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REVIEW ARTICLE

Imaging and Oncologic Drug Development

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ABSTRACT

For decades anatomic imaging with computed tomography or magnetic resonance imaging has facilitated drug development in medical oncology by providing quantifiable and objective evidence of response to cancer therapy. In recent years metabolic imaging with [^{18}F]fluorodeoxyglucose–positron emission tomography has added an important component to the oncologist's armamentarium for earlier detection of response that is now widely used and appreciated. These modalities along with ultrasound and optical imaging (bioluminescence, fluorescence, near-infrared imaging, multispectral imaging) have become used increasingly in preclinical studies in animal models to document the effects of genetic alterations on cancer progression or metastases, the detection of minimal residual disease, and response to various therapeutics including radiation, chemotherapy, or biologic agents. The field of molecular imaging offers potential to deliver a variety of probes that can image noninvasively drug targets, drug distribution, cancer gene expression, cell surface receptor or oncoprotein levels, and biomarker predictors of prognosis, therapeutic response, or failure. Some applications are best suited to accelerate preclinical anticancer drug development, whereas other technologies may be directly transferable to the clinic. Efforts are underway to apply noninvasive in vivo imaging to specific preclinical or clinical problems to accelerate progress in the field. Because resources are limited, and patient suffering from failed or ineffective therapy continues, a concerted effort is being made to address these issues. Many simultaneous activities involving academia; the pharmaceutical, device, and biotechnology industries; US Food and Drug Administration; National Cancer Institute; Centers for Medicare and Medicaid Services; and specialized networks sponsored by the National Institutes of Health are beginning to address these issues to develop consensus recommendations and progress in this important area.

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INTRODUCTION

The 5-year survival from the most common cancers affecting people living in the United States has changed little in the last two decades, with some notable exceptions.[1] According to 2005 American Cancer Society data, the death rate per 100,000 people from cancer in the United States was 193.9 in 1950 and 193.4 in 2002. Although molecularly targeted therapy has provided some promise in the last few years, it has become clear that all too commonly the impact on survival is limited due to drug resistance and disease progression.[2-5] Although intensive efforts continue to develop strategies to reverse resistance, significant progress has been made in improving survival by earlier detection of breast and colon cancer, allowing surgery and adjuvant therapies with associated better outcomes at an earlier stage of disease. Another area where progress has been made is with use of neoadjuvant therapies to allow response before surgery and additional therapies. In all cases imaging has been crucial for staging as well as monitoring response to therapy.[6] Molecular imaging has great potential to become incorporated into the drug development and monitoring process (Table 1).[7,8]

In recent years, advances in the molecular understanding of cancer pathogenesis have allowed additional insights into the determinants of disease progression and therapeutic response. For nearly a decade comprehensive gene expression profiling has been defining molecular signatures that have yet to find their way into standard clinical practice with rapid turnaround at an affordable price.[9] In the same way that serum markers of disease (eg, alpha-fetoprotein, carcinoembryonic antigen, CA-125, or lactate dehydrogenase) assist clinicians in monitoring disease burden or therapeutic response in real time, analysis through imaging of molecular changes within human tumors in vivo offers the opportunity for earlier modifications of therapy based on assessment of prognostic or therapeutic determinants. Such imaging will greatly complement assessments based on tissue pathology, genomics, and the emerging field of serum proteomics.[10] Imaging technology can detect noninvasively specific molecular changes associated with the presence of early malignant disease, disease recurrence, or likelihood of response or the lack thereof to continued therapy. These advances also have great potential to accelerate drug development in the clinic by allowing the testing of new therapeutics earlier in many patients based on knowledge of specific molecular alterations, and by providing earlier predictive information regarding response to therapy.

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Table 1. Promise of Imaging Science

Features of imaging
Noninvasive, optical biopsy
Sequential/multiple sampling
Quantitative localization
Molecular target expression
Levels
Patterns
Applications
Screening/early detection
Early diagnosis
Staging and therapy monitoring
Drug development tool
Molecular target–based drug screening
Imaging of drug biodistribution, in 3D and within tumor microenvironment
Target-based validation in animal models
Imaging of drug-target interaction in vivo
Coregister drug distribution with drug target expression
Coregister drug distribution with drug effect

Considerable progress has been made in the in vitro and preclinical development of molecular imaging agents. Small molecule imaging agents that can detect specific proteins or nucleic acids within living human cells, including mutant oncoproteins, have been studied, and some progress has been made in preclinical models.[11] Antibody therapeutics represent a class for which imaging is particularly promising. Cell surface receptors, such as those bound by epidermal growth factor or vascular endothelial growth factor (VEGF), offer opportunities for antibody-mediated target imaging as well as radiochemical or other cargo delivery directly to the malignant cells or their vascular supply.[12-14] Molecularly targeted agents that bind tightly to kinase active sites or other targets within tumor cells provide another approach for target imaging after conjugation to radioisotopes or optical imaging agents.[15-17]

In the preclinical area, using the same applications discussed above, imaging provides a powerful tool for every aspect of the drug development process. This includes using the ability to image molecular changes to design targeted screens for identification of candidate small molecule therapeutics (Fig 1).[18] It also includes the development of animal models of human tumors that can allow drug target validation as well as monitoring of therapeutic efficacy in tumors with different combinations of genetic alterations.[19,20] Such information can be efficiently obtained from animal models in preclinical testing and can be extremely useful as in vivo molecularly targeted imaging in cancer patients evolves to a point where the presence of the same targets can be visualized. Bioluminescence is particularly useful in preclinical drug development strategies because of its ability to image gene transcription or protein-protein interactions as well as tumor volume or tumor microenvironment.[21-25] These techniques should allow cell-based drug screens targeting specific molecular changes relevant to tumor progression or therapeutic resistance to become amenable to high-throughput approaches. In addition to molecular targets such as deregulated oncogenes, their oncoprotein products, and tumor suppressor genes, the expression or function of which is deficient in human cancers, imaging offers the opportunity to detect other important aspects of tumor progression including metabolic or physiologic changes, vascular changes, or tumor cell death. Changes in gene expression and protein levels after therapy that can be detected noninvasively through imaging offer additional opportunities to develop tests for prognostic or therapeutic monitoring.

