Building the Bridge: Molecular Imaging Biomarkers for 21st Century Cancer Therapies

Mark A. Sellmyer1,2*, Iris K. Lee*1,3, and David A. Mankoff1

1Department of Radiology, University of Pennsylvania, Philadelphia, Pennsylvania; 2Department of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, Pennsylvania; and 3Department of Bioengineering, University of Pennsylvania, Philadelphia, Pennsylvania

FOCUS ON MOLECULAR IMAGING

Precision medicine, in which the molecular underpinnings of the disease are assessed for tailored therapies, has greatly impacted cancer care. In parallel, a new pillar of therapeutics has emerged with profound success, including immunotherapies such as checkpoint inhibitors and cell-based therapies. Nonetheless, it remains essential to develop paradigms to predict and monitor for therapeutic response. Molecular imaging has the potential to add substantially to all phases of cancer patient care: predictive, companion diagnostics can illuminate therapeutic target density within a tumor, and pharmacodynamic imaging biomarkers can complement traditional modalities to judge a favorable treatment response. This “Focus on Molecular Imaging” article discusses the current role of molecular imaging in oncology and highlights an additional step in the clinical paradigm termed a therapeutic biomarker, which serves to assess whether next-generation drugs reach their target to elicit a favorable clinical response.

Key Words: molecular imaging; PET; companion diagnostics; personalized medicine; targeted cancer therapy; cancer biomarkers

DOI: 10.2967/jnumed.121.262484

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here has been rapid progress in the development of targeted cancer therapies over the past 20 years. Cytotoxic chemotherapeutic regimens are still effective and often used but lack specificity and frequently result in significant side effects. One of the goals of the precision medicine era is to better tailor treatments to the individual’s particular cancer. This tailoring has evolved to include targeted treatments that are based on biomarkers present in the tumor. The therapeutic strategies have diversified in the postgenomic era, taking advantage of biologic agents, recombinant proteins, chimeric approaches, radiotherapies, and even so-called “living drugs” based on engineered cells or viruses that can sense and respond to a particular pathology. As the diversity of approaches has advanced, molecular imaging is uniquely positioned to broaden its critical role in modern therapy development, therapeutic monitoring, and response assessment (2,3).

Molecular diagnostics such as tissue or blood-based biomarkers continue to play an important role and are often a gold standard in terms of being both predictive and prognostic biomarkers. To ensure that the therapeutic potential of targeted drugs is realized, there has been a push for a drug–diagnostic codevelopment model in which diagnostic tests and drugs are developed in parallel (4). For example, immunohistochemistry for estrogen receptor (ER) expression plays an important role as a biomarker forecasting tumor aggressivity and response to estrogen pathway therapies in breast cancer (5), and molecular characterization of hematologic malignancies such as diffuse large B-cell lymphoma guides the use of modern therapies such as chimeric antigen receptor (CAR) T cells and bispecific antibodies targeting cell surface markers, such as CD19 and CD20 (6). Other recent examples include peripheral blood sampling for cytokines associated with T-cell activation (e.g., IL-12) and circulating tumor DNA (7,8). These examples highlight how tissue-sampling approaches can complement circulating biomarkers that capture the state of the pathology or therapy in action. However, despite their benefits, all these biomarkers are currently being assessed using in vitro assays with biopsied tissues and blood samples. Direct-sampling approaches are limited by their invasive nature, which makes repeated sampling impractical. Furthermore, direct sampling comes with the potential trade-off of failing to capture tumor heterogeneity, or assess multifocal disease, and can be prone to sampling errors and artifacts (9). Indeed, even robust gold standard techniques with biologically relevant results can be misleading as to whether a therapy may be successful. For example, programmed-death ligand 1 (PD-L1) expression was not predictive of overall response to immune checkpoint therapy with nivolumab in patients with recurrent metastatic urothelial carcinoma (10). This result highlights that current methods of assessing biomarkers not only are invasive and impractical but also might not yet be powerful enough to accurately predict whether the patient will respond to certain treatments. Thus, key developments in molecular imaging are needed to address the current limitations and to provide clinicians with the information to best tailor cancer therapies.

