without disrupting the body's biochemistry and physiology. It is unique in the use of the tracer principle and the concept of the magic bullet, which were developed in the early 1900s.

Nuclear medicine is based on the tracer principle and a targeted molecule/magic bullet

George Charles de Hevesy is usually considered the first one to identify the tracer principle, which is the basis of nuclear imaging [5]. In 1923, he used 10.6-hour half-life lead-212 to study the uptake of solutions in bean plants. Although lead was generally considered toxic at the level needed to detect it with laboratory instruments of the era, he was able to use small, nontoxic amounts of the radioisotope of lead because of the sensitivity of the detection technique. The following year he carried out the first experiment in animals using Bi-210 to label and follow the circulation of Bi in rabbits after intramuscular injection of bismuth-containing antisyphilitic drugs. In a book co-authored with Fritz Paneth [6], the tracer method was introduced with the use of radioelements as indicators.

Paul Ehrlich coined the expression 'magic bullet' [7,8]. The German word 'Zauberkugel' appeared earlier in his publications, on the basis of his view of 'side chains', the precursor of the concept of receptors, and on the desirable properties of drugs that do not harm the host but do attack the parasitic invader. Ehrlich's first magic bullet, arsphenamine (Salvarsan), was discovered in 1909 that provided cure only for syphilis at that time. Obviously, arsphenamine was developed without the benefit of knowing the target or being able to image a target *in vivo*. Today, the target is known and external imaging can be used to monitor the reaction of the drug with its target [9].

On the basis of these two principles, many radiotracers have been developed over the past 50 years for imaging human subjects. The experience in applying these two principles in the pregenomic era has enabled investigators to incorporate the advances of the postgenomic era rapidly. Techniques for developing radiotracers do not differ between the pregenomic and postgenomic era, but the approach of choosing the targets has altered. Most of the leads for targeted radiotracers from the pregenomic era were based on autopsy data and drug efficacy studies, whereas in the postgenomic era the increase in targets and potential drugs to bind to these targets (druggable targets) comes from the understanding of genomics, proteomics and single nucleotide polymorphisms (SNPs). Autopsy studies are still mentioned in the literature as a rationale for target choice in introductions on radiopharmaceutical design, but are obviously not an ideal source of targets for early disease detection. The pregenomic era is not without its successes. One of the most successful radiotracers today, F-18 Fludeoxyglucose, was developed in the pregenomic era, based on the understanding of the biology elucidated by Sokoloff *et al.* using C-14 deoxyglucose [9]. Radiotracers from both of these approaches are based on targeted imaging that parallels the magic bullet concept in the world of biomarker identification and development for disease pathogenesis.

Comparing imaging techniques: the mass associated with radionuclides

There are now a number of imaging techniques that can be used for the external detection of either anatomical or physiologic

TABLE 1

The advantage of radionuclides in targeted imaging *in vivo*, especially low density sites (<20 nM)

Imaging modality[a]	Concentration at the target	Resolution
For PET, 2–10 nmol/70 kg	<0.1 nM	1–2 mm (animal); 4–6 mm (human)
For SPECT, <2 nmol/70 kg	<0.03 nM	1–2 mm (animal); 4–12 mm (human)
For Gd MRI	10–100 μM	25–100 μm (animal); 0.5–2 mm (human)

[a] Positron emission tomography (PET), single photon emission tomography (SPECT) and magnetic resonance imaging (MRI).

changes in animals [10] and humans [11] (Table 1). The anatomical techniques are important for determining the size, shape and position of abnormalities with high spatial resolution, for example, the longest dimension of the tumor as measured by CT or magnetic resonance imaging (MRI) is still the gold standard for solid tumor response to treatment (RECIST) [12]. The nontargeted agents, those distributed in or excreted by high capacity systems (e.g. blood pool, kidney, liver and bone), have been used to better define either blood flow or tissue permeability, or the excretory processes.

The targeted radiotracers are generally considered as those radioligands binding to well-defined receptors, enzymes and other protein sites [13]. The separation between targeted and nontargeted radioligands is arbitrarily given with the broad range of target densities. In general, receptors are present at the lowest density, followed by enzymes, which are most often detected using inhibitors rather than substrates. It is with these low density targets that nuclear imaging has a distinct advantage. There are exceptions, however, for example the asialoglycoprotein receptor in the liver, which is present at a high density (~500 nM) to permit the use of MRI contrast agents [14,15]. These generalities apply mainly to mechanisms where first-order kinetics prevail, that is, where cellular metabolic trapping of enzyme substrates is not a major component of the biochemical localization or where cell surface internalization is not a predominant mechanism during the time of measurement. In these cases, more mass can be localized in the target, and care must still be taken to avoid pharmacologic effects. For example, fluorine-19 can be detected externally using MRI. The glucose analog, 2-[^{18}F]fluoro-2-deoxyglucose (FDG) is commonly used to measure a parameter related to glucose metabolism at human doses of 10–20 mCi (370–740 MBq) and specific activities from the original 1 Ci/mmol (37 GBq/mmol) to the present 5000 Ci/mmol (185 TBq/mmol) at the end of synthesis. If 2-[^{19}F]fluoro-2-deoxyglucose is used with MRI the amount of glucose analog needed to give an external image causes severe pharmacologic effects [16]. Likewise, to measure the metabolism of fluorouracil using MRI requires a reduction in the spatial and temporal resolution and an increase in the given dose beyond the amounts of fluorouracil used routinely in chemotherapy [17]. Another example, which is one of the first proofs of principle for targeted MRI, investigated the detection of the transferrin receptors by external imaging using the T2 agent, iron oxide, linked to transferrin. To obtain an external image of mouse tumors, the transferrin receptor was genetically upregulated and the feedback loop was blocked to prevent the accumulation of excess iron. Indeed, *in vivo* imaging of a mouse bearing a transferrin-receptor-containing-tumor identified the tumor by external MRI, but not the control tumor. This landmark publication set the parameters for subsequent efforts in targeted MRI [18].

The experience with the allometric effect on percentage injected dose per gram of tissue (%ID/g) going from rodents to man with radiolabeled antibodies suggests that the injected mass for a targeted MRI contrast agent–antibody conjugate will need to be increased in humans over the amount used in rodents to obtain external images [19].

Validation of radiotracers

Because nuclear imaging records gamma ray emissions and not chemical structure, the new radiotracer must be validated as binding to the target with specificity and selectivity representative of the parent compound such that the recorded gamma rays reflect the biodistribution of the parent compound at the target protein. Furthermore, the radiotracer needs to be shown to be sensitive to changes in the target protein as a function of disease. A biomarker of personalized medicine must be monitoring the 'personalized' target protein by external imaging in spite of the complications set forth by metabolism, protein binding, and flow and permeability changes. This rigor is demanded of all biomarkers if they are to be involved in the treatment decision.