At the clinical level, the value of molecular imaging has also become evident. Target imaging can be extremely helpful in determining why a therapy is working or not working, in addition to a potential clinical use in stratifying patients to receive or not receive a specific therapeutic agent based on imaging of the therapeutic target. Combining molecular target imaging with other biomarker determinants of prognosis or therapeutic response is likely to increase predictive value for decision making because of tumor heterogeneity as well

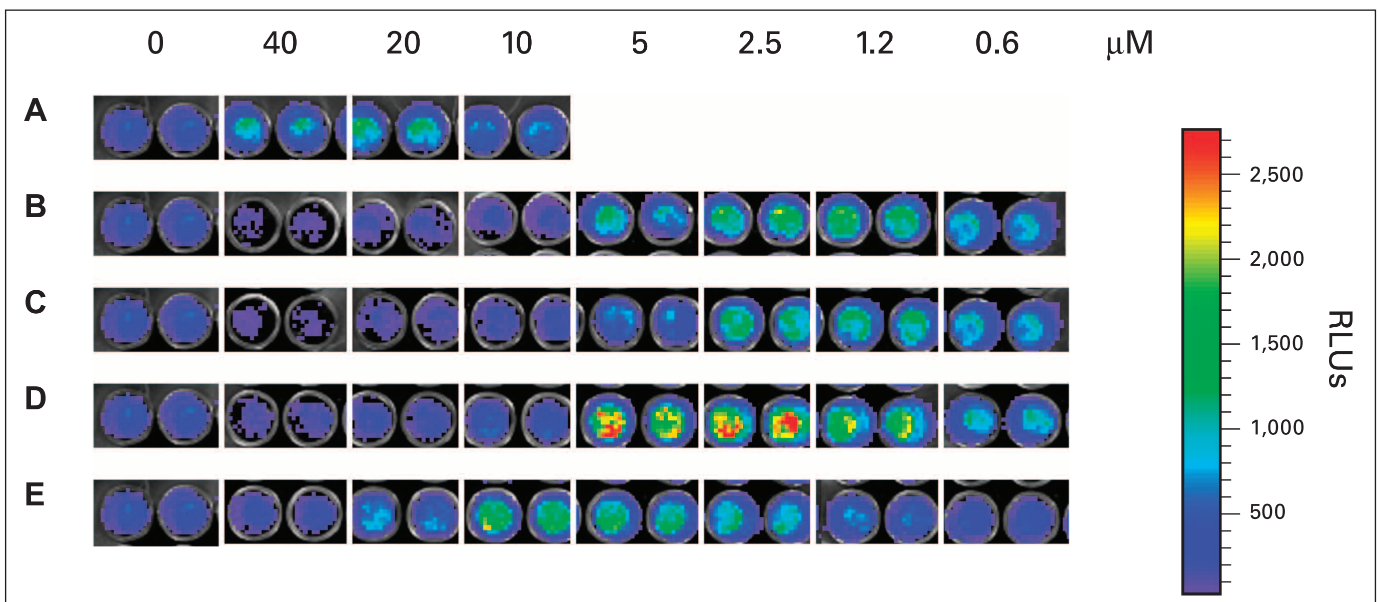

Fig 1. Imaging target activation in live cell-based assay. (A) CP-31398; (B-E) CP-31398 derivatives were found to activate p53 family signaling in the nanomolar range of drug concentration suggesting potential use of lower doses in efficacy studies. The color gradient at the right shows relative light units (RLUs). Data are from reference 18.

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as the predilection of cancers to evolve genetic or epigenetic alterations to evade therapy. Such strategies include classical anatomic imaging techniques combined with novel functional imaging approaches, including coregistration between computed tomography (CT) or magnetic resonance imaging (MRI) and [^{18}F]fluorodeoxyglucose–positron emission tomography or fluorothymidine–positron emission tomography,[26-32] the latter measuring cell proliferation, which is a downstream effect mediated by molecular targets.

For the newer imaging probes developed for specific molecular targets, formidable developmental hurdles still exist for their clinical use. For example, delivery of adequate tracer to the target to allow noninvasive visualization is often a problem. Radiotracer labeling is not readily available for most of the new drugs, and in those instances where labeled drugs are available, delivery to the target at concentrations adequate for noninvasive visualization is still not often possible. There are many factors that contribute to successful probe development, as listed in Table 2.[7]

MOLECULAR TARGETS FOR ANTICANCER DRUG DEVELOPMENT

Overview

A number of well-defined genetic alterations contribute to the immortalization, transformation, and progression of human cancers.[33-35] These include telomerase activation, overexpression or mutation of a number of cellular oncogenes, loss, hypermethylation or mutation of tumor suppressor genes, cell cycle deregulation, suppression of apoptosis, angiogenesis, activation of proteases that promote invasion and metastasis, and evasion of the host immune system. All of these alterations provide molecular targets that are potential candidates for imaging (Table 3). Classically, transformation involves changes in cell shape, loss of contact inhibition in monolayer cell culture, and anchorage-independent growth in soft agar. In vivo, transformed cells acquire the ability to form tumors in immunocompromised mice. The host immune system provides a powerful tumor suppressor mechanism through the action of the tumor necrosis factor family of cytotoxic ligands and cytotoxic T cells, whereas immune suppression contributes to cancer development.

Inflammation has become increasingly recognized as a contributor to cancer development, and components of the stroma also support tumor growth and survival.[36] A number of tyrosine kinase cell surface receptors promote cell proliferation and survival, and include ErbB2/HER2/*neu*, epidermal growth factor receptor (EGFR) and insulin-like growth factor receptor. These receptors trigger a number of intracellular signaling cascades including the RAS-RAF-MAPK-ERK-c-JUN (*RAS* oncogene product–v-*RAF* murine sarcoma viral oncogene homolog product–mitogen-activated protein kinase–extracellular signal-regulated kinase–v-*JUN* avian sarcoma virus 17 oncogene homolog product–phosphatidylinositol-3-kinase–thymoma viral proto-oncogene product, also known as protein kinase B) and PI3K-AKT pathways.[37,38] The RAS pathway ultimately influences cell proliferation and cell migration, whereas the AKT pathway leads to cell survival. Targets of AKT include MDM2 (mouse double minute 2 homolog), mTOR (mammalian target of rapamycin), p27, p21, Forkhead, and others that promote cell survival and proliferation. In addition to gene amplification, which can increase gene copy number and gene expression (for example, in the case of HER-2/*neu* or EGFR), mutations frequently lead to oncogene activation. The *RAS* genes are mutated frequently in human cancer, including colon cancer, adenocarcinoma of the pancreas and esophagus, among many others. v-*Myc* avain myelocytomatosis viral oncogene homolog product (c-MYC) is frequently overexpressed or mutated in epithelial malignancies and is involved in activating chromosomal translocations in hematopoietic malignancies such as Burkitt's lymphoma. One of the hallmarks of cancer is genomic instability, which remains poorly understood but could provide a key target for therapy in the future.[39-41] Microsatellite instability has been linked to mutations or silencing of mismatch repair genes and cancer development in both inherited (hereditary nonpolyposis colorectal cancer) and sporadic tumors.[42]

Colon Cancer

Colorectal cancer provides one of the best-understood models of human cancer progression because the progressive lesions have been accessible for histochemical and molecular analysis, and genetic studies have led to the identification of important genes and target pathways.[43] The earliest change involves mutations of the adenomatous polyposis coli (*APC*) gene located on human chromosome 5q. *APC* mutations lead to familial adenomatous polyposis and are also found frequently in sporadic colorectal cancers. *APC* gene mutation leads to release of oncogenic β-catenin suppression that normally leads to its proteasomal degradation in the cytoplasm. *APC* mutation or β-catenin mutation can target increased levels of β-catenin to the

