BIOENGINEERING

Imaging approaches to optimize molecular therapies

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Imaging, including its use for innovative tissue sampling, is slowly being recognized as playing a pivotal role in drug development, clinical trial design, and more effective delivery and monitoring of molecular therapies. The challenge is that, while a considerable number of new imaging technologies and new targeted tracers have been developed for cancer imaging in recent years, the technologies are neither evenly distributed nor evenly implemented. Furthermore, many imaging innovations are not validated and are not ready for widespread use in drug development or in clinical trial designs. Inconsistent and often erroneous use of terminology related to quantitative imaging biomarkers has also played a role in slowing their development and implementation. We examine opportunities for, and challenges of, the use of imaging biomarkers to facilitate development of molecular therapies and to accelerate progress in clinical trial design. In the future, in vivo molecular imaging, image-guided tissue sampling for mutational analyses ("high-content biopsies"), and noninvasive in vitro tests ("liquid biopsies") will likely be used in various combinations to provide the best possible monitoring and individualized treatment plans for cancer patients.

NEEDS, HAVES, AND WANTS

Precision oncology aims to adapt treatment decisions to an individual tumor's molecular and genetic characteristics, thereby increasing the chance of a successful outcome (*1*, *2*). Imaging biomarkers have the potential to contribute to both preclinical and clinical cancer drug development, for instance, by knowing target behavior and location [reviewed in (*2*–*5*)]. However, there are other opportunities for the latest imaging technologies in precision medicine, such as revamping clinical trials by stratifying patients to enhance enrollment, tailoring dosing, evaluating therapeutic efficacy, and lowering costs (*6*), and in routine patient care through improved efficacy and closely monitored toxicity. Despite new opportunities and ongoing developments, future strategic efforts are necessary to better synchronize national and international development efforts, prioritize biological targets for imaging, and define the most beneficial clinical applications. Here, we highlight six important areas for imaging in the era of precision oncology.

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Sampling tumors comprehensively and frequently

Image guidance is essential in procuring high-quality, representative biopsy samples for (i) mutational analyses so that drugs can be matched to the right patients (precision medicine), (ii) analyses of tumor and host cell components (toward immunotherapy), (iii) establishing patient-derived cell lines and mouse models (avatars), and (iv) cellular and molecular tumor phenotyping. Fueled by technological innovations, including the capacity to use reduced sample amounts, next-generation image-guided cell harvesting and biopsy procedures are becoming faster and safer through technical advances in imaging equipment and biopsy devices so that they can be performed serially. Such sampling strategies may complement imaging biomarkers and "liquid biopsies," more effectively linking genetic with proteomic, metabolomic, and imaging data.

A recent trend in image-guided biopsies has been to collect more than the typical one to two cores necessary for the traditional diagnostic pathology workup. The use of coaxial systems allows repeat sampling through a 16- to 18-gauge core needle placed under image guidance. With this approach, sufficient amounts of tissue fragments can be harvested to allow genotyping, snapshot analysis, immunohistochemistry, establishment of cell lines for drug development, "avatar" development [for example, patient-derived xenografts (PDXs), organoids, and cell lines for drug testing], and additional testing mandated by drug sponsors. Such high-content biopsies are thus essential in initiating genotype-centric drug trials (Fig. 1) and in frequent reassessment of efficacy. Additional advances that allow much lower CT doses, shorter image acquisition times, systems for acquiring smaller samples, and improved molecular analysis platforms will undoubtedly further expand the use and reach of high-content biopsies.

With newer analytical approaches, it is now possible to perform multiplexed analyses of hundreds of protein markers spanning entire pathways (*7*–*10*). These newer analytical technologies require far fewer cells, which allows sampling with 21-gauge fine needles rather than performing cutting-core biopsies, resulting in less invasive procedures, shorter procedure times, and lower complication rates. According to the U.S. National Cancer Institute, advanced interventional centers have >95% success rates in procuring tissue to identify actionable mutations with multicore biopsies validated histologically, whereas the yields can be considerably lower using traditional single-core approaches.

Selecting patients for a specific therapy

Now, patient selection is largely based on pathological and molecular analysis of tumor material from surgical specimen or image-guided biopsies. Neither of these approaches captures the spatial and temporal heterogeneity of cancer. Drug targets may no longer be present, or new drug targets may have developed since the initial diagnosis. Furthermore, genetic alterations conferring sensitivity or resistance to a specific drug may not be present in all metastases (due to intratumoral and intertumoral heterogeneity); therefore, the findings from biopsies may be misleading. Whole-body positron emission tomography–computed tomography (PET-CT) studies that cover all possible metastatic sites can address both issues and potentially identify patient populations that are most likely to respond to a molecularly targeted treatment. In addition to molecular imaging with PET-CT, there is also the possibility that quantitative feature analysis of imaging data sets in CT or magnetic resonance imaging (MRI) ("radiomics") can be used to describe molecular tumor phenotypes (*11*, *12*). Finally, better patient selection by molecular imaging could also reduce the number of patients required for early-phase clinical trials (*13*, *14*).