In vitro and *in vivo* competitive binding experiments

An often-raised concern in radiopharmaceutical design is whether the measurement of an *in vitro* affinity constant will define the necessary, but not sufficient, potential of a targeted radiotracer *in vivo*. This is only part of the question because an affinity constant cannot be evaluated without considering the target density [i.e. the equilibrium association affinity constant (K_a, in nM^{-1}) or the reciprocal of K_a, the dissociation constant (K_d in nM) must be combined with the target density (in nM) for a meaningful prediction]. The receptor density from *in vitro* or *ex vivo* experiments is often quoted in femtomoles per milligram of protein, or in picomoles per gram of wet tissue weight. Because tomographic imaging is a voxel measurement, the conversion of femtomoles per milligram of protein to a volumetric density is often carried out assuming 10% protein per gram of tissue.

In the early 1980s, when external imaging using a receptor-binding radiotracer was initiated in humans, the main design goal was to find radioligands with the highest affinity [20]. By the mid-1980s, a number of receptor-binding radiotracers had been identified and studied in humans (Table 2). At that time, the proposed model for choosing compounds and for predicting the maximal bound-to-free (B/F) ratio *in vivo* was borrowed from the *in vitro* analysis of receptor binding. It used equilibrium equations where higher association affinity constants (K_a) and higher receptor concentrations (B_{max}) give higher B/F ratios, which represent maximum target-to-nontarget (T/NT) ratios that are rarely met *in vivo* [21]. Of course, these *in vitro* measurements are made in the absence of metabolites, involved very low nonspecific binding (because it can be subtracted) and were conducted under true equilibrium conditions in which the amount of added ligand

TABLE 2

The year of the publication of the first human studies of enzyme/receptor targeting in humans using PET and SPECT

Date	PET	SPECT
1977	2-[^{18}F]fluoro-2-deoxyglucose	
1983	[^{11}C]*N*-MeSpiperone	3-Quinuclidinyl-4-[^{123}I]iodobenzilate
1983	[^{18}F]FDOPA	
1984	[^{18}F]Cyclofoxy	
1985	[^{11}C]Raclopride	[^{99m}Tc]Neoglycoalbumin
1985	[^{11}C]Carfentanil	
1985	[^{11}C]Flumazenil	
>1985	Many targeted radiotracers	Many targeted radiotracers

relative to the number of binding sites was carefully and systematically controlled. Furthermore, delivery and permeability are not confounding factors *in vitro*. Therefore, *in vitro* B/F ratios provide best-condition values, which have never been achieved *in vivo* [22]. When different volumes of distribution were considered (i.e. the receptors were in a small volume and the radioligand was in a larger volume, usually the extracellular fluid) the equilibrium equation was a more complicated quadratic equation [23].

What then is the value of determining a maximal B/F ratio *in vitro*? It can certainly be used to dismiss certain radioligands that, due to a combination of an affinity constant and a receptor density, will not allow external imaging of the target. In other words, the use of these *in vitro* equations can be useful as a necessary criterion, but is far from a sufficient criterion.

The relationship between affinity constants given varying transporter densities are well described by Kung and Kung [22] who discussed the inhibitors of three transporters: dopamine transporter (DAT), serotonin transporter (SERT) and norepinephrine transporter (NET) (Table 3). Of the three transporters analyzed, NET has the lowest transporter density in the brain and, therefore, requires a lower K_d compared to SERT, which has a higher transporter density. DAT is one of the highest density targets identified for external imaging and external imaging can still be effective with binding as weak as a K_d of 10 nM. For [^{18}F]fallypride, the B_{max}/K_d determined *in vitro* (which ranged from a high of 900 to a low of 30) was observed *in vivo* to be in the range from 24 to 2 [24]. This reflects the extra complexity *in vivo* compared to the experiments *in vitro* where metabolism, protein binding, flow and permeability are not confounding factors.

The use of gene-manipulated (knockout) mice to support subtype specificity

The traditional pharmacologic proof for a receptor subtype binding radiotracer (i.e. the use of increasingly larger doses of subtype-specific drugs) can be unconvincing if the blocking doses cause changes in blood flow, permeability, metabolism or any other pharmacokinetic property that alters biodistribution. The use of knockout mice has been very effective in predicting and validating targeting at a receptor subtype, but has been less effective in serving as a model for disease. Zambrowicz and Sands [25] reviewed drugs with the largest market size in 2001 and found that they targeted 43 proteins out of the 500 or more druggable proteins identified in the pregenomic era. Over 20 radioligands have been validated using knockout mice to determine subtype-specific receptor binding [26].

Radiolabeled 3-(3-(3-fluoropropyl)thio)-1,2,5-(thiadiazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyridine ([^{18}F]FP-TZTP) provides an example of the value of the knockout mice model because the muscarinic-2 (M2) receptor subtype is distributed homogeneously throughout the gray matter and there are no high-affinity agonists that can be used to block the binding. Furthermore, competitive binding studies using nonradioactive agonists can produce pharmacologic changes [27].

Given that large decreases in [^{18}F]FP-TZTP brain uptakes were seen only in M2 knockout versus wild-type (WT) mice, but not in knockout mice of other receptor subtypes, [^{18}F]FP-TZTP preferentially labels M2 receptors *in vivo*. Knockout mice are a valuable tool for validating the subtype selectivity and hold great promise for accelerating radioligand validation [28].

Tissue with and without measurable target density

One of the key methods to determine target density is to incorporate the ratio of the target radioactivity to tissue without measurable target density. In many cases, the cerebellum does not contain the targeted protein, for example, it is devoid of dopamine D2 and 5-HT$_{1A}$ receptors. In this case, the cerebellum can be used as a reference tissue and a decrease in target brain region to cerebellum as a function of increased nonradioactive ligand for

TABLE 3

The affinity constants, transporter densities and B_{max}/K_d for transporters in the rat brain (from Ref. [7])

	B_{max} fmol/mg protein (nM)[a]	K_d (nM)	Compound	B_{max}/K_d
DAT	2000 (200) [rat striatum]	10	[^{99m}Tc]TRODAT	20
SERT	194 (19.4) [rat frontal cortex]	0.13	[^{125}I]ADAM	149
NET	55 (5.5) [rat frontal cortex]	0.05	2-[^{125}I]INXT	110

TRODAT: technetium, 2-[[2-[[[3-(4-chlorophenyl)-8-methyl-8-azabicyclo[3.2.1]oct-2-yl]methyl](2-mercaptoethyl)amino]ethyl]amino]-ethanethiolato(3-)-oxo-[1*R*-(exo–exo)]. ADAM: 2-[2-(dimethylaminomethyl-phenylthio)]-5-[^{125}I]iodophenylamine. INXT: (*R*)-*N*-methyl-(2-[^{125}I]iodo-phenoxy)-3-phenylpropylamine.