Table 2. Considerations for Successful Probe Development

Targets have low nanomolar to micromolar concentrations
Intracellular delivery usually necessary
Amplification of signal-to-noise ratio usually necessary
Imaging probes should have a mass in tissues in a range of 0.1 to 0.01 of the target concentration so as not to exert mass or pharmacologic effects
Probe must have low nonspecific binding
Washout of probe must be sufficiently slow to allow accumulation at target site, but rapid enough to provide adequate target to background contrast
Imaging probe development often occurs late in the drug development process
Probe development requires rigorous pharmacokinetics characterization
Radiolabeling can change the binding properties of small molecular probes
Metabolites of the radiolabeled probe can lead to inaccurate ADME of the probe
Pharmacokinetics characterization can be essential to evaluating kinetic models that reflect the target
Peptides afford specificity and binding affinity, but are cleared and degraded rapidly
Antibodies afford specificity and binding affinity but can cause immunogenic reactions

Abbreviation: ADME, absorption/distribution/metabolism/excretion.

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Table 3. Molecular Target Candidates for Molecular Probe Development

HER-2/*neu* (*erb*B-2)	BCR-ABL	APC	HDACs
EGFR (*erb*B-1)	RAS	BRCA1, BRCA2	CpG islands
EGFRviii	B-RAF	P53	COX-2
*erb*B-1/*erb*B-2	MEK	MDM2	RARβ
Pan-*erb*B	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	Metalloproteinases	EWS-FLI	
XIAP	Proteases	PTEN	
FLIP		NBS	
Decoy receptors		TCFs	

Abbreviations: HER-2, human epidermal growth factor receptor 2; BCR-ABL, breakpoint cluster region—Abelson murine leukemia fusion protein; APC, adenomatous polyposis coli tumor suppressor; HDAC, histone deactylace; EGFR, epidermal growth factor receptor; CpG, cytosine phosphate guanine; B-RAF, v-*RAF* murine sarcoma vital oncogene homolog B1 product; COX-2, cyclo-oxygenase-2; MEK, mitogen-activated protein kinase; MDM2, mouse double minute 2, human homolog of p53 binding protein; RARβ, mitogen activated protein kinase; MDM2, mouse double minute 2, human homolog of p53 binding protein; ERK, extracellular signal-regulated kinase; RXR, retinoic X receptor; VEGF, vascular endothelial growth factor; AKT, thymoma viral proto-oncogene product, also known as protein kinase B; VEGFR, vascular endothelial growth factor receptor; c-KIT, v-*KIT* Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog product; IGFR, insulin-like growth factor receptor; TGF-β, transforming growth factor beta; PDGFR, platelet-derived growth factor receptor; NFκB, nuclear factor kappa B; *DCC*, tumor suppressor gene deleted in colon cancer; iNOS, induced nitric oxide synthase; TNF, tumor necrosis factor; mTOR, mammalian target of rapamycin; c-MYC, v-*MYC* avian myelocytomatosis viral oncogene homolog product; ER, estrogen receptor; c-JUN, v-*JUN* avian sarcoma virus 17 oncogene homolog product; AR, androgen receptor; CHK-2, checkpoint-like protein-2 (serine/threonine kinase); CDK, cyclin-dependent kinase; IAP, inhibitor of apoptosis; HIF, hypoxia-inducible factor; *DPC4*, deleted in pancreatic carcinoma locus 4; PPARγ, peroxisome proliferator-activated receptor γ; BAX, B-cell protein-2 (BCL2)-associated X protein; E2F1, E2F transcription factor 1, a retinoblastoma tumor suppressor binding protein; PAPR, poly(adenosine diphospate-ribose) polymerase; BCL, B-cell protein-2 (facilitator of apoptosis); BCL_{XL}, extracellular BCL protein; ATM, ataxia telangiectasia mutated gene product; hTERT, human telomerase reverse transcriptase; EWS-FLI, Ewing sarcoma-Friend leukemia virus integration fusion gene product; XIAP, X-linked inhibitor of apoptosis protein; PTEN, phosphatase and tensin homologue deleted on chromosome 10; FLIP, FLICE (FADD [FAS-associated death domain]-like interleukin-1β-converting enzyme)-like inhibitory protein; NBS, Nijmegen breakage syndrome; TCF, T-cell factor.

nucleus to associate with T-cell factor transcription factors and activate growth-promoting genes such as c-MYC or cyclin D. *APC* mutations promote polyp formation, but for colorectal cancer and metastases to occur, additional events are involved. For colorectal cancers to develop, the additional well-known events include methylation changes, *RAS* gene mutations, mutations in the *P53* tumor suppressor gene on chromosome 17p, or alterations on chromosome 18q that involve the deleted in pancreatic cancer gene (*DPC4*) or the deleted in colon cancer gene (*DCC*); overexpression of the phosphatase gene *PRL3* has also been associated with metastases from colorectal cancer.[44,45]

Angiogenesis is another important contributor to colorectal cancer progression, and recently the US Food and Drug Administration approved the antibody bevacizumab (Avastin; Genentech, South San Francisco, CA), which targets VEGF, for use with irinotecan, fluorouracil, and leucovorin as first-line therapy in advanced colorectal cancer due to a highly significant prolongation in patient survival in this patient population.[46] Deregulation of cell cycle control and altered topological expression of cyclin-dependent kinase (CDK) inhibitors such as p21 (WAF1/CIP1) occur in polyps and carcinomas.[47] Cyclooxygenase-2 (COX-2) and prostaglandins contribute to colorectal cancer and COX-2 inhibitors have been shown to reduce polyp number and size. Recent studies have implicated β-catenin as a mediator in COX-2 signaling pathways and prostaglandin E2 mediation of colon cancer.[48] In addition to DPC4, the transforming growth factor-β pathway has been implicated in colorectal cancer through mutation of the RII receptor in mismatch repair–deficient tumors.[49] Such tumors are also prone to developing *BAX* gene mutations or loss of expression.[50] Although *BAX* mutations have not been shown to be initiating events in colorectal cancer, they can contribute to resistance to therapy.