Predicting therapeutic response

In the past 5 years, 51 oncologic drugs were approved by the U.S. Food and Drug Administration (FDA) (www.centerwatch.com). There were roughly 4800 early-phase and ~2300 late-phase clinical trials. The average cost of oncologic drug development has increased to $3 billion, and overall drug costs per patient are on the rise, prompting the search for efficient biomarkers (*2*). Moving forward, with a confluence of imaging and other biomarker data, we now have the opportunity to determine whether a given therapy will lead to a response in a patient and whether the patient is receiving the optimal dose (Fig. 1). A quantitative imaging biomarker, usually derived from an in vivo image (often CT, MRI, or PET), is measured on a ratio or interval scale and provides an indicator of a normal biological process, a pathogenic process, or a response to a therapeutic intervention (*15*). The early identification of treatment response is particularly important, especially for a high-cost treatment regimen. As demonstrated for 2-deoxy-2-[^{18}F] fluoro-D-glucose (^{18}F-FDG) (*16*–*18*), early decreases in metabolic activity can serve as a marker of chemosensitivity, whereas paradoxical increases of signals may indicate the activation of an immunologic response. Although some imaging biomarkers exist today (Fig. 1), many more are in development—or yet to be discovered for certain cancers—and will have to be subsequently validated in large clinical cohorts. An array of new imaging agents developed by academia and industry (Table 1) will likely enable the measurement of target engagement and not just downstream effects of tumor shrinkage at relatively late time points. Co-preclinical testing—in other words, the use of mouse models to inform clinical trials—will play an essential role in the discovery phase (*19*, *20*), as will new cancer models ranging from PDXs and humanized mouse models to in vitro approaches including organoids and tumor slice cultures (*21*).

A clinical example of an imaging-based predictive biomarker in breast cancer is estrogen receptor (ER) imaging with [^{18}F]fluoroestradiol (^{18}F-FES) (Table 1). Because 20% of patients may demonstrate heterogeneity of ER expression at different sites of malignancy, molecular imaging of ER status has been actively pursued. Furthermore, receptor expression is not static and may change over time during therapy and/or because of hypoxia. ^{18}F-FES uptake correlates with ER expression, as measured by immunohistochemistry, and has shown ER heterogeneity in vivo (*22*). ^{18}F-FES has been used as a pharmacodynamic biomarker to monitor ER binding by ER-targeted therapies in humans. In a proof-of-concept study, ^{18}F-FES PET-CT was used to demonstrate target engagement by fulvestrant, which is an ER antagonist, and to select the dose of fulvestrant needed to abolish ER availability (*23*). Similar work has been successfully applied to the ER antagonist and the ER degrader GDC-0810 (Genentech) (*24*).

Finding the optimal drug dose

Traditionally, cytotoxic agents have been evaluated in clinical trials by using the maximum

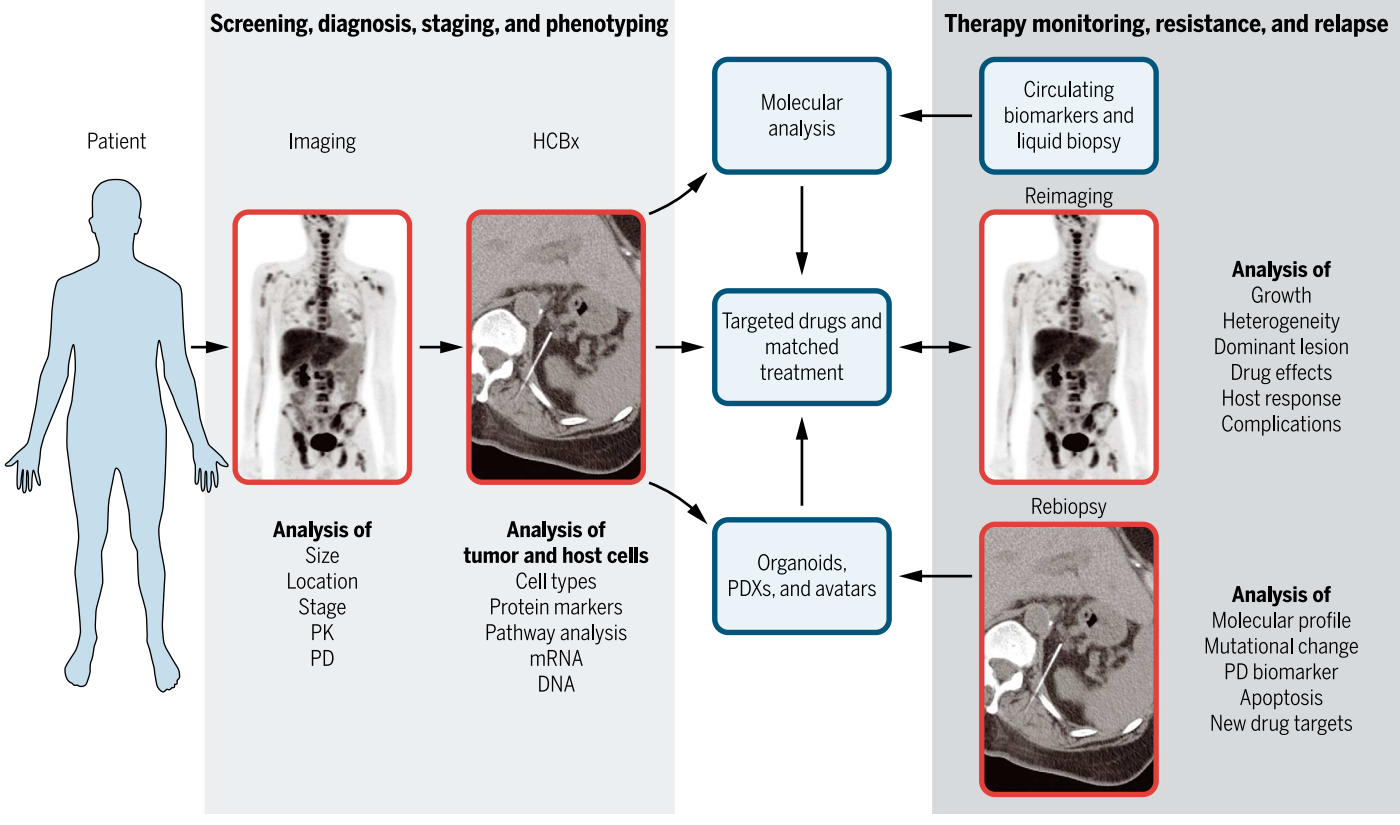

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Fig. 1. Advanced imaging for high-content biopsy (HCBx) and molecular phenotyping. In modern cancer therapy, the tumor specimen is subjected to detailed molecular analysis, including protein-, mRNA-, and DNA-based analyses. These molecular biomarkers are interpreted within the relevant signaling pathway to identify a potential therapeutic approach for the cancer. Selection of an appropriate therapy is dependent on detailed analysis of drug-target interactions. Drug response and development of resistance are monitored by repeat analyses. Cell lines, organoids, and PDX models are established at different time points to serve as avatars for drug testing and/or to understand tumor biology. Molecular imaging, imaging biomarkers, and image-guided biopsies (red boxes) play a critical role in precision oncology. PK, pharmacokinetic; PD, pharmacodynamic.