[a] Assuming 10% proteins per gram of tissue.

the same receptor validates the specificity and saturability of binding to the receptor [29,30].

Extracting biochemical information from gamma emissions *in vivo*: mathematical analysis of radiotracer biodistribution

Because positron emission tomography (PET) and, to an increasing extent SPECT, are quantitative imaging techniques that are capable of transforming collected gamma rays into quantitative terms (Ci/voxel or TBq/voxel), it is important to further transform these data into biochemical parameters. This is especially important for studies that assess target protein densities. Many publications propose detailed mathematical approaches to extract these biochemical parameters. One such approach starts with mathematical modeling and compartmental analysis and ends with the single scan technique [31], which illustrates the path from validation of radiotracers to the requirements for clinical use. There are two popular plotting methods named after the senior author developing the approach. The 'Patlak' plot was developed for radioligands that tend toward irreversible binding [32], whereas the 'Logan' plot was developed for radioligands that tend toward reversible binding [33]. If the binding to the protein target is much larger than the efflux from the tissue, then the binding in the target tissue can be due to perfusion or permeability, but not due to protein target density. If the goal is to develop a radioligand to measure protein target density, the efflux from the tissue must be faster than the binding to the target protein. Both the Patlak and Logan plot approaches have been adapted to use reference regions with little or no target protein rather than unmetabolized plasma, which requires rapid blood drawing and sophisticated chromatographic separations with minimal radioactivity.

To advance to an efficient and cost-effective clinical procedure a single scan technique that gives a measure of the density of target tissue or enzyme activity must be developed. Hoh [34] presented this as a key criterion for clinical acceptance of Fludeoxyglucose F-18 injection as a diagnostic radiotracer. Two examples of the evolution from compartmental analysis to the single scan technique are Fludeoxyglucose F-18 injection for PET and beta-CIT I-123 injection for SPECT. Both have undergone extensive pharmacokinetic analysis in animals and humans and both are now used in the clinic with single scans at times chosen from the pharmacokinetic analysis to give a relative measure of glucose metabolism and dopamine transporter density, respectively.

Separating flow, permeability and target protein binding

In vitro experiments are more straightforward to interpret than *in vivo* experiments because binding is the only metric being measured and flow and permeability are not encountered. The separation of flow, permeability and target protein binding is an important consideration when analyzing the biodistribution of a new radiotracer. In one case, the radiolabeled control protein F-18 albumin (Alb) outperformed the new radiopharmaceutical F-18 labeled transferrin (Tf) in targeting Tf-receptor-containing tumors. Tumor Tf uptake values remained below those of the Alb tracer, and tumor-to-blood ratios of [^{18}F]Tf in each xenograft increased in parallel with those of the Alb tracer. The tissue permeabilities of [^{14}C]Alb and [^{18}F]Tf in LS1747T were calculated to be 1.29 ± 0.49 and 1.03 ± 0.38 μl/(min g) (mean ± SD), respectively, whereas the tissue permeabilities of the two tracers in A431 tumors were $0.79b \pm 0.24$ and $0.44b \pm 0.04$ μl/(min g). Pharmacokinetic modeling of the data showed that the observed uptake values in the two xenografts are consistent with a nonreceptor-mediated distribution. In the liver the biodistribution is controlled by specific [^{18}F]Tf binding to receptors compared with the [^{14}C]Alb control because of the absence of permeability barriers. Accumulation of [^{18}F]Tf in tumors, by contrast, is controlled by permeability up to six hours postadministration.

Applications of molecular imaging to drug discovery and patient outcome: target protein occupancy studies in humans

The appropriate pharmaceutical dose to study clinically or to individualize the dose after approval can best be determined by carrying out occupancy studies with either a radiolabeled form of the drug or with another radiolabeled compound that binds specifically to the same site. The saturation of the target site is best determined in humans using either PET or SPECT, especially for drugs that are targeted to the central nervous system (CNS). The optimal technique is to titrate the free receptor before and after a dose of the drug. With the use of several dose levels, the saturation of the target protein can be determined quantitatively and a dose that balances efficiency and toxicity can be chosen. There are several pharmacokinetic situations where neither the plasma levels nor indirect measurements, such as electroencephalogram (EEG) and functional MRI (fMRI), will reflect target occupancy of the drug. Nevertheless, the radiotracer must be validated, as outlined in previous sections, and assurances must be available that the radioactivity at the target reflects binding between the drug and a single target protein. There are examples in the literature where a drug can bind to more than one target, but unless they are anatomically separated the analysis of the radioactivity distribution is complicated.

The nuclear imaging technique is best suited for drugs targeting a single protein. This technique can also be used to measure the half-life of the drug at the target by using serial determinations of the free target density. Passchier *et al.* have outlined the properties of the ideal radioligand and various methods of calculating the drug-related target protein occupancy [35]. Many radiopharmaceuticals, especially those designed for CNS applications, have been used in occupancy studies but fewer have been used to predict clinical outcome. For example, there have been a number of clinical studies using 3-*R* quinuclidinyl 4-*S* [^{123}I]iodobenzilate (*RS* IQNB) that suggest the ligand is responsive to changes in receptor concentration in normal subjects and patients with Alzheimer's disease. Nevertheless, the primary clinical use of RS [^{123}I]IQNB has been to rule in or rule out binding to the muscarinic receptor. Examples in the literature document the interaction of olanzapine, risperidone, clozapine, donepezil and phenserine with the muscarinic receptor [36].

Reductionist approach to imaging for single gene diseases and a single control point

For more than 50 years, radiopharmaceutical scientists have targeted specific proteins and therefore conducted mechanistic targeting, a reductionist approach defined by Sams-Dodd [37]. The

first reference given for nonradioactive-targeted probes is attributed to Paul Ehrlich. His concept, however, was derived from a toxicology point of view rather than a specific target-to-nontarget advantage needed for successful external imaging of a radioligand [7].

Although those involved in genomics research correlate several SNPs with a disease, molecular imaging is usually limited to monitoring one or two protein expression products because of the strict limits on administered radioactivity and the time involved in sequential studies. SPECT may have an advantage because single photon emitting radionuclide emissions can be separated based on their different photon energies and, therefore, simultaneous study of two or more protein expression products is a technical possibility.