Molecular Targets in Specific Tumor Types

A number of molecular targets are found in several tumor types as well as those that are unique or highly relevant to specific tumor types. Chromosomal translocations that arise as a consequence of genomic instability or DNA repair defects contribute to the formation of oncogenic fusion proteins that provide ideal targets for therapy. These fusion protein targets include Ewing sarcoma–Friend leukemia virus integration fusion gene product (EWS-FLI) in Ewing's sarcoma[51] and BCR-ABL in chronic myelocytic leukemia,[52] among others. Mutations in the lipid and protein phosphatase and tensin homologue deleted on chromosome 10 (PTEN) occur commonly in brain tumors, prostate, and other tumors. PTEN mutations lead to PI3K-mediated activation of oncogenic AKT.[53] AKT can also become activated through the action of a number of oncogenic tyrosine kinase receptors including HER-2/*neu*[54] and EGFR.[55] EGFR frequently is overexpressed in a wide range of tumors including non–small-cell lung cancer (NSCLC), esophageal cancer, and colorectal cancer. An oncogenic isoform of EGFR is found solely in gliomas, and this isoform, EGFR vIII, represents a unique target in that tumor type.[56,57] EGFR mutations have been found in approximately 10% of NSCLC,

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in the subgroup of patients that have shown objective responses to the kinase inhibitors gefitinib (Iressa; AstraZeneca, London, UK) or erlotinib (Tarceva; OSI Pharmaceutials Inc, Melville, NY).[58] *BRAF* gene mutations frequently are found in melanoma, and targeted therapy has been developed against the RAF kinase. Interestingly, the RAF kinase inhibitor sorafenib was approved in 2005 for therapy of renal cell cancers, where it appears to have antiangiogenic effects through additional kinase targets including a VEGF receptor.[59] RAS mutations are extremely common in pancreatic cancer, and farnesyl transferase inhibitors have been developed to block their activity in this target organ. Because RAS mutations are also common in other tumor types, farnesyl transferase inhibitors are being tested alone or in combination therapies in many tumor types.[60]

Importance of DNA Repair Defects

In addition to mismatch repair defects that occur in colorectal tumors,[61] other repair defects contribute to cancer development and provide additional molecular targets for therapeutic development. Mutations in the *XP* genes occur in xeroderma pigmentosum and contribute to cancer susceptibility.[62] Cells from xeroderma pigmentosum patients are actually also more susceptible to being killed by DNA-damaging therapeutics. Mutations in the *BRCA1* and *BRCA2* genes occur in hereditary breast cancer and result in defective repair that is mediated through homologous recombination.[63] BRCA1-deficient cells have high sensitivity to ionizing radiation and DNA-damaging chemotherapeutics such as cisplatin. BRCA2-deficient cells have been identified in Fanconi anemia and have been found to be sensitive to mitomycin.[64] In pancreatic cancer, *BRCA2* mutations have been described, and alterations in other Fanconi genes have also been found to correlate with mitomycin sensitivity due to repair defects.[65] Sonic hedgehog has been proposed as a target for therapy in pancreatic and other cancers.[66] Recently PARP inhibitors have been proposed for clinical testing in BRCA2-deficient tumors.[67] *ATM* gene mutations that occur in the cancer-prone ataxia telangiectasia syndrome, *NBS* mutations in Nijmegin breakage syndrome, and *CHK2* and *P53* mutations in Li-Fraumeni syndrome affect the DNA damage response and repair pathways that can promote tumor formation and altered sensitivity to therapy.[68]

Some Universal Targets

Activation of nuclear factor kappa B (NFκB) signaling has been widely observed in human cancer and can contribute to resistance to therapy.[69] NFκB activation results in transcription of genes that promote cell survival and cell proliferation. NFκB transcriptional targets include the prosurvival genes that produce IAP (inhibitor of apoptosis) 1, IAP2, and BCL_{XL} (extracellular B-cell protein), as well as the cell cycle regulatory gene cyclin D1. Both NFκB and its transcriptionally activated genes represent molecular targets for therapeutic development. Proteasome inhibitors including bortezomib (Velcade; Millenium Pharmaceuticals, Cambridge, MA), which is approved for treating multiple myeloma,[70] ultimately inhibit the degradation of IκB (inhibitor of κB), which normally keeps NFκB in the cytoplasm and inactive with regard to transcriptional activation. Another universal target is the hypoxia-inducible factor (HIF) that is involved in transcriptional activation of genes that promote cell survival in response to hypoxic stress.[71] Targets of HIF include erythropoietin, the glucose transporter Glut1, and VEGF, which promotes angiogenesis and tumor survival. Histone deacetylases have also been targeted in recent years for therapy in cancer with histone deacetylase inhibitors.[72] An emerging target for therapy is micro-RNA, the overexpression of which can modulate gene expression and contribute to oncogenic transformation.[73]

A number of additional targets exist that are the subjects of ongoing efforts for drug targeting. Cell cycle deregulation is a hallmark of cancer, and provides several therapeutic targets, including CDKs (CDK4, CDK2, cdc2) as well as E2F1 (E2F transcription factor 1). Tumor cells have increased E2F1 activity, and an E2F1 promoter driving a suicide gene has been proposed as a therapeutic agent.[74] The epithelial-to-mesenchymal transition that has been observed in vivo as tumors gain invasive and metastatic potential provides another opportunity for therapeutic intervention. Transcription factors such as Snail or Slug have been implicated in epithelial-to-mesenchymal transition and may predict clinical outcome.[75] Integrins, chemokine receptors, and matrix metalloproteases provide additional therapeutic targets in cancer.[76]

IMAGING IN DIFFERENT PHASES OF DRUG DEVELOPMENT

Drug Screening

Virtually every gene or protein target relevant to cancer development, progression, or therapeutic response can be exploited in therapeutic design through approaches involving molecular imaging. Noninvasive optical imaging provides a platform for cell-based assays with high sensitivity, high throughput capability, and the potential to modulate molecular events using small molecules or other types of therapeutic agents (Figs 1 and 2).

Various luciferase genes including firefly luciferase and Renilla luciferase encode proteins that use distinct substrates such as *D*-luciferin and coelenterazine, respectively, resulting in light emission. Introduction of constitutively active or gene-specific promoter-driven luciferase genes into cells allows for screening of modulation of cell number or transcriptional activity.[80] Because a number of targets in cancer are transcription factors, including HIF, p53, NFκB, β-catenin, and other oncogenic transcription factors such as those that result from chromosomal translocations, it is possible to identify activators or blockers to modulate therapeutic response.[18,20,81,82]

The technology exists to use in vivo imaging to detect protein-protein interactions based on reporter reconstitution through the bringing together of split domains or through designs similar to mammalian two-hybrid strategies.[83] There are also strategies that rely on bioluminescence resonance energy transfer.[23] Such imaging makes it possible to identify small molecules that can promote or disrupt protein-protein interactions for therapeutic gain.[24,84] In addition, a number of oncogenic proteins require interaction with other cellular proteins to signal cell proliferation, survival, or tumor progression. Disruption of these interactions is expected to lead to growth arrest, cell death, or tumor regression. Dual-reporter designs make it possible to monitor and manipulate multiple events simultaneously (Fig 3).[21,85]

Imaging of CDK2 activity and its inhibition has been possible through the use of a p27-luciferase fusion that is degraded on phosphorylation by CDK2.[86] Accumulation of bioluminescence in vivo has been demonstrated after exposure of tumor cells to the CDK inhibitors flavopiridol or roscovitine. The approach used illustrates how a drug target that affects the stability of another protein