Here we briefly review the molecular imaging paradigm that has evolved in recent years and consider new ways of applying molecular imaging to predict and assess response to 21st century cancer therapeutics, including the unique ability of molecular imaging to capture targeted therapy delivery to tumor sites. Concepts such as integrated and integral biomarkers and the evolving use of biomarkers in oncology clinical trials—early response indicators and surrogate endpoints—were discussed in more depth previously (3).

INTRODUCTION ON TYPES OF BIOMARKERS

We consider a clinical imaging paradigm of informed decision making using several branch points that include predictive
markers, therapeutic markers, and pharmacodynamic markers (2), guided by definitions used in oncology (11). Predictive markers measure the therapeutic target and ideally give quantitative measures of target expression at the disease site. We define the term therapeutic biomarker as a marker that can measure target engagement or occupancy to guide drug dosing, for example. Pharmacodynamic biomarkers measure biochemical processes or phenotypic outcomes that are downstream from the target to assess whether the drug has had its intended action after treatment. This approach, illustrated in Figure 1, highlights serial branch points in treatment decision making and adds the important strength of molecular imaging markers to assess therapeutic target engagement.

Before discussing each type of biomarker in more depth and with relevant examples, we should note that there are numerous pathologic biomarkers and their partner radiotracers that can be used in different capacities at multiple points in the paradigm. For example, the ER PET imaging described below can be used as both a predictive and a therapeutic biomarker. A key concept in our framework (Fig. 1) is that the timing of imaging dictates the primary capacity in which the biomarker is functioning and providing useful information. In general, a predictive biomarker describes an assessment before treatment. Therapeutic and pharmacodynamic biomarkers are assessed during treatment, typically early after treatment has been applied. Although all markers are in some sense predictive of later response, a distinction is made between markers that predict response in advance of treatment (predictive) and those that require a short exposure to treatment (therapeutic, pharmacodynamic).

**PREDICTIVE MARKERS**

A well-known example of a predictive biomarker is PET imaging of ER expression using 18F-fluorodeoxyglucose. 18F-fluorodeoxyglucose uptake has been shown to strongly correlate with ER expression measured by conventional tissue-based assays (12). Clinical studies have demonstrated a robust correlation between baseline 18F-fluorodeoxyglucose uptake levels and therapeutic response, with 18F-fluorodeoxyglucose uptake being highly predictive of breast cancer responsiveness to ER-targeted endocrine therapies and aromatase inhibitors. More importantly, 18F-fluorodeoxyglucose PET has a high negative predictive value, with the lack of uptake strongly suggesting a lack of response, demonstrating how the assessment of ER status with 18F-fluorodeoxyglucose can thus be used to select patients whose tumor expresses the therapeutic target and to guide therapy.

Predictive marker imaging can also be used to guide radionuclide therapy. An example includes the theranostic pairing of 68Ga-DOTATATE (Netspot; Advanced Accelerator Applications) with 177Lu-DOTATATE (Lutathera; Advanced Accelerator Applications), a somatostatin-targeted peptide receptor radionuclide therapy for the treatment of neuroendocrine tumors (13). For radionuclide therapy, one can also consider radiopharmaceutical dosimetry, specifically the estimation of radiation dose to normal organs from the radiopharmaceutical, as an important predictive marker for guiding therapy (14). An important area of ongoing dosimetry research is the ability of imaging to assess tumor dose in addition to normal organ exposure, as a key predictive measure of tumor response. For this task, closely paired diagnostic agents and quantitative tomographic imaging can provide a good estimate of disease targeting to optimize treatment choices and radiopharmaceutical dose selection (14,15), in an elegant use of paired diagnostic–therapeutic radiopharmaceuticals—that is, a theranostic approach.

Although the above examples have been in development for over a decade, many promising predictive markers are in the preclinical stage for emerging therapies. Poly[adenosine diphosphate ribose] polymerase (PARP) 1 has emerged as an attractive anticancer target given its role in DNA damage repair, and the development of PARP inhibitors is on the rise for the treatment of various types of cancers (16). A radiotracer based on the PARP inhibitor AG14699, 18F-fluorothantrace, is currently at the stage of validation against tissue-based studies for breast and ovarian cancer to assess its predictive value and has the potential to be a clinical predictor of response to PARP inhibitor therapies (17–19).