Molecular imaging with radiopharmaceuticals has a long history of developing radiolabeled compounds for specific targets at the molecular level using pregenomic techniques to identify the target include biochemical probes such as iodide (~50 years), receptor-binding radiotracers and radiolabeled monoclonal antibodies (both ~25 years). The progress in proteomics and genomics has led to the discovery of targets that are important in early disease detection. Radiolabeled probes for genes, mRNA, antisense and protein–protein interactions have been prepared and studied *in vitro* and *in vivo* in animals, but it is the reporter gene approach that has progressed the furthest toward clinical studies [38,39].

These advances have led to four major approaches for developing radiopharmaceuticals for use with pharmaceuticals:

(i) Monitoring general disease control points such as proliferation, hypoxia, apoptosis, angiogenesis, inflammation and metastasis.
(ii) Monitoring the targeting of a therapeutic radiopharmaceutical with a radiolabeled imaging pharmaceutical for the same target.
(iii) Using external imaging to monitor the same control point that the pharmaceutical companies are targeting.
(iv) Monitoring a downstream biochemical process affected by the drug.

The first approach is analogous to the pharmaceutical approach of developing a blockbuster such as an angiotensin-converting enzyme (ACE) inhibitor or a statin that is therapeutically effective in a wide range of diseases and patients, as opposed to developing a targeted molecule that is effective in a small subpopulation of patients. The latter three approaches represent so-called individualized or personalized medicine that is the hope of higher therapeutic efficiency.

If a single disease control point is identified as the drug target, then a single control point will be available as a target for the imaging agent. This reduces drug targeting of an organism to drug targeting a single protein expression product. This is clearly a reductionist approach to drug discovery, driven by the explosion in the identification of new targets and additional information in the postgenomic era. Moreover, because most nuclear imaging procedures are limited to one or two radiotracer studies per patient per session, the reductionist concept of drug development is ideal as an imaging approach and follows implicitly what radiopharmaceutical scientists have been pursuing for many years. In addition, following the lead of the pharmaceutical companies increases the probability that the radiotracer will have an impact on clinical studies [26]. Clearly, there are still lesions to be learned from drug development, but there is a clear convergence of goals [40]. It is safe to say that earlier radiopharmaceutical design based on tissue samples obtained during an autopsy did not often lead to clinical use, especially in complicated neurological and psychiatric diseases.

Monitoring general disease control points

Imaging of general disease control points such as proliferation, hypoxia, apoptosis, angiogenesis, inflammation and metastasis is more advanced compared to targeting a single receptor, enzyme or transporter, although the first generation of radiopharmaceuticals probably will undergo further validation and refinement in terms of pharmacokinetics, pharmacodynamics and sensitivity (Table 4). It is important that the research efforts demonstrate that radiotracers labeled with either a metallic radionuclide or a radiohalogen undergo a thorough validation to demonstrate what biochemical is traced by the analog. Krohn *et al.* [41] define this validation as an ongoing process given that using a fluorinated analog as a tracer for a specific biochemical such as glucose or thymidine is susceptible to nonparallel changes in flow, permeability, metabolism, flux, receptor density and the associated binding rate constants for the analog compared to the parent compound. This has not been investigated uniformly for F-18 labeled radiotracers and is seldom addressed for radiometallic tracers.

In oncology these represent fundamental properties of neoplasia, rather than a single protein expression product [42]. Although F-18 Fludeoxyglucose is the radiopharmaceutical most often used in treatment planning for high-precision radiotherapy, Lecchi *et al.* [43] describe a series of radiotracers that have been used in treatment planning, but more often in response assessment (Table 4).

Groves *et al.* [44] have reviewed PET radiotracers including those proposed for radiotherapy by Lecchi *et al.* [43]. This class of radiotracers measures molecular characteristics of specific tumors, their

TABLE 4

Examples of radiotracers used specifically in concert with high-precision radiotherapy

Radiotracer	Mechanism	Disease target
[^{18}F]Choline, [^{11}C]choline	Lipid metabolism	Prostate cancer (CA)
[^{11}C]Methionine	Amino acid metabolism	Brain tumor
[^{18}F]Fluoromisonidazole	Hypoxia marker	Head-neck and lung CA
[^{11}C]Acetate	Cell proliferation	Head-neck and prostate CA
3′-Deoxy-3′-[^{18}F]fluorothymidine (FLT)	Cell proliferation	Rapidly growing CA

biological signature and the extent of post-therapy response. Groves *et al.* include the study of osteoblast metabolism using F-18 fluoride and Tc-99m MDP, and [^{18}F]fluoro-L-dihydroxyphenylalanine ([^{18}F]FDOPA) in neuroendocrine tumors. Studies have shown that [^{18}F]FDOPA is a useful tracer for detecting primary and metastatic carcinoid tumors and low-grade brain tumors. The same tumors can contain a variety of somatostatin-subtype (STT) receptors. Examples of such somatostatin radioligands include ^{68}Ga-DOTATATE and ^{68}Ga-DOTATOC, which target the SSTr2, SSTr4 and SSTr5 receptor subtypes. They also include an estrogen receptor binding radiotracer, 16a-[^{18}F]fluoroestradiol ([^{18}F]FES), which binds specifically to estrogen receptors in patients with breast cancer. This radiotracer can be used before and after aromatase inhibitor therapy. Additional PET-tracer markers of amino acid metabolism have been developed. *In vivo* evidence from animal studies shows that [^{18}F]fluoromethyltyrosine and [^{18}F]fluoroethyltyrosine can successfully image tumors. Data from human studies have also become available that show that [^{18}F]fluoroethyltyrosine uptake predicts poor outcome in patients with glioma [45].

Monitoring the targeting of a therapeutic radiopharmaceutical with a radiolabeled imaging pharmaceutical for the same target

If the therapeutic radiopharmaceutical truly occupies a small percentage of the protein target, which will occur if the therapeutic radiopharmaceutical is at or near the theoretical-specific activity, the diagnostic radiopharmaceutical can be used not only to judge if the biodistribution is favorable for therapeutic studies but also to monitor the effect of the radiotherapy after treatment using reduced-binding sites as the metric.