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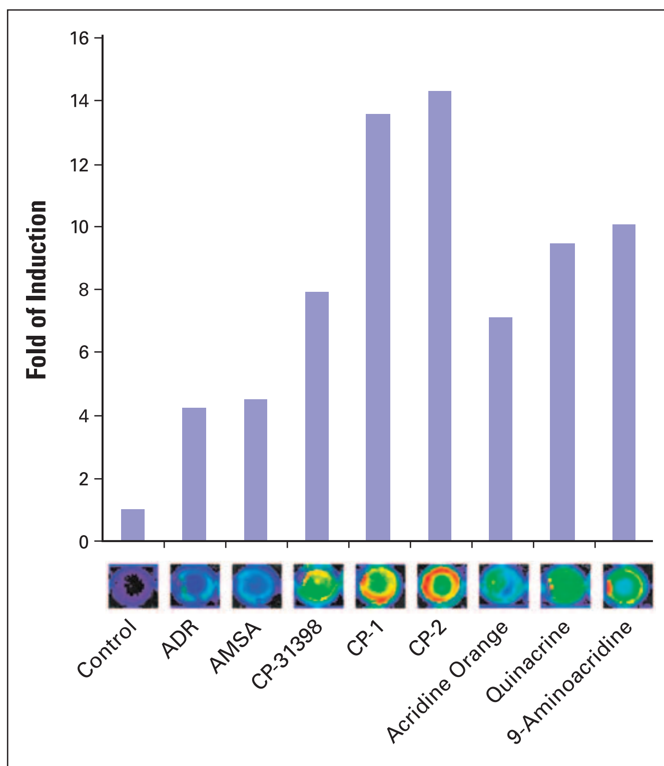

Fig 2. Acridine derivatives were found to activate p53 family signaling suggesting a novel molecular target. The color gradient at the right shows relative light units. Data are from reference 18. ADR, adriamycin; AMSA, amsacrine or 4'-(9-A cridinylamino)-3'-methoxymethanesulfonanilide; CP-1 and -2 and CP-31398, acridine analogs.

that can be imaged may be used to identify or investigate the effects of target inhibition.[86]

It is also possible to use imaging to screen for activators of apoptosis, for example, using the binding of fluorescent quantum dot-tagged annexin V to the cell surface of dying cells or through use of DEVD-modified amino-luciferin to detect caspase 3 activation in response to candidate small molecule therapeutics.[77,78,87] Molecular beacons, either DNA based or peptide based, can be used to identify small molecules with potential to alter gene expression (Fig 4) or enzymatic activity, including caspase or protease activity.[89,90] A cathepsin-sensitive probe has been described to detect increased cathepsin activity associated with colonic polyps and carcinomas.[88] Such a fluorescent or near-infrared probe can also be used to help identify inhibitors of cathepsin activity that may have therapeutic utility.

Genetic approaches can be used at the screening stage to control for a specific molecular target of interest in a particular screen.[19] In addition, if the target of interest for disruption by a small molecule is an inhibitor of apoptosis such as XIAP (X-linked inhibior of apoptosis) or BCL_{XL}, use of siRNA (small interfering RNA) can facilitate analysis of genetically matched cells except for the target of interest that is affected by the small molecules in a particular screen. As such, imaging can be used to compare rapidly the matched cell lines in a high-throughput screen.

It is difficult to overstate the impact of noninvasive imaging of molecular events in tumor cells on translational research and drug development research, beginning with the small molecule-screening phase. Visualization of molecular events and their modulation by candidate therapeutics can be performed with several thousand compounds simultaneously providing an image of the results that can be analyzed qualitatively or quantitatively before additional screening or validation. Multiple 96-well plates can be analyzed simultaneously at specific time points and over several days to follow the effects of candidate small molecules on target pathways or cell viability.[18]

Preclinical Testing

Noninvasive in vivo imaging offers great potential to facilitate translational drug development research at the animal testing phase. With some information about the maximum-tolerated dose of a given small molecule, one can begin to test efficacy in animal models of human cancer. In this regard, National Cancer Institute (NCI) databases contain toxicity information for thousands of compounds that have been tested during the last two decades.

There are a number of available models for preclinical efficacy testing. Classically, tumor xenografts growing either subcutaneously or orthotopically in immunodeficient mice have been used to investigate the effects of candidate therapeutics on tumor growth or tumor regression. There are also syngeneic models that rely on mouse tumors that grow in immunocompetent mice. All of these models are amenable to the use of noninvasive optical imaging using bioluminescence or fluorescence to assess tumor volume. Although the imaging has some limitations (for example, there is lower signal obtained from deeper tumors), there are predictable relationships between cell number, tumor volume, and optical signal.[20] Performing controlled experiments and obtaining multiple images longitudinally provides reliable information regarding response to therapy; at the same time, money on animal costs is saved because fewer animals are required.

With xenograft models it is possible to obtain information using multiple reporters to evaluate tumor volume as well as effects on the molecular target of interest.[19,21,91] This is useful because molecularly targeted therapy often ends up affecting additional targets in vivo. A good example is the antiangiogenic effect of the recently approved RAF kinase inhibitor in renal cell cancer. The use of multiple reporters also offers the potential to monitor molecular changes within tumors, including aspects of the tumor microenvironment that may be relevant to therapeutic response.

A number of transgenic mouse models have emerged in recent years that may also be useful for testing therapeutics. Some of the models include knockouts of various tumor suppressor genes (eg, *P53*, mismatch repair *MSH2*, *APC*, *VHL*, and p16/Arf knockouts). There are also tissue-specific knockouts of tumor suppressor genes such as *BRCA1* or *PTEN*, among many others.[92-94] Other transgenics include tissue-specific oncogene activation (eg, RAS activation in the lung or pancreas, with or without mutant p53 expression),[95-99] or various oncogenes activated in the mammary gland, including *HER-2/neu*, *RAS*, *MYC*, or cyclin D1.[100,101] Several of the transgenic models offer the potential to turn off the oncogenic signal to determine effects on tumor maintenance or ultimate resistance to therapy.[102-105] Two applications have been described where imaging was performed in transgenic mice. Deletion of the Rb tumor suppressor gene in the pituitary was performed in cells that only expressed luciferase in the Rb-deficient state, and so pituitary tumors could be imaged and the effects of therapeutics could be tested.[106] A second example involved transplantation of constitutive luciferase-expressing tumor cells