Table 1. Examples of clinical imaging probes that will enable oncologic precision medicine. An overview of select, currently active investigational new drugs for precision oncological imaging. The list of agents is illustrative rather than comprehensive (see www.ncbi.nlm.nih.gov/books/NBK5330/ for a more complete list of agents under development). ^{18}F-FLT, [^{18}F]fluorothymidine; CSNAT, caspase-sensitive nanoaggregation tracer; ^{18}F-RGD-K5, 2-((2*S*,5*R*,8*S*,11*S*)-5-benzyl-8-(4-((2*S*,3*R*,4*R*,5*R*,6*S*)-6-((2-(4-(3-(18)F-fluoropropyl)-1*H*-1,2,3-triazol-1-yl)acetamido)methyl)-3,4,5-trihydroxytetrahydro-2*H*-pyran-2-carboxamido) butyl)-11-(3-guanidinopropyl)-3,6,9,12,15-pentaoxo-1,4,7,10,13-pentaazacyclopentadecan-2-yl)acetic acid; EF5, 2-(2-nitro-(1)*H*-imidazol-1-yl)-*N*-(2,2,3,3,3-pentafluoropropyl)-acetamide; ^{18}F-FMISO, [^{18}F]fluoromisonidazole; PD-L1, programmed death ligand 1; CTLA4, cytotoxic T lymphocyte–associated antigen 4; CART, chimeric antigen receptor T cell; FIAU, 2′-fluoro-2′-deoxy-1-beta-D-arabinofuranosyl-5-iodouracil; FMAU, 2′-deoxy-2′-fluoro-5-methyl-1-β-D-arabinofuranosyluracil; TAM, tumor-associated macrophage; ^{18}F-AraG, 2′-deoxy-2′-[^{18}F]fluoro-9-β-D-arabinofuranosylguanine; dCK/dGK, deoxycytidine kinase/deoxyguanosine kinase; ^{18}F-FAC, 1-(2′-deoxy-2′-[^{18}F]fluoroarabinofuranosyl)cytosine; PSMA, prostate-specific membrane antigen; DFO, desferrioxamine B; ER, estrogen receptor; AR, androgen receptor; HER2, human epidermal growth factor receptor 2; STEAP1, six transmembrane epithelial antigen of the prostate 1; ^{18}F-FDHT, 16β-^{18}F-fluoro-5α-dihydrotestosterone; ^{18}F-DOPA, [^{18}F]-L-dihydroxyphenylalanine; PI3K, phosphatidylinositol 3-kinase; ^{18}F-FDG, 2-deoxy-2-[^{18}F]fluoro-D-glucose; PARP, poly(ADP-ribose) polymerase; PARPi-FL, fluorescent PARP1 inhibitor; SSTR2, somatostatin receptor 2; HSP-90, heat shock protein 90; PUH71, (8-{(6- iodo-1,3-benzodioxol-5-yl)sulfanyl}-9-{3-(propan-2-ylamino)propyl}purin-6-amine).

Target	Imaging agent	Primary application
Generic pharmacodynamic readouts		
Tumor-cell proliferation	^{18}F-FLT	Lymphoma and prostate, head and neck, and non–small cell lung cancers
Apoptosis	^{18}F-CSNAT	Apoptosis
Multidrug resistance	^{99m}Tc-Sestamibi	MDR1 expression
Angiogenesis	^{18}F-RGD-K5 and ^{89}Zr-bevacizumab	Angiogenesis inhibitors
Hypoxia	^{18}F-EF5 and ^{18}F-FMISO	Radiation planning
Metabolism	^{18}F-Fluoroglutamine	Glutamine metabolism
Physiology	^{11}C-labeled drugs	In vivo pharmacokinetic measurements
Immunotherapy		
PD-L1 inhibition	Labeled antibody	Checkpoint inhibition
CTLA4 inhibition	^{18}F-FLT	Checkpoint inhibition
CART	^{18}F-FIAU and ^{18}F-FMAU	T cell labeling
TAM	Ferumoxytol nanoparticle	TAM recruitment and nanotherapeutics
Activated T cells	^{18}F-AraG	
dCK/dGK	^{18}F-FAC	Imaging tumors and immune cells
Antibody fragments		
PSMA	^{89}Zr-DFO-huJ591	Prostate and brain cancers
HER2	^{89}Zr-DFO-trastuzumab	Breast and gastric cancers
CD20	^{64}Cu-Rituximab	B cell lymphoma
STEAP1	^{89}Zr-MSTP2109A	Prostate cancer
Small-molecule inhibitors		
ER	^{18}F-FES	Breast cancer
AR	^{18}F-FDHT	Prostate cancer
EGFR	^{11}C-Erlotinib	Lung and pancreatic cancers
B-RAF inhibition	^{18}F-DOPA and ^{18}F-FDG	Melanoma and colon cancers
PI3K inhibition	^{18}F-FDG	Breast and lung cancers
BCR-ABL tyrosine kinases	^{18}F-Dasatinib	Prostate and breast cancers
PARP	PARPi-FL	Head and neck cancers
Theranostics (imaging and/or therapy pairs)		
SSTR2	^{68}Ga/^{177}Lu-DOTATATE	Neuroendocrine tumors