Over the past two decades, Wieland's group at the University of Michigan has been developing radiotracers that can be used noninvasively to assess cardiac sympathetic innervation in the living human [46]. Sympathetic neuronal imaging agents have been developed to monitor different aspects of the norepinephrine pathway. [^{131}I]- or [^{123}I]-*meta*-iodobenzylguanidine (MIBG) is taken up more efficiently by the vesicles than [^{11}C]-*meta*-hydroxyephedrine (HED) via the vesicular monoamine transporter (VMAT), but HED is released from the vesicles more efficiently. Neither are substrates for monoamine oxidase (MAO). These studies were carried out using the heart as the target and not neuroendocrine tumors [47].

Since the early 1980s, radiolabeled forms of MIBG have been more widely used for the diagnosis and radiotherapy of various neuroendocrine tumors, such as neuroblastoma and pheochromocytoma. Clinical studies using commercially available [^{131}I]MIBG demonstrate that it undergoes little *in vivo* metabolism with most of it being excreted, unchanged, in the urine. This rapid clearance from nontarget tissues makes it an excellent agent for imaging and radiotherapy [48]. A [^{131}I]MIBG or [^{123}I]MIBG imaging study is performed before the [^{131}I]MIBG radiotherapy study to assure the presence of the target protein, the NET.

Somatostatin binds to the five subtypes of the somatostatin receptor. This receptor is upregulated on neuroendocrine tumors and this upregulation leads to a signal to background ratio that can be detected by external imaging. It also enables tumor selectivity in therapeutic applications. The cyclic peptide octreotide is the analog most frequently labeled with metal chelates such as [^{111}In]DTPA, [^{68}Ga]DOTA and various ^{99m}Tc chelates for imaging and [^{177}Lu]DTPA and [^{90}Y]DOTA for therapy, all of which were selective for the SSTR2 with IC_{50} values of <10 nM, whereas native somatostatin binds to all five subtypes with equally high affinity. Because these analogs have neurotransmitter properties they are internalized, which further increases the localization beneficial to imaging and therapy [48,49]. The general design criteria have been to increase renal clearance, but minimize renal retention so that the radiolabeled probe can be used to monitor tumors anywhere in the body at a low absorbed radiation dose. At present, an imaging study using [^{111}In]octreotide is carried out first, followed by nonradioactive octreotide chemotherapy, which is followed by either therapeutic [^{90}Y]DOTA-TOC [50] or [^{177}Lu]DOTA-TATE [51]. Since the presence of NET and SSR appear to be inversely related and depended on cell differentiation, imaging both pathways can be instrumental in choosing therapy.

The American College of Radiology has recently set practice guidelines for [^{90}Y]ibritumomab tiuxetan (Zevalin) and [^{131}I]tositumomab (Bexxar), which are approved by the FDA for radioimmunotherapy of non-Hodgkin's lymphoma [52]. Both antibodies are directed against the CD20 antigen, which is found on the surface of normal and malignant B lymphocytes. [^{90}Y]ibritumomab tiuxetan consists of ibritumomab, a murine IgG1-kappa monoclonal antibody and tiuxetan, which chelates ^{111}In for imaging and ^{90}Y for therapy. [^{131}I]Tositumomab, murine IgG_{2a}-lambda monoclonal antibody, is covalently linked to ^{131}I. The preliminary imaging studies are to determine dosimetry or assess biodistribution before the radiotherapeutic is administered. The package insert for these two radiotherapeutics has guidelines for interpreting the imaging study and these guidelines must be met before the therapy can commence. These two imaging studies differ from [^{131}I]MIBG and [^{111}In]octreotide in that the two radiolabeled antibody imaging studies are designed to protect the normal tissue whereas [^{131}I]MIBG and [^{111}In]octreotide imaging studies are designed to assure the presence of the upregulated tumor target.

Using external imaging to monitor the same control point that the pharmaceutical companies are targeting

In some diseases, blocking a single molecular mechanism is sufficient to obtain a significant therapeutic effect. This target type is not as restrictive as the genetic target but is based on the assumption that there still are situations where a single mechanistic target is controlling the disease process (a control point) [37]. There are several examples of such control points. Gamma camera imaging with ^{99m}Tc-maEEE-Z(HER2:342), an Affibody molecule labeled with Tc-99m, detected HER2-expressing tumors in xenografts at 1-hour postinjection. Coupling of different chelator sequences to Affibody molecules favorably modified the *in vivo* kinetics by increasing the hydrophilicity and introducing more stable chelates for Tc-99m [53].

Imaging can also be used to monitor pharmacologic changes due to treatments such as anthracycline, a chemotherapy drug. De Korte *et al.* imaged upregulated myocardial human epidermal growth factor receptor 2 (HER2) expression following cardiac stress using [^{111}In]DTPA-trastuzumab. [^{111}In]DTPA-trastuzumab scans detected increased HER2 in 50% of the patients shortly after anthracyclines and with nonanthracycline-related heart failure.

[^{111}In]DTPA-trastuzumab scintigraphy after anthracyclines but prior to adjuvant trastuzumab can potentially identify patients susceptible to trastuzumab-related cardiotoxicity [54]. This same radiotracer with DOTA as the chelating agent rather than DTPA (diethylenetriaminepentaacetic acid) has also been evaluated in animal models of HER2 over-expression and found to localize with specificity with high tumor to background ratios [55]. Minibodies have also been radiolabeled and studied in mice with the goal of maximizing the whole body kinetics without kidney retention. However, immunohistochemical staining of kidney proximal tubules indicated that this uptake in kidney is a specific process and will not be easily decreased in the presence of increased tumor uptake [56].

Monitoring a downstream biochemical process affected by the drug

Another approach to radiolabeling a key protein expression product affected by the experimental therapeutic drug requires close collaboration with either a university pharmacologist or a pharmaceutical company. Producing a radiolabeled analog of the drug with the proper validation studies will lead to an important preclinical parameter, namely drug occupancy and also the concomitant duration of drug occupancy. However, radiolabeling an analog of the drug itself will not lead to an understanding of the effectiveness of the drug because this two-variable experiment will make it difficult to interpret the changes in radioactivity at the target protein site. Unless drug washout is assured, something that is not likely to be possible during chemotherapy, changes in the radioactivity at the target site could be caused by changes in the number of binding sites or changes in the drug occupancy of the target. If a change in a downstream expression product is monitored by external imaging, a clearer interpretation of the imaging results is achieved.