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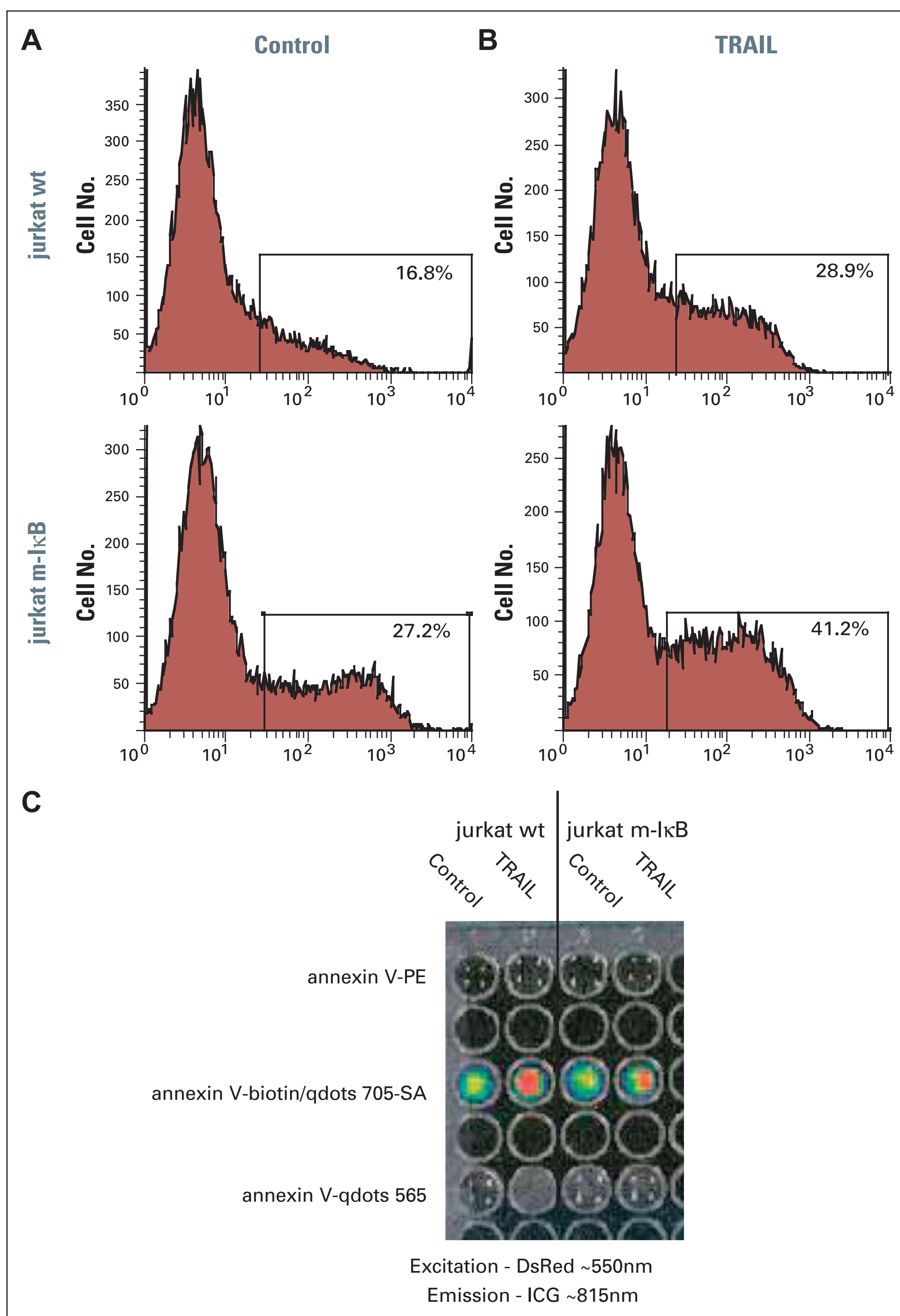

Fig 3. Fluorescence imaging of apoptosis in human tumor cells using Annexin tagged with Q-dots. (A-B) Human wild-type (wt) or mutant IκB expressing Jurkat cells were untreated (A) or treated (B) with TRAIL, stained with Q-dot tagged Annexin V and analyzed by flow cytometry. Brightly fluorescent cells are enclosed in a box and quantified (%). (C) Fluorescence cell-based imaging of the same cells in A-B using fluorescently tagged Annexin V as indicated. Data are from reference 87. IκB, inhibitor of κB; TRAIL, tumor necrosis factor–related apoptosis-induced ligand.

from an inducible MYC-transgenic model into mice that did not express luciferase.[107]

It is important to realize the limitations of transgenic models when they are used for therapeutic testing. A particular genetic alteration, although it leads to tumor development in mice, may only mimic rare situations in humans, and the tumors may lack the environmental influences as well as genomic instability associated with human cancers. The transgenic models include a limited number of genetic alterations in specific cell types. The order of events and their occurrence in specific cell types in humans in vivo is the subject of intensive investigation, and the role of such alterations in stem cells and tumor cells is still being unraveled. The technology exists to validate transgenic mouse models further with respect to their relevance to human cancer with all its genetic and epigenetic alterations. Human cancer is complex and different from mouse cancer in its pathogenesis, and thus it is likely that all available models will continue to be evaluated in the coming years in therapeutic testing. It is clear that useful information can be obtained from the simpler xenograft

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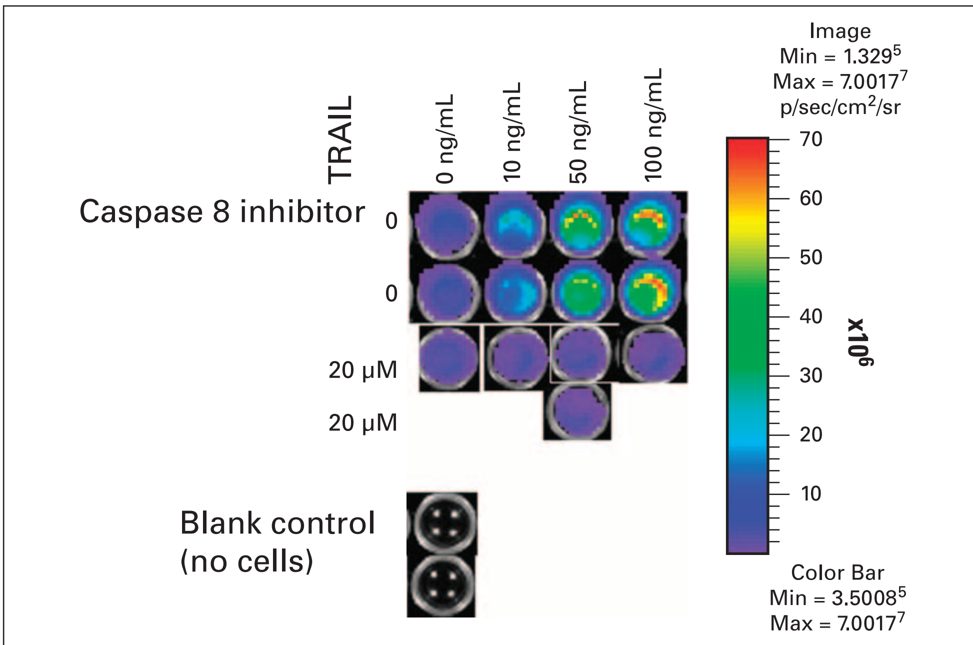

Fig 4. Cell-based bioluminescence imaging of apoptosis using DEVD-aminoluciferin. p53−/− HCT116 colon cancer cells were untreated or treated with various doses of TRAIL (as indicated) for 6 hours and apoptosis was imaged following incubation of the cells in the presence of DEVD-aminoluciferin. Cleavage of DEVD by TRAIL-activated caspases releases luciferin that is then used by luciferase as a substrate to emit bioluminescence that is imaged. Data are from reference 77. TRAIL, tumor necrosis factor–related apoptosis-inducing ligand.

models before investing in extensive preclinical development, given that agents that are not efficacious in these models are unlikely to be useful in humans.