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Target	Imaging agent	Primary application
PSMA	^{68}Ga/^{177}Lu-HBED-CC	Prostate cancer
CXCR4	^{68}Ga/^{177}Lu-Pentixafor	Myeloma and lymphoma
HSP-90	^{124}I-PUH71	Solid tumors and lymphoma
Disialoganglioside GD2	^{124}I-3F8/^{131}I-3F8/^{124}I-hu3F8	Neuroblastoma (pediatrics)
8H9 antigen	^{124}I-8H9/^{131}I-8H9	Leptomeninges (pediatrics)

tolerated dose (MTD) as the basis for dose selection. However, given the introduction of molecularly targeted agents and immunotherapies, new emphasis has been placed on the use of pharmacodynamic end points. Pharmacodynamic assessment seeks to characterize whether a drug inhibits the intended target and leads to the desired physiologic effect. There is a need to move from MTD to biologically relevant dose concepts. Newer quantitative, multimodality imaging approaches provide the means for accurate assessment of dose-response relationships and offer parametric representation of regional changes in tumor biology in relation to therapeutic effects.

One example of using imaging for dose finding is a recent first-in-human study of apalutamide (ARN-509; Aragon Pharmaceuticals) conducted in patients with progressive metastatic castration-resistant prostate cancer (*25*). The authors compared the traditional MTD to the biologically relevant dose, which was assessed using the imaging pharmacodynamic biomarker ^{18}F-fluorodihydrotestosterone (FDHT). The uptake of FDHT, an analog of endogenous dihydrotestosterone, reflects AR expression and binding capacity. FDHT PET-CT imaging was used to measure pharmacodynamic response to ARN-509 and to capture the biological diversity of multifocal metastatic disease through visualization of in situ AR binding (*25*, *26*). The response reached a plateau at a dose of 120 mg (with FDHT uptake near background), indicating saturation of AR binding and thus achievement of the optimal drug concentration (*25*, *26*). The safety margin based on dose escalation was reached at 480 mg. After further measurements, the authors recommended a daily dose of 240 mg, which achieved maximal AR inhibition based on FDHT PET-CT accompanied by robust and lasting declines in prostate-specific antigen. All activities were consistent with preclinical modeling, and ultimately, the recommended—and FDA-accepted—dose was much lower than the traditional MTD.

Alternative and surrogate clinical trial end points

Several different end points have been used in cancer drug development to establish drug efficacy and obtain regulatory approval. These have mainly consisted of overall survival, progression-free survival, and radiologic response rates based on tumor size measurements and/or enhancement characteristics. Given the limitations of these traditional end points (cost, insensitivity, and variability), clinical trials have become expensive and time-consuming. The development of molecular biomarkers that could be used as surrogate end points could reduce the size, complexity, and duration of future clinical trials; accelerate drug development; and improve patient outcomes. Furthermore, combining multiple quantitative biomarkers to arrive at surrogate end points may provide new strategies in the drug development process (*7*).

Immunotherapies can paradoxically lead to an increase in tumor size and/or the appearance of new lesions, due to immune cell infiltration, rendering conventional size measurements of limited value to inform clinical management. Translational examples of emerging imaging in immunotherapy include the use of imaging strategies to track the location(s) and viability of T cells (*27*) or therapeutics (*28*). For example, T cells can be labeled ex vivo and then used to image the homing of those cells to areas of inflammation (Table 1). Several alternative strategies are being developed to image subsets of immune cells, including CD3 (*29*) and CD8 (*30*), CD206 (*31*), innate immune cells (*28*, *32*), or checkpoint inhibitors such as CTLA4, PD1, and PD-L1 (*33*).

In current clinical practice, findings from radiology and pathology are largely uncoordinated and presented in silos. These often lengthen the time required for end users to resolve or properly integrate findings, adversely affecting treatment decisions. In the future, integration of biopsy data, imaging data, and other serum biomarkers into longitudinal, visual patient data will hopefully occur. RadPath (*34*, *35*) is one recent example of a system for interfacing clinical information systems and obtaining an interactive compendium for a diagnostic patient case. It is hoped that future efforts will expand on this important integration point (Table 2).

Theranostics

The combined use of closely related therapeutic and diagnostic molecules ("theranostics") has been explored for different drug classes, including biologicals and nanomaterials; however, peptide-based approaches based on radiolabeled peptide show particular promise in the clinic. For example, SSTR2-targeting therapeutic radiopharmaceuticals (such as, ^{177}Lu) are well established for patients with neuroendocrine tumors (*36*) and have been shown to improve patient outcome in randomized controlled trials. On the basis of the intensity and extent of target expression documented by quantitative imaging, therapies can then be adjusted by individualized dosimetry. These tailored approaches are quite attractive and have spurred the development of more advanced targeted agents, which are about to enter the clinic (for example, PEN-221 from Tarveda Therapeutics). Other examples of theranostic agents with encouraging clinical results are those targeting PSMA in patients with metastatic prostate cancer. Although larger systematic studies are still lacking, ongoing clinical trials have shown major and durable responses in patients for whom all approved therapies have failed (*37*, *38*). Additional examples of theranostics are provided in Table 1.

NEW MOLECULAR BIOMARKERS: WHO DRIVES DEVELOPMENT AND VALIDATION?