The abnormal Philadelphia chromosome was identified in the early 1960s, but not until the early 1970s did improved cytogenetic techniques lead to the demonstration that a translocation occurred between chromosomes 9 and 22. Molecular techniques later identified the critical genes involved as v-abl Abelson murine leukemia viral oncogene homolog (*ABL*) on chromosome 9 and breakpoint cluster region (*BCR*) on chromosome 22. Subsequently,

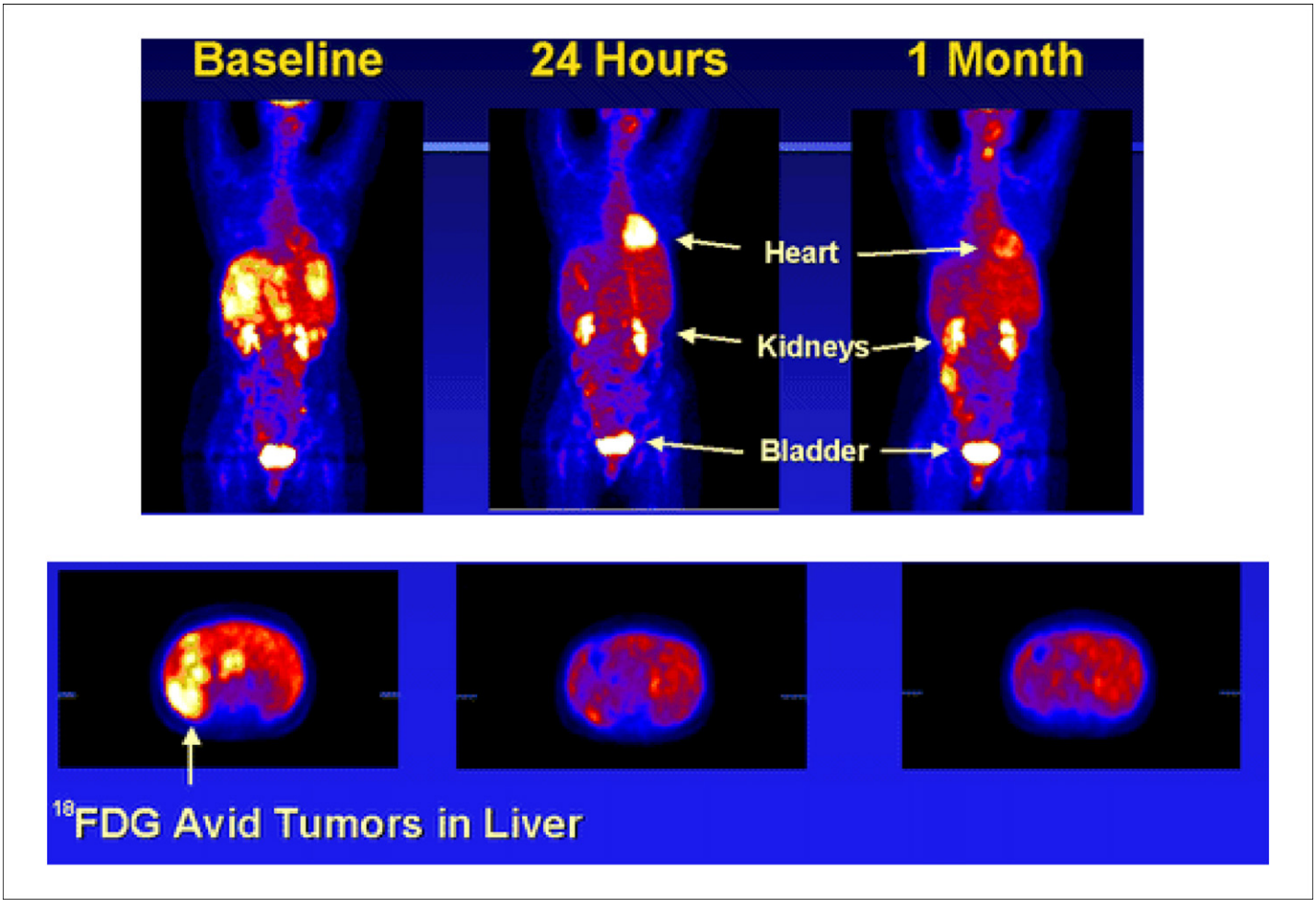

FIGURE 1

In vivo proteomics using F-18 Fludeoxyglucose (FDG) positron emission tomography (PET). Sequential PET scans obtained in the same patient at baseline (before treatment), one day and one month after imatinib treatment began. The images at each point include a two-dimensional PET scan of the body (top) and an axial PET scan of a slice through the site of the pelvic tumor (bottom). The uptake in the cardiac blood pool, the myocardium, the liver, the bowel, the bilateral renal collecting system and the bladder is within physiologic limits in this patient. Images were obtained with the use of similar doses of FDG, acquisition times and protocols at the three time points. The patient also had similar blood glucose concentrations at each of these three time points. Taken from Ref. [60]. Permissions to use material from the *New England Journal of Medicine* or another publication produced by the Massachusetts Medical Society has been granted.

it was shown that the product of the *BCR–ABL* fusion was an abnormal kinase that apparently was the stimulant for the proliferation of myeloid cells to produce chronic myelogenous leukemia (CML) [57]. This then led to the discovery of pharmaceuticals that inhibited the mutant tyrosine kinase by binding to its ATP-binding site.

The bcr–abl translocation in patients with CML and activated mutations in the c-kit tyrosine-kinase receptor in patients with gastrointestinal stromal tumors (GIST) have been treated successfully with the first FDA-approved small-molecule drug for this indication, imatinib (Gleevec™). Imatinib was radiolabeled with the short-lived isotope C-11 to determine the initial biodistribution of imatinib. The radiotracer was cleared rapidly from the plasma, heart, lung and spleen, with a higher and more-sustained concentration in the liver and excretion through the gastrointestinal tract [58]. However, neither C-11 imatinib nor other radioligands that bind to the ATP site have been used in patients to monitor progression of disease. Instead, decreased uptake of Fludeoxyglucose F-18 injection has proven to be an effective predictor of the success of the imatinib therapy. One example of the importance of [^{18}F]FDG as a downstream measure is the early predictive effect of an FDG scan immediately after the start of imatinib chemotherapy [59,60] (Fig. 1). Several studies have shown that the early imaging results with FDG predict treatment outcome although this is not yet an FDA-approved indication [61]. There have been isolated reports of patients with GIST that demonstrated decreased [^{18}F]FDG uptake after treatment but no response. This suggests that treatment with imatinib was sufficient to inhibit [^{18}F]FDG uptake or phosphorylation but was not sufficient to inhibit tumor cell proliferation [62].