Efforts have been underway to image physiologic parameters within tumor masses exposed to therapeutic agents in vivo. Tumor vasculature has been imaged after exposure of syngeneic tumors to antiangiogenic agents using ultrasound to detect blood flow.[108,109] Classical methods including CT, MRI, and more recently positron emission tomography (PET) imaging are providing extremely valuable information regarding tumor volume, tumor response, and the metabolic state of the treated tumors. Coregistration of various methods (for example, combining anatomic imaging, such as CT, with functional imaging, such as PET) is currently under investigation to determine the most efficient use of multiple methods for specific applications. Integration of genetic approaches in the preclinical testing phase is useful for target validation, just as it is in the screening phase.

Noninvasive imaging technologies offer applications that can be used to gain additional information in preclinical models. These include imaging drug targets in tumors or normal tissues and the development of in vivo assays for imaging drug toxicity. Some progress has been made recently toward fluorescence-based imaging of apoptosis in response to therapy in freshly resected human colon.[110] This approach provides some indication of sensitivity and feasibility of doing this type of imaging endoscopically in living human patients. The approach also provides a unique reference point for toxicity determination

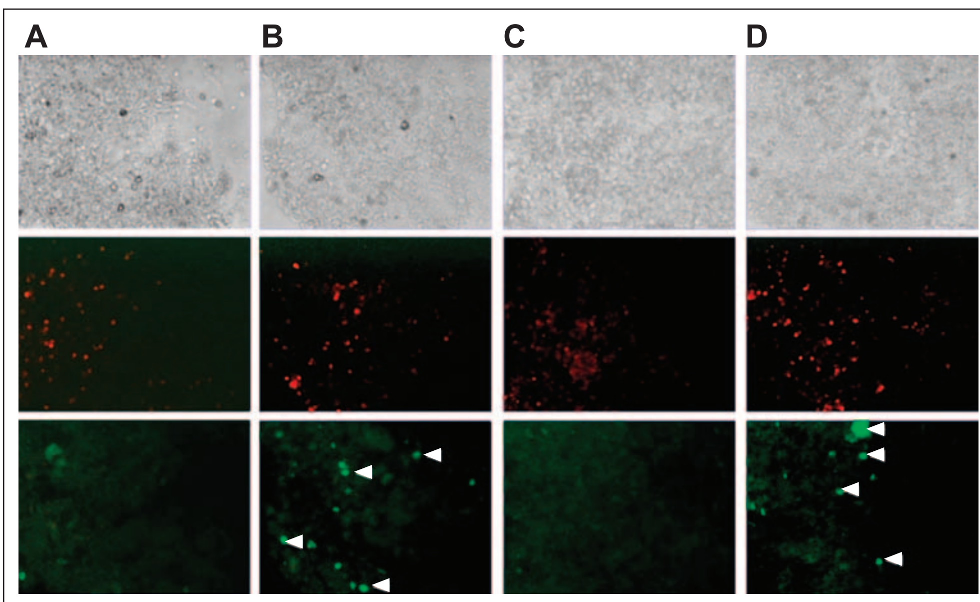

Fig 5. Fluorescence imaging of apoptosis in freshly-resected human colon. Human ascending colon explants were either untreated (A), treated with 200 μg/mL fluorouracil for 24 hours (B), 100 ng/mL TRAIL for 18 hours (C), or the combination of FU plus TRAIL (D). Bright field (upper panels), propidium iodide (middle panels), or FLICA (FITC-VAD-FMK; lower panels) stained images were obtained. Data are from reference 110. FU, fluorouracil; FLICA, fluorochrome-labeled inhibitors of caspases (eg, FITC, fluoroscein reactive isothiocyanate; VAD, valine, aspartic acid, dexamethasone; FMK, fluoromethylketone); TRAIL, tumor necrosis factor–related apoptosis-inducing ligand; Ad, adenovirus.

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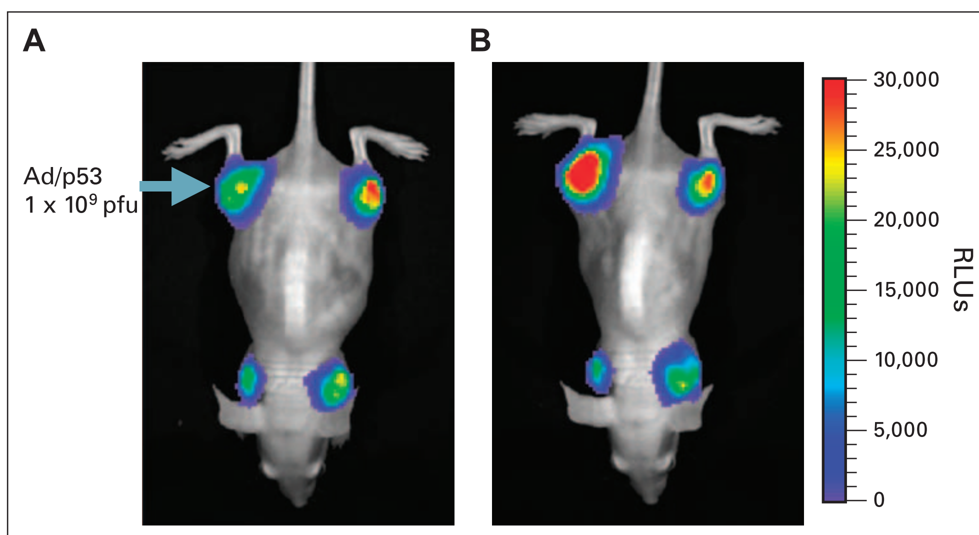

Fig 6. Imaging drug molecular target activation in vivo. Imaging gene expression before (A) or after (B) gene delivery into an estabished human tumor xenograft. A molecular response due to tumor suppressor gene delivery by adenovirus into one of four (arrow) established tumors is evident (B) as a bright bioluminescence signal emitted due to transcriptional activation in the injected tumor. The color gradient at the right shows relative light units. Data are from reference 20.

and may complement other existing approaches that rely on cell lines or xenografts. Other applications involve imaging of minimal residual disease in transplantable mouse models, and are important in understanding drug efficacy and resistance mechanisms.[79]