The use of predictive markers has extended into new classes of therapies to support patient selection and response prediction. The emergence of immunotherapies has motivated the development of antibody-based, immuno-PET probes for imaging therapeutic targets. For example, 89Zr-atezolizumab, an anti-PD-L1 antibody, has been developed to assess PD-L1 expression on cancer cells to predict benefit from PD-L1/PD-L1 checkpoint blockade therapy (Fig. 2A) (20). Initial results from clinical studies have demonstrated that 89Zr-atezolizumab tumor uptake positively correlates to the responsiveness of tumor to PD-L1 blockade therapy with atezolizumab and to both progression-free survival and overall survival. Furthermore, PD-L1 status evaluated by PET imaging has been shown to better predict clinical response than can immunohistochemistry or RNA-sequencing–based biomarkers (20). Imaging of checkpoint protein receptor cytotoxic T-lymphocyte–associated antigen 4 (CTLA4) with 89Zr-ipilimumab is currently being studied in a clinical trial setting (NCT03313323) to determine the correlation between tumor uptake of radiolabeled ipilimumab and response to ipilimumab therapy.

To date, tumor-specific and therapeutic-specific markers have been the dominant classes of predictive PET imaging biomarkers. A deviation in this trend is noted with the emergence of PET tracers developed to image pan-tumor markers, such as fibroblast activation protein (FAP), in the tumor microenvironment (21). FAP-specific enzyme inhibitor has been radiolabeled with 68Ga to image FAP-positive reactive stromal content in various solid tumors with high contrast (68Ga-FAP inhibitor) (22). The DOTA-coupled, chelated nature of the radiotracer highlights that the radioisotope can easily be switched with a therapeutic isotope such as 177Lu, enabling potential therapeutic pairing of 68Ga-FAP inhibitor imaging with 177Lu-FAP inhibitor therapy. Given its selective expression in tumor stromal cells, FAP has also emerged as a promising stromal cell target for solid tumor immunotherapy, including CAR T-cell therapy, portending the use of FAP PET imaging as a companion diagnostic for FAP CAR T-cell therapy to assess the biodistribution of the target (23).

![FIGURE 1. Clinical decision pathway incorporating imaging biomarkers. This path highlights 3 different potential roles of molecular imaging as predictive, therapeutic, and pharmacodynamic biomarker. (Adapted from (2)).](image-url)
THERAPEUTIC BIOMARKERS

Therapeutic biomarkers, which are focused on assessing whether the therapy has reached the target, are a rapidly evolving area in molecular imaging. Traditional examples include diagnostic and therapeutic radiopharmaceuticals (e.g., neuroendocrine tumor agents), including therapeutic agents that intrinsically emit a measurable signal, such as $^{131}$I therapy (24). Dosimetry with $^{131}$I allows optimal dosing in refractory thyroid cancers, yielding measurements that optimize the radiotherapy dose to the target without surpassing toxicity limits to key organs such as the lungs and the bone marrow. With the advent of quantitative molecular imaging biomarkers, imaging can also assess drug dosing for standard therapeutics. An initial study performed to measure ER availability with $^{18}$F-fluoroestradiol before and during fulvestrant therapy demonstrated that residual $^{18}$F-fluoroestradiol uptake after treatment is associated with early clinical disease progression (12). This observation motivated the use of $^{18}$F-fluoroestradiol to measure target engagement of selective ER degrader drugs for determining the optimal dose to achieve clinically significant ER inhibition (Fig. 2B) and is now increasingly applied in the development of new selective ER degrader agents (25).