Preliminary data suggest that [^{18}F]FDG monitoring may be used for monitoring not only imatinib but also other kinase inhibitors. On the basis of animal studies, Su *et al.* proposed that gefitinib, an epidermal growth factor (EGF) receptor tyrosine-kinase inhibitor should be effective in predicting outcome in nonsmall-cell lung cancer [63]. By contrast, Smith-Jones *et al.* found that, in mice bearing BT-474 breast tumor xenografts and undergoing treatment with heat shock protein 90 (Hsp90) inhibitors, [^{18}F]FDG distribution was not instructive compared to Ga-68 radiolabeled F(ab′)$_2$ fragment of trastuzumab (Herceptin™) [64]. Dimitrakopoulou-Strauss proposed that [^{18}F]FDG may be a promising method for monitoring the therapeutic effect of mTOR inhibitors [65]. Clearly, [^{18}F]FDG is not useful in all cancer treatments but has shown great promise in certain situations.

One example for the latter approach of targeting a single control point in a particular disease is the external imaging of HER2 expression as a function of treatment using an inhibitor of Hsp90. 17-Allylaminogeldanamycin (17-AAG) is the first Hsp90 inhibitor to be tested in a clinical trial. This drug induces proteasomal degradation of HER2 by binding to the Hsp90 chaperone protein. The challenge is to monitor this treatment using external imaging. The lead for targeting the HER2 protein comes from the clinical trials of trastuzumab, an antibody for HER2 (also known as erbB-2 and Neu), which is a cell surface glycoprotein with tyrosine-kinase activity. On the basis of the clinical trials, HER2 over-expression is now an entry criterion and amplification/over-expression is predictive for treatment response in breast cancer. Larson's group at Sloan Kettering Memorial Cancer Center has developed an antibody fragment for HER2 radiolabeled with Ga-68. The Ga-68 radiolabeled F(ab′)$_2$ monitors the change in the protein expression product as a function of treatment with 17-AAG. The effectiveness of 17-AAG was demonstrated using small animal imaging in BT-474 tumor bearing mice [66] (Fig. 2). This approach does not monitor the effectiveness of 17-AAG binding to Hsp90 directly, but rather to the protein expression product and therefore does not suffer from the criticism that the change in radioactivity distribution could result from either drug occupancy or reduced number of HER2 proteins per cell or a reduction in the number of cells. Only a combination of the latter two is measured with this approach.

Another occupancy study that is unique to nuclear imaging is the measurement of endogenous neuroreceptor. The concentration of neurotransmitter at the target, either a receptor or transporter, can

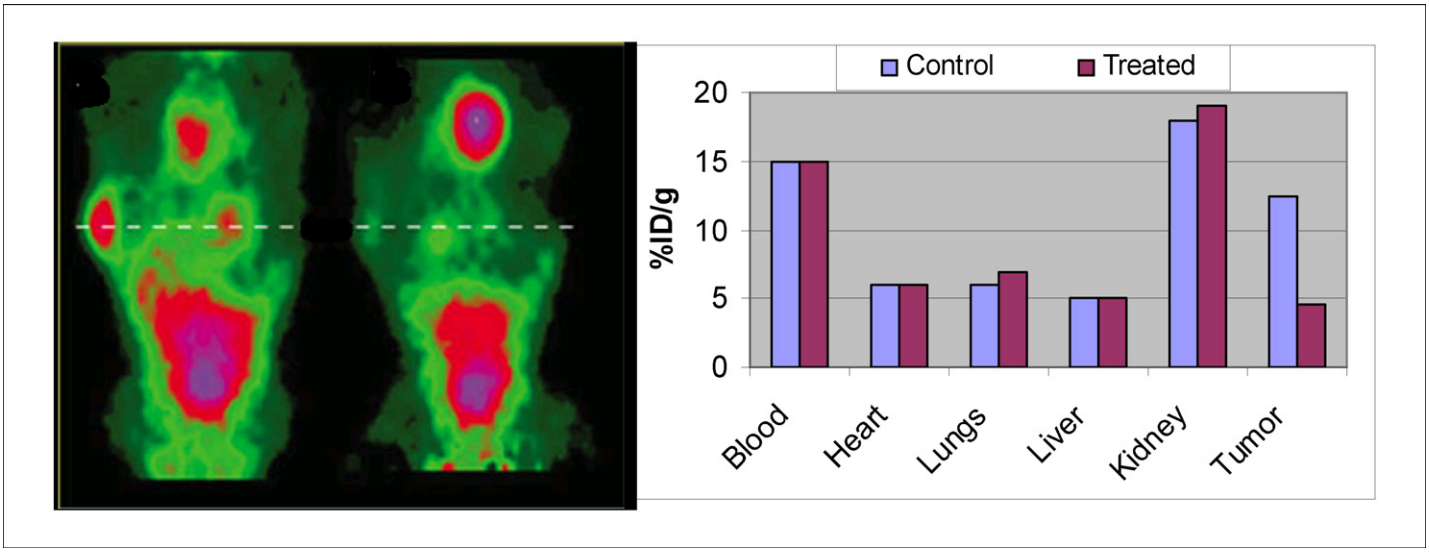

FIGURE 2

MicroPET (positron emission tomography) images. MicroPET images (coronal) of mice with BT-474 tumors with Ga-68-DOTA labeled F(ab′)$_2$ fragment of the anti-HER2 antibody Herceptin™ at 3 hours before and 24 hours after 17-allylaminogeldanamycin treatment (left panel). The quantitative data were obtained from dissection studies performed after the 24-hour image (right panel). Permission to use this figure has been granted by Dr Steven Larson.

be measured indirectly by the competition with a radiolabeled probe for the same target protein. Several radiotracers (e.g. [^{11}C]Raclopride for the D2/D2 receptor and [^{11}C]DASB for the SERT) have been tested in humans and shown to be sensitive to the concentration of neurotransmitter in the synapse. The sensitivity of a M2 receptor specific antagonist, [^{18}F]FP-TZTP (thiadiazolyltetrahydro-1-methyl-pyridine), binding to changing levels in brain acetylcholine (ACh) was assessed in monkeys by administering physostigmine, an acetylcholinesterase inhibitor, by intravenous infusion beginning 30 min before tracer injection. Inhibition of acetylcholinesterase decreases the metabolism of ACh in the synapse, which decreases the binding of [^{18}F]FP-TZTP by competition for the M2 receptor. In a comparison of two groups that were clinically normal at the time of study, Cohen *et al.* found that the gray matter radiotracer concentration for [^{18}F]FP-TZTP was significantly higher in the normal subjects with the APOE-epsilon4+ allele than in the normal subjects without the allele (APOE-epsilon 4−), whereas there were no differences in global cerebral blood flow. Given that [^{18}F]FP-TZTP measures the muscarinic system rather than just receptor density because of its agonist properties and competition with ACh, changes in receptor-binding affinity caused by changes in G-protein binding and/or competition with ACh at the muscarinic receptor can be monitored. A reasonable hypothesis to explain the increased radioactivity concentration in elderly normal subjects with APOE-epsilon 4+ is a decreased concentration of ACh in the synapse, which would lead to higher binding of [^{18}F]FP-TZTP. This type of competition was also shown to be possible in the studies in monkeys using physostigmine. As a result, the use of [^{18}F]FP-TZTP can be considered an *in vivo* measurement of muscarinic systems biology, rather than the receptor density alone [67]. This approach could be used to compare the drugs that block acetylcholinesterase (e.g. donepezil, rivastigmine and tacrine) to measure which one is more efficient in increasing synaptic ACh.