Strategic efforts are needed to prioritize targets and more effectively link companion diagnostics to drugs during clinical trials and to design larger multicenter trials (Table 2). This effort

Table 2. Advancing the appropriate use of imaging procedures for molecularly targeted therapies. We define eight major needs and propose some possible solutions. SNMMI, Society for Nuclear Medicine and Molecular Imaging; EANM, European Association of Nuclear Medicine; ECOG, Eastern Cooperative Oncology Group; ACRIN, American College of Radiology Imaging Network.

Need	Goal	Progress
Defined utility of imaging biomarkers	Establish common standards of clinical utility of imaging biomarkers using evidence-based approaches	
Standardized analytical practices	Develop standards for specimen acquisition, processing, analysis, and quality to maximize accuracy of imaging biomarker results	EANM/SNMMI have published a guideline on acquisition of FDG PET-CT studies that also includes quantitative analysis
Defined indications and benefits	Enhance communication to providers and patients about the performance characteristics and evidence for use of specific imaging biomarkers for molecularly targeted therapies	
Reimbursement for proven imaging biomarkers	Develop a coordinated, transparent federal process for regulatory and reimbursement decisions for imaging biomarkers for molecularly targeted therapies	
National imaging databases	Enhance the development of sustainable national databases for imaging biomarkers and enhance biomedical informatics to query databases	ECOG-ACRIN store the images from their trials with a process to make images available to researchers
National use guidelines	Develop and update clinical practice guidelines for the effective use of imaging biomarkers for molecularly targeted therapies	
Integration into major national cancer initiatives	Integrate the development of new imaging biomarkers and image-guided biopsy approaches into the U.S. precision medicine initiative	
Comprehensive evaluation of diagnostic test results	Integrate imaging, biopsy, pathology, and serum biomarker data into longitudinal, visual patient data records	Nascent efforts under way at some institutions and in certain trials (ECOG-ACRIN)

will have to be a multidisciplinary attempt, because no single commercial entity seems to have all the components to efficiently drive this effort. The traditional stakeholders (academia, imaging device manufacturers, imaging agent companies, and pharmaceutical industries) have occasionally collaborated, but progress has been sluggish over the last decade.

Three new molecular imaging agents have been FDA-approved since 2012 (two of them in 2016). Development of ^{11}C-choline for imaging of prostate cancer was largely driven by one academic institution, Mayo Clinic, submitting a new drug application (NDA), which was approved by the FDA in 2012. Development of the somatostatin receptor ligand ^{68}Ga-DOTATATE for PET imaging was mostly driven by academia, with published data in humans as the basis for an NDA submitted by Advanced Accelerator Applications; it was FDA-approved in 2016. Development of ^{18}F fluciclovine (FACBC), a non-natural amino acid for imaging of prostate cancer, was also initiated by academia, but clinical trials were performed by industry (GE Healthcare and, later, Blue Earth Diagnostics). Although these three examples show that new molecular imaging agents can be translated to the clinic, they also show the long development times: more than 15 years for choline (*39*) and DOTATATE, and 8 years for FACBC.

Companion in vitro diagnostics will likely be part of FDA NDA processes in the future, given their potential for therapeutic response assessment. Development of predictive imaging biomarkers, on the other hand, requires a new model of cooperation among academic centers and health care providers who can play a more active role in their development. Primary academic centers and large health care networks often have direct access to large patient cohorts, advanced imaging, molecular analytical capabilities, and sophisticated research enterprises. They are, therefore, at the epicenter of where new developments could occur. In addition, patient advocacy groups, as well as foundations, could play promoting roles and their future engagement will be critical to advance imaging biomarkers to the clinic.

Another challenge will be to develop internal standards that could then be adapted globally. For example, PERCIST (PET response criteria in solid tumors) has been developed to monitor tumor response with ^{18}F-FDG PET-CT, and the criteria are now commonly used in oncology clinical trials (*40*). However, standardized approaches for how to validate imaging biomarkers for patient selection and to assess pharmacodynamics effects are still lacking. Such standards will be essential to determine a biomarker's predictive value and its ability to monitor early response to therapy and, in turn, to tailor further therapies.

COST CONTROLS

Cancer care is hugely expensive, now averaging >$10,000/month per patient and $91 billion per year globally (*41*). Most of the expenses for end-of-life care are due to drug costs and hospitalization. The introduction of molecularly targeted therapies and immunotherapies has further increased the costs. In parallel, advanced imaging and image-guided interventions by themselves are also expensive. However, the judicious use of imaging can actually save costs by defining which patients will benefit from expensive therapies and which will not.

There is no question that the indiscriminate use of expensive drugs and imaging tests is wasteful. Members of primary care teams have to do a better job of clarifying when and how to use new technology so that money is actually saved (*42*)—or so that any additional money spent leads to a worthwhile increase in survival and/or quality of life. This is possible but will require some prospective studies, perhaps in trials in large health care networks. Reducing costs will involve treating the right patients with the right drugs, eliminating treatments that do not work, and keeping patients out of hospitals—all goals of precision and personalized medicine (*21*, *43*). The development of decision models that make it possible to understand the role of a given imaging strategy in clinical management—notably, without having to perform a clinical trial—will be critical

(*44*). Such an approach could estimate the cost-effectiveness of a trial even if the actual clinical trial is cost-prohibitive or too lengthy to actually be conducted. For example, although large clinical trials using ^{18}F-FDG PET could not initially be performed to show how it might be cost-effective in the management of solitary pulmonary lung nodules, decision models predicted its potential utility (*45*). Over time, these models were proved correct as many physicians used ^{18}F-FDG PET to manage their patients. Furthermore, these models were used to convince Medicare to reimburse ^{18}F-FDG for this application.