The expansion and translation of imaging-based therapeutic monitoring may be even more critical for 21st century therapies, including immunotherapies and so-called living drugs that entail gene and cell therapies. Living drugs are uniquely challenging to monitor because of their dynamic behavior in vivo. For example, a cell therapy may undergo autonomous regulation, with dynamic expansion over the entire lifetime of the cell, with faithful signal amplification with each cell division (28). Although relatively straightforward and inexpensive, direct labeling strategies are hampered by dilution of signal on cell division and death and is therefore not amenable to medium- or long-term monitoring of therapy (28). Two alternative approaches include using cell surface labels (e.g., inducible T-cell costimulator [ICOS] or CD8) coupled with immuno-PET radioprobes or reporter gene imaging platforms. In the latter, living drugs such as CAR T cells are transduced with a reporter gene of interest that can be specifically targeted by a complementary radioligand, allowing for imaging over the entire lifetime of the cell, with faithful signal amplification with each cell division (28). One of the most extensively studied PET reporter genes is HSV1-tk. HSV1-tk–engineered cytotoxic T lymphocytes expressing interleukin-13 CAR to target human gliomas after surgical resection were monitored using a radiolabeled analog of the anti-herpes drug penciclovir, $^{18}$F-FHBG.
(9-4\(^\text{18F}\)-fluoro-3-[hydroxy(methyl)butyl]guanine). PET imaging of 
\(^{18}\text{F}-\text{FHBG} \) demonstrated cytotoxic T-lymphocyte accumulation in 
the areas of the tumor, noting that \(^{18}\text{F}-\text{FHBG} \) does not naturally 
cross the intact blood–brain barrier (30).

FOLH1 encodes for prostate-specific membrane antigen and is 
an example of a PET reporter gene that has gained a lot of attention, 
given its human origin. It has been engineered into CD19 
CAR T cells to assess its trafficking to CD19-expressing tumor 
cells in mice, with high sensitivity (31). An elegant finding from 
this work was the demonstration that the number of intratumoral 
CAR T cells derived from the PET images did not correlate with 
the T-cell counts in the blood, suggesting that the peripheral blood 
may not reflect the degree to which tumors are infiltrated with 
CAR T cells, which is therapeutically relevant information.

We recently described \textit{E. coli} dihydrofolate reductase and radiotracer 
derivatives of its highly specific small-molecule inhibitor tri-
methoprim as a promising reporter gene–probe pair for monitoring 
CAR T cells. \(^{18}\text{F}-\text{trimethoprim} \) imaging showed early residence of 
\textit{E. coli} dihydrofolate reductase–expressing disialoganglioside 
2–targeted CAR T cells in the spleen by day 7, followed by on-target 
accumulation in disialoganglioside 2–positive tumor by day 13 (32). 
\textit{Ex vivo} anti-human CD8 immunohistochemistry showed that as 
less than 11,000 \textit{CD8 E. coli} dihydrofolate reductase–expressing CAR T 
cells per cubic millimeter of tumor tissue could be detected in the 
PET images.

Beyond CAR T cells, approaches using reporter gene imaging 
have been established as a common platform to monitor other 21st 
century therapies. Notably, gene therapy/gene replacement with 
adeno-associated virus, and cancer therapies using oncolytic 
viruses, are important fields in which the penetrance and durability 
of the viral vector often have an impact on the therapeutic out-
come (33,34).

**PHARMACODYNAMIC BIOMARKERS**

As the above paradigms are geared for making proper clinical 
management choices from the beginning of a therapeutic interven-
tion (predictive) and measuring whether the drug reaches its target 
(therapeutic), crucial downstream measures of efficacy can be 
assessed with pharmacodynamic biomarkers. These markers are 
well known to the nuclear medicine community and have been 
studied for decades. Two key classes of pharmacodynamic 
markers are metabolic and proliferative measures. The most 
widely adopted metabolic radiotracer is \(^{18}\text{F}-\text{FDG} \), which functions 
as a measure of glycolysis (35). Many clinical patient streams rely 
on PET/CT imaging with \(^{18}\text{F}-\text{FDG} \) for diagnosis, including lymph-
oma, head and neck tumors, high-risk skin cancer (e.g., mela-
noma), and breast cancer (36). These patients are treated with 
diverse chemotherapeutic, biologic, immunotherapeutic, and now 
cell therapy approaches, and \(^{18}\text{F}-\text{FDG} \) remains a crucial tool to 
understand the glycolytic response. Not only have National Com-
prehensive Cancer Network guidelines been developed to include 
PET/CT with input from referring clinicians, but also routine 
response criteria such as PERCIST have been developed to stan-
dardize results communication (37). On the horizon, metabolic 
biomarkers such as amino acid derivatives related to acetate and 
glutamine are in development. These may be applied in certain 
clinical situations as surrogates for understanding specific intracel-
lular processes such as fatty acid synthesis or transport of amino 
acids, particularly for tumors that are not \(^{18}\text{F}-\text{FDG} \)-avid and for 
metabolically targeted therapies (38,39).