From genome to molecular imaging: oncology as a promising case study

A comprehensive application of the genomic advances should start with the genomic profile or a proteomic profile of a disease and then proceed to identification of the protein expression product followed by the development of a specific probe for the single control point of the disease. Previous publications have discussed criteria that would allow a conclusion that the genotype and phenotype (imaging target) are linked in a causal relationship. Among these criteria are the points that phenotypic differences are correlated with no more than three gene differences and that these phenotypic differences are discrete (the area where imaging is most likely to contribute) [68].

The complexity of the human genome with an estimated 10 million SNPs in the 3 billion genomic complement that defines the inherited diversity of the human population, combined with the acquired genetic lesions that occur with carcinogenesis and neoplastic progression, suggests that this causal relationship between the genotype and phenotype cannot be practically established. This is further suggested by the speculation that few diseases are related to a few genes with up to 40 genes playing major roles in a complex disease [69]. Such a complex dependence could not be studied by targeted imaging, given that only one or two targets can be monitored in a clinical situation. The emerging data, however, at least in oncologic disease, suggest that this genotypic/phenotypic causal linkage can be made since, despite the genetic complexity for a specific cancer, a predominant single control point is often present.

The case studies for this so far include the regulatory approval of drugs such as imatinib (Gleevec^{TM}), transtuzumab (Herceptin^{TM}), gefitinib (Iressa^{TM}), erlotinib (Tarceva^{TM}), bevacizumab (Avastin^{TM}), cetuximab (Erbitux^{TM}), sorafenib (Nexavar^{TM}) and sunitinib (Sutent^{TM}) where one protein is predominant in controlling the disease in certain patients. These successes have stimulated an exponential expansion of research effort to define key molecular targets that drive neoplastic progression and therefore become candidates for drug development. Table 5 is a growing list of such targets that are being studied and for which drugs are being developed in industry [70].

There are also drugs for therapeutic applications other than cancer that appear to have single control points for the disease. For example, cyclooxygenase (COX)-2 inhibitors as nonsteroidal anti-inflammatory drugs and ACE inhibitors for congestive heart failure appear to target a single disease control point.

It is important to emphasize that, although there are many examples of single control points for a disease, only a variable percentage of a cohort with any given disease will respond and not have this control point over-ridden or by-passed by alternate pathways that can occur from mutated receptors, drug-induced resistance, alternate signaling pathways, among others. The above-stated drug successes only work in a percentage of patients and the challenge for personalized medicine, to which imaging is contributing, is to define responders and nonresponders. One strategy for this is combining targeted imaging probes to define the patients that might respond with a second imaging probe, measuring a downstream biologic property of neoplasia (e.g. proliferation,

TABLE 5

Molecular target candidates for molecular probe development

HER-2/neu (erbB-2)	BCR–ABL	APC	HDACs
EGFR (erbB-1)	RAS	BRCA1, BRCA2	CpG islands
EGFRviii	B-RAF	P53	COX-2
erbB-1/erbB-2	MEK	MDM2	RARβ
Pan-erbB	ERK	P27	RXR
VEGF	PI3K/AKT	P21	RXR/RAR
VEGFR	c-kit	Forkhead	Snail
IGFR	TGFβ	β-Catenin	Slug
PDGFR	NFκB	DCC	iNOS
TNF	mTOR	c-MYC	ER
Death receptors	Proteasome	c-JUN	AR
CHK-2	Hsp90	CDKs/cyclins	Aromatase
IAP1, IAP2	HIF-1α	DPC4	PPARγ
BAX	E2F1	PARP	PRL3
BCL/BCL$_{XL}$	Integrins	ATM	hTERT
Caspases	MMPs	EWS-FLI	
XIAP	Proteases	PTEN	
FLIP		NBS	
Decoy receptors		TCFs	

angiogenesis, hypoxia, cell death) indicating which subsets with the receptor are responding to therapy.

It is important to note that this progress is incremental and that molecular targeted drugs being used in combination with standard therapies are yielding better responses than standard therapies in patients who have the defined molecular targets. This expanding knowledge obtained from basic and clinical research and treatment data will allow a continuing improvement in development and applied use of the new probes.

The progress and promise of molecular imaging for oncology drug development and personalized cancer patient management is therefore very high. Once the imaging probe has been validated for specificity and selectivity and target density has been established, as discussed in this manuscript, targeted imaging will be valuable in drug development for cohort selection (in combination with biopsies and proteomics), and certainly for drug dosing and monitoring therapy. Once a drug is approved the imaging probe will be very important for personalized medicine in deciding which patients should receive the drug, possibly in modifying dose and certainly in monitoring therapy and decisions to continue therapy or change a drug in the individual patient.

Concluding remarks

Many probes radiolabeled with short-lived PET and SPECT radionuclides are available and many more can be prepared. In addition, there are many other applications of nuclear imaging not directly associated with drug discovery or individualized drug treatments. But an important role for targeted imaging has been shown to be in drug development and individualizing treatment. The key is to choose the right radiolabeled probe for the right target for the right disease, just as it is in pharmaceutical research and development [71]. The imaging approach differs depending on whether one is pursuing a single disease target based on either a single gene disease, whether pursuing a single disease control point or whether pursuing a more general target that will be applicable to a number of treatment paradigms. Regardless of the choice, the radiotracer must be validated as binding to the target with the appropriate pharmacokinetics and pharmacodynamics for effective external imaging. Furthermore, the imaging procedure should uniquely affect either the drug development process or clinical care. This latter aspect of probe design and development must be the primary goal of radiopharmaceutical scientists and clinicians alike. For personalized medicine that encompasses a combination of a key genetic abnormality, an identified protein expression product and a radioligand that is specific for that protein and for those drugs that target a more general property of a disease, nuclear imaging is invaluable because it can be transferred from animals to humans in Phases 0–III and then after approval to individualize the treatment of patients.

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