REIMBURSEMENT POLICIES LIMIT ROUTINE CLINICAL USE

There is an abundance of promising imaging agents and single center–derived data regarding new imaging biomarkers. So, how is it that we have not been able to move beyond ^{18}F-FDG imaging and bone scanning in the routine clinical setting? A major hurdle has been the lack of reimbursement for new molecular imaging agents and the absence of reimbursement codes for high-content biopsy approaches. For example, new radiopharmaceuticals targeting prostate cancer may significantly affect diagnostic and therapeutic approaches, but without close cooperation between academia, foundations, the National Institutes of Health, industry, and regulatory agencies, the translation of such new imaging probes remains challenging and lengthy. At times, industry has supported imaging as part of a clinical trial (such as FES with a new estrogen oral drug), but the approval of the matched imaging biomarker is usually not pursued for economic reasons. There are some instances where philanthropy, foundations, and academia have come together, for example, in the Movember FDHT international multicenter trial, but these instances are rare.

New regulatory models are needed to distinguish between one-time use of radiopharmaceuticals in subpharmacological doses (microdosing) and possibly lifelong applications of regular drugs. In addition, prospective multicenter validation studies of new imaging protocols are needed to provide the required evidence for reimbursement by national health care systems. Unless new and validated imaging tests are reimbursed, they will not be used and providers will continue to rely on more costly and less efficient methods. One solution to address this problem is to allow prospectively acquired registry data (beyond classical phase 1 to 3 studies) to be used for the NDA process; this would lead to a more timely and less costly introduction of new imaging agents. In the end, patients will benefit most from new technology, particularly when it is delivered in a justified setting and developed by multidisciplinary teams of physicians.