Alternatively, a therapy may impact the phenotype of the dis-
ease process, a feature for which quantitative molecular imaging is 
ideally suited. An exciting recent example of imaging a pharma-
dynamic response to an estrogen challenge was illustrated by Deh-
dashiti et al. (40). This approach leverages the observation that 
stimulation of pathways downstream from the ER in breast cancer 
leads to increased expression of the closely related progesterone 
receptor. The investigators showed that an increase in uptake of 
the progesterone receptor radiotracer \(^{18}\text{F}-\text{florouracil} \) in response to an 
estradiol challenge is a potent way to assess for ER receptor function 
and can predict breast cancer response to endocrine therapy. Pharmacodynamic biomarkers for 
biologics and living drugs also have great potential, especially 
with respect to immunooncology.

The focus of imaging pharmacodynamic markers is no longer 
solely on the tumor itself but rather on the immune system. For 
example, CD8 minibodies image immune cells in inflammatory 
conditions and cancer and can be used in conjunction with a base-
line image to understand how the immune system responds to 
immunotherapy such as a checkpoint inhibitor (anti-CTLA4 or anti-
PD1) (41). Another notable example that is nearing clinical transla-
tion is the use of ICOS to monitor “stimulator-of-interferon-genes” 
protein agonist immune activation or CAR T-cell trafficking 
(42,43). Xiao et al. (43) showed that immuno-PET imaging of 
ICOS with \(^{89}\text{Zr}-\text{desferrioxamine-ICOS} \) enabled specific detection of 
activated T cells and their coordinated immune response to stimula-
tor-of-interferon-genes protein and programmed cell death protein 
1 checkpoint blockade in the setting of Lewis lung cancer models, 
highlighting the promising potential of ICOS imaging as a way to 
monitor T-cell–mediated immune response to various immunothera-
pies (Fig. 2C). Xiao et al. (43) also demonstrated that the ICOS sig-
nal is detectable before changes in tumor volume, suggesting that 
ICOS imaging will allow for highly sensitive, early detection of 
response, compared with traditional anatomic imaging approaches.

Despite the success of biologic therapies and antibodies (including 
bispecifics), integration of similar such protein-based imaging tools 
into the clinical paradigm has been challenging, in part because of 
the long circulation time of many of these therapies themselves 
and the practical challenges of imaging full-length antibodies, for 
example. For 21st century therapy imaging, small molecules or immune 
molecules engineered specifically for imaging will continue to play a 
large role, and efforts to make smaller biologic probes using radiol-
sotopes with shorter half-lives have shown promise (41).

**CONCLUSION**

The use of imaging biomarkers, in their diverse capacities, can 
impact and improve on a one-size-fits-all approach to medical diag-
nosis and treatment. Precision medicine promises that with a deep 
understanding of the molecular mechanisms and pathology heteroge-
nity, tailored therapies can be prescribed for the improved treatment 
and health of patients. This molecular imaging biomarker paradigm 
for both cancer clinical trials and future clinical applications serves 
as a reference for basic scientific developments in the field of cancer 
molecular imaging and a formulaic approach to guide clinical trials. 
Imaging serves to complement diagnostics based on in vitro assays 
and tissue sampling, especially in terms of predictive biomarkers. 
Optimizing the dose regimen and understanding whether the drug 
reaches the pathology is a key component of therapeutic biomarkers, 
and finally, pharmacodynamic biomarkers are used to assess the
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