REFERENCES AND NOTES

1. J. Mendelsohn, Personalizing oncology: Perspectives and prospects. *J. Clin. Oncol.* **31**, 1904–1911 (2013).
2. L. A. Graig, J. K. Phillips, H. L. Moses, *Biomarker Tests for Molecularly Targeted Therapies—Key to Unlocking Precision Medicine* (The National Academies Press, Washington, DC, 2016).
3. B. M. Seddon, P. Workman, The role of functional and molecular imaging in cancer drug discovery and development. *Br. J. Radiol.* **76** (suppl. 2), S128–S138 (2003).
4. M. Rudin, R. Weissleder, Molecular imaging in drug discovery and development. *Nat. Rev. Drug Discov.* **2**, 123–131 (2003).
5. J. K. Willmann, N. van Bruggen, L. M. Dinkelborg, S. S. Gambhir, Molecular imaging in drug development. *Nat. Rev. Drug Discov.* **7**, 591–607 (2008).
6. A. Falconi, G. Lopes, J. L. Parker, Biomarkers and receptor targeted therapies reduce clinical trial risk in non–small-cell lung cancer. *J. Thorac. Oncol.* **9**, 163–169 (2014).
7. A. V. Ullal, V. Peterson, S. S. Agasti, S. Tuang, D. Juric, C. M. Castro, R. Weissleder, Cancer cell profiling by barcoding allows multiplexed protein analysis in fine-needle aspirates. *Sci. Transl. Med.* **6**, 219ra9 (2014).
8. J.-R. Lin, M. Fallahi-Sichani, P. K. Sorger, Highly multiplexed imaging of single cells using a high-throughput cyclic immunofluorescence method. *Nat. Commun.* **6**, 8390 (2015).
9. A. P. Frei, F.-A. Bava, E. R. Zunder, E. W. Y. Hsieh, S.-Y. Chen, G. P. Nolan, P. F. Gherardini, Highly multiplexed simultaneous detection of RNAs and proteins in single cells. *Nat. Methods* **13**, 269–275 (2016).
10. J. R. Heath, A. Ribas, P. S. Mischel, Single-cell analysis tools for drug discovery and development. *Nat. Rev. Drug Discov.* **15**, 204–216 (2016).
11. R. J. Gillies, P. E. Kinahan, H. Hricak, Radiomics: Images are more than pictures, they are data. *Radiology* **278**, 563–577 (2016).
12. H. J. Yoon, I. Sohn, J. H. Cho, H. Y. Lee, J.-H. Kim, Y.-L. Choi, H. Kim, G. Lee, K. S. Lee, J. Kim, Decoding tumor phenotypes for ALK, ROS1, and RET fusions in lung adenocarcinoma using a radiomics approach. *Medicine* **94**, e1753 (2015).
13. J. Menis, B. Hasan, B. Besse, New clinical research strategies in thoracic oncology: Clinical trial design, adaptive, basket and umbrella trials, new end-points, and new evaluations of response. *Eur. Respir. Rev.* **23**, 367–378 (2014).
14. M. A. Miller, S. Gadde, C. Pfirschke, C. Engblom, M. M. Sprachman, R. H. Kohler, K. S. Yang, A. M. Laughney, G. Wojtkiewicz, N. Kamaly, S. Bhonagiri, M. J. Pittet, O. C. Farokhzad, R. Weissleder, Predicting therapeutic nanomedicine efficacy using a companion magnetic resonance imaging nanoparticle. *Sci. Transl. Med.* **7**, 314ra183 (2015).
15. L. G. Kessler, H. X. Barnhart, A. J. Buckler, K. R. Choudhury, M. V. Kondratovich, A. Toledano, A. R. Guimaraes, R. Filice, Z. Zhang, D. C. Sullivan; QIBA Terminology Working Group, The emerging science of quantitative imaging biomarkers terminology and definitions for scientific studies and regulatory submissions. *Stat. Methods Med. Res.* **24**, 9–26 (2015).
16. B. Coudert, J.-Y. Pierga, M.-A. Mouret-Reynier, K. Kerrou, J.-M. Ferrero, T. Petit, P. Kerbrat, P.-F. Dupré, T. Bachelot, P. Gabelle, S. Giard, D. Coeffic, P. Bougnoux, J.-B. Prevost, G. Paintaud, G. Thibault, J. Hernandez, M. Coudert, L. Arnould, A. Berriolo-Riedinger, Use of [^{18}F]-FDG PET to predict response to neoadjuvant trastuzumab and docetaxel in patients with HER2-positive breast cancer, and addition of bevacizumab to neoadjuvant trastuzumab and docetaxel in [^{18}F]-FDG PET-predicted non-responders (AVATAXHER): An open-label, randomised phase 2 trial. *Lancet Oncol.* **15**, 1493–1502 (2014).
17. O. W. Press, H. Li, H. Schöder, D. J. Straus, C. H. Moskowitz, M. LeBlanc, L. M. Rimsza, N. L. Bartlett, A. M. Evens, E. S. Mittra, A. S. LaCasce, J. W. Sweetenham, P. M. Barr, M. A. Fanale, M. V. Knopp, A. Noy, E. D. Hsi, J. R. Cook, M. J. Lechowicz, R. D. Gascoyne, J. P. Leonard, B. S. Kahl, B. D. Cheson, R. I. Fisher, J. W. Friedberg, US Intergroup Trial of response-adapted therapy for stage III to IV Hodgkin lymphoma using early interim fluorodeoxyglucose-positron emission tomography imaging: Southwest Oncology Group S0816. *J. Clin. Oncol.* **34**, 2020–2027 (2016).
18. O. Humbert, A. Cochet, B. Coudert, A. Berriolo-Riedinger, S. Kanoun, F. Brunotte, P. Fumoleau, Role of positron emission tomography for the monitoring of response to therapy in breast cancer. *Oncologist* **20**, 94–104 (2015).
19. M. J. Whitley, D. M. Cardona, A. L. Lazarides, I. Spasojevic, J. M. Ferrer, J. Cahill, C.-L. Lee, M. Snuderl, D. G. Blazer III, E. S. Hwang, R. A. Greenup, P. J. Mosca, J. K. Mito, K. C. Cuneo, N. A. Larrier, E. K. O'Reilly, R. F. Riedel, W. C. Eward, D. B. Strasfeld, D. Fukumura, R. K. Jain, W. D. Lee, L. G. Griffith, M. G. Bawendi, D. G. Kirsch, B. E. Brigman, A mouse-human phase 1 co-clinical trial of a protease-activated fluorescent probe for imaging cancer. *Sci. Transl. Med.* **8**, 320ra4 (2016).
20. A. M. Laughney, E. Kim, M. M. Sprachman, M. A. Miller, R. H. Kohler, K. S. Yang, J. D. Orth, T. J. Mitchison, R. Weissleder, Single-cell pharmacokinetic imaging reveals a therapeutic strategy to overcome drug resistance to the microtubule inhibitor eribulin. *Sci. Transl. Med.* **6**, 261ra152 (2014).
21. A. A. Friedman, A. Letai, D. E. Fisher, K. T. Flaherty, Precision medicine for cancer with next-generation functional diagnostics. *Nat. Rev. Cancer* **15**, 747–756 (2015).
22. E. Currin, L. M. Peterson, E. K. Schubert, J. M. Link, K. A. Krohn, R. B. Livingston, D. A. Mankoff, H. M. Linden, Temporal heterogeneity of estrogen receptor expression in bone-dominant breast cancer: ^{18}F-fluoroestradiol PET imaging shows return of ER expression. *J. Natl. Compr. Canc. Netw.* **14**, 144–147 (2016).
23. H. M. Linden, B. F. Kurland, L. M. Peterson, E. K. Schubert, J. R. Gralow, J. M. Specht, G. K. Ellis, T. J. Lawton, R. B. Livingston, P. H. Petra, J. M. Link, K. A. Krohn, D. A. Mankoff, Fluoroestradiol positron emission tomography reveals differences in pharmacodynamics of aromatase inhibitors, tamoxifen, and fulvestrant in patients with metastatic breast cancer. *Clin. Cancer Res.* **17**, 4799–4805 (2011).
24. A. Lai, M. Kahraman, S. Govek, J. Nagasawa, C. Bonnefous, J. Julien, K. Douglas, J. Sensintaffar, N. Lu, K.-J. Lee, A. Aparicio, J. Kaufman, J. Qian, G. Shao, R. Prudente, M. J. Moon, J. D. Joseph, B. Darimont, D. Brigham, K. Grillot, R. Heyman, P. J. Rix, J. H. Hager, N. D. Smith, Identification of GDC-0810 (ARN-810), an orally bioavailable selective estrogen receptor degrader (SERD) that demonstrates robust activity in tamoxifen-resistant breast cancer xenografts. *J. Med. Chem.* **58**, 4888–4904 (2015).
25. M. R. Smith, E. S. Antonarakis, C. J. Ryan, W. R. Berry, N. D. Shore, G. Liu, J. J. Alumkal, C. S. Higano, E. Chow Maneval, R. Bandekar, C. J. de Boer, M. K. Yu, D. E. Rathkopf, Phase 2 study of the safety and antitumor activity of apalutamide (ARN-509), a potent androgen receptor antagonist, in the high-risk non-metastatic castration-resistant prostate cancer cohort. *Eur. Urol.* S0302-2838(16)30133-6 (2016).
26. D. E. Rathkopf, M. J. Morris, J. J. Fox, D. C. Danila, S. F. Slovin, J. H. Hager, P. J. Rix, E. Chow Maneval, I. Chen, M. Gönen, M. Fleisher, S. M. Larson, C. L. Sawyers, H. I. Scher, Phase I

study of ARN-509, a novel antiandrogen, in the treatment of castration-resistant prostate cancer. *J. Clin. Oncol.* **31**, 3525–3530 (2013).
27. M. F. Kircher, S. S. Gambhir, J. Grimm, Noninvasive cell-tracking methods. *Nat. Rev. Clin. Oncol.* **8**, 677–688 (2011).
28. M. Rashidian, E. J. Keliher, A. M. Bilate, J. N. Duarte, G. R. Wojtkiewicz, J. T. Jacobsen, J. Cragnolini, L. K. Swee, G. D. Victora, R. Weissleder, H. L. Ploegh, Noninvasive imaging of immune responses. *Proc. Natl. Acad. Sci. U.S.A.* **112**, 6146–6151 (2015).
29. B. M. Larimer, E. Wehrenberg-Klee, A. Caraballo, U. Mahmood, Quantitative CD3 PET imaging predicts tumor growth response to Anti-CTLA-4 therapy. *J. Nucl. Med.* jnumed.116.173930 (2016).
30. R. Tavaré, H. Escuin-Ordinas, S. Mok, M. N. McCracken, K. A. Zettlitz, F. B. Salazar, O. N. Witte, A. Ribas, A. M. Wu, An effective immuno-PET imaging method to monitor CD8-dependent responses to immunotherapy. *Cancer Res.* **76**, 73–82 (2016).
31. F. O. Cope, B. Abbruzzese, J. Sanders, W. Metz, K. Sturms, D. Ralph, M. Blue, J. Zhang, P. Bracci, W. Bshara, S. Behr, T. Maurer, K. Williams, J. Walker, A. Beverly, B. Blay, A. Damughatla, M. Larsen, C. Mountain, E. Neylon, K. Parcel, K. Raghuraman, K. Ricks, L. Rose, A. Sivakumar, N. Streck, B. Wang, C. Wasco, A. Williams, M. McGrath, The inextricable axis of targeted diagnostic imaging and therapy: An immunological natural history approach. *Nucl. Med. Biol.* **43**, 215–225 (2016).
32. C. Engblom, C. Pfirschke, M. J. Pittet, The role of myeloid cells in cancer therapies. *Nat. Rev. Cancer* **16**, 447–462 (2016).
33. E. B. Ehlerding, C. G. England, D. G. McNeel, W. Cai, Molecular imaging of immunotherapy targets in cancer. *J. Nucl. Med.* jnumed.116.177493 (2016).
34. W. Hsu, S. X. Han, C. W. Arnold, A. A. Bui, D. R. Enzmann, A data-driven approach for quality assessment of radiologic interpretations. *J. Am. Med. Inform. Assoc.* **23**, e152–e156 (2016).
35. C. W. Arnold, W. D. Wallace, S. Chen, A. Oh, F. Abtin, S. Genshaft, S. Binder, D. Aberle, D. Enzmann, RadPath: A web-based system for integrating and correlating radiology and pathology findings during cancer diagnosis. *Acad. Radiol.* **23**, 90–100 (2016).
36. W. A. van der Zwan, L. Bodei, J. Mueller-Brand, W. W. de Herder, L. K. Kvols, D. J. Kwekkeboom, GEPNETs update: Radionuclide therapy in neuroendocrine tumors. *Eur. J. Endocrinol.* **172**, R1–R8 (2015).
37. M. M. Heck, M. Retz, C. D'Alessandria, I. Rauscher, K. Scheidhauer, T. Maurer, E. Storz, F. Janssen, M. Schottelius, H.-J. Wester, J. E. Gschwend, M. Schwaiger, R. Tauber, M. Eiber, Systemic radioligand therapy with ^{177}Lu labeled prostate specific membrane antigen ligand for imaging and therapy in patients with metastatic castration resistant prostate cancer. *J. Urol.* **196**, 382–391 (2016).
38. C. Kratochwil, F. L. Giesel, M. Stefanova, M. Benešová, M. Bronzel, A. Afshar-Oromieh, W. Mier, M. Eder, K. Kopka, U. Haberkorn, PSMA-targeted radionuclide therapy of metastatic castration-resistant prostate cancer with ^{177}Lu-labeled PSMA-617. *J. Nucl. Med.* **57**, 1170–1176 (2016).
39. T. Hara, N. Kosaka, H. Kishi, PET imaging of prostate cancer using carbon-11-choline. *J. Nucl. Med.* **39**, 990–995 (1998).
40. S. J. Min, H. J. Jang, J. H. Kim, Comparison of the RECIST and PERCIST criteria in solid tumors: A pooled analysis and review. *Oncotarget* **7**, 27848–27854 (2016).
41. Experts in Chronic Myeloid Leukemia, The price of drugs for chronic myeloid leukemia (CML) is a reflection of the unsustainable prices of cancer drugs: From the perspective of a large group of CML experts. *Blood* **121**, 4439–4442 (2013).
42. P. B. Bach, L. B. Saltz, R. E. Wittes. "In cancer care, cost matters," *New York Times*, 14 October 2012, p. A25.
43. K. T. Schmidt, C. H. Chau, D. K. Price, W. D. Figg, Precision oncology medicine: The clinical relevance of patient-specific biomarkers used to optimize cancer treatment. *J. Clin. Pharmacol.* 10.1002/jcph.765 (2016).
44. R. G. Koleva-Kolarova, M. J. W. Greuter, M. van Kruchten, K. M. Vermeulen, T. Feenstra, E. Buskens, A. W. J. M. Glaudemans, E. F. J. de Vries, E. G. E. de Vries, G. A. P. Hospers, G. H. de Bock, The value of PET/CT with FES or FDG tracers in metastatic breast cancer: A computer simulation study in ER-positive patients. *Br. J. Cancer* **112**, 1617–1625 (2015).
45. S. S. Gambhir, J. E. Shepherd, B. D. Shah, E. Hart, C. K. Hoh, P. E. Valk, T. Emi, M. E. Phelps, Analytical decision model for the cost-effective management of solitary pulmonary nodules. *J. Clin. Oncol.* **16**, 2113–2125 (1998).

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