Clinical Trials

Classical imaging methods using x-rays, ultrasound, bone scans, PET scans, CT scans, or MRI are widely used by clinicians in clinical trials and in clinical practice. Anatomic imaging has been possible for many years and provides a basis to document objective response to therapy. However, one of the major limitations in current imaging strategies is that significant periods of time often must elapse while patients are treated for two to three cycles of chemotherapy before it is possible to document objective responses. The field of molecular imaging offers strategies that in the future may provide scans that can detect molecular events shortly after therapy and that can predict response or resistance to therapy.[6,7] At present, there are some serum markers, such as alpha-fetoprotein, lactate dehydrogenase, CA-125, immunoglobulin, prostate-specific antigen, or carcinoembryonic antigen, that are useful in monitoring therapeutic response when they are elevated in specific cancer types. However, for the majority of the common tumors, including lung cancer, breast cancer, CNS cancer, or a large fraction of colorectal cancers, there are no serum markers that can assist with response monitoring acutely after therapy. Clearly,

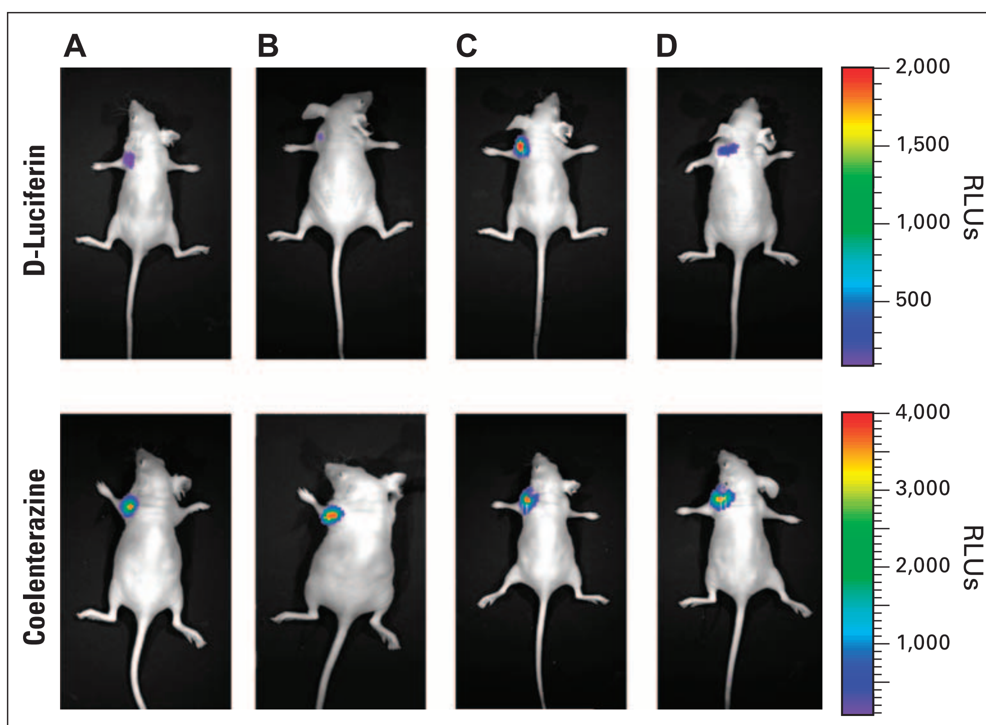

Fig 7. In vivo imaging of endogenous tumor suppressor p53-dependent transcriptional activity after systemic delivery of CPT11 (C). A dual-reporter in vivo experiment was performed to image tumor volume (lower panels) using a renilla luciferase reporter or chemotherapy-modulated transcriptional activity (upper panels) using a firefly luciferase gene regulated by the endogenous p53 stablized by CPT11. Untreated control tumors (A-B) or treated tumors lacking the drug target (D) are shown. The color gradients at the right show relative light units. Data are from reference 21.

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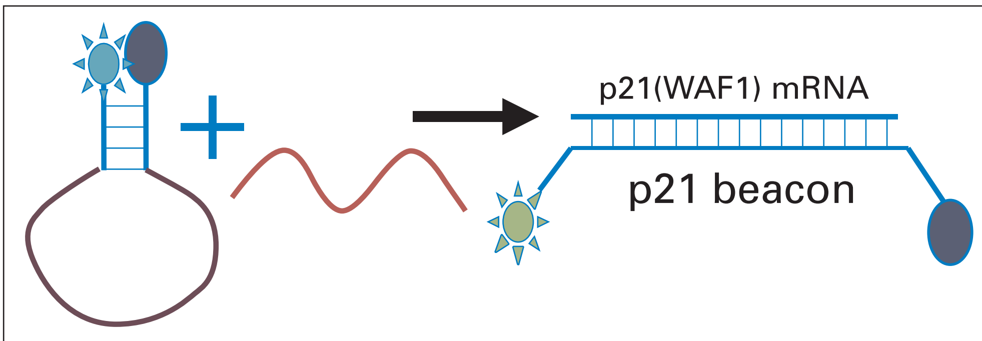

Fig 8. Principle of fluorescence emission from an activatable molecular beacon that senses gene induction. When the quenched beacon (left) hybridizes with its target mRNA, it opens up separating the quencher from the fluorophor (right) resulting in fluorescence emission.

as the field of serum proteomics progresses there will be an interface between serum biomarkers and imaging technologies that can complement each other in response detection.

Current approaches to use optical imaging in humans to monitor therapeutic response involve the use of near-IR imaging to detect oxy- and deoxy-hemoglobin, fat, and water content in breast tissue in patients that have received chemotherapy for advanced breast cancer.[111-113] There are early indications that such imaging may provide useful adjunctive information that appears worth additional testing in larger patient cohorts.[114] Such methodologies are being combined with classical methods including MRI or mammography, where coregistration offers additional important information.

Endoscopic approaches are being developed to image changes in the surface or subsurface epithelium throughout the GI tract. Using such fluorescence imaging technologies that incorporate fluorescent probes and specialized microscopes attached to fiberoptic devices of endoscopes, it is possible to image normal as well as neoplastic epithelium in the esophagus and colon.[115] Molecular beacons have potential for transferability to the clinic to image molecular changes associated with the potential to respond to therapy. Photodynamic therapy is an important area for which probes are being developed. Probes that can be activated by the tumor cells or the tumor microenvironment offer the potential for targeted therapy using light and photosensitizers.[116-118] Molecular imaging is furthest away from current clinical use, in part because few probes are available and the predictive molecular changes are still being discovered.[7,90]

OBSTACLES TO DRUG DEVELOPMENT AND HOW IMAGING MAY FACILITATE

The emergence of molecular imaging technologies offers great potential to facilitate drug discovery and development research. As described above there are numerous pathogenetic targets that are cancer-specific that can be exploited by novel therapeutics. However, this process requires multidisciplinary interactions and some collaboration between academia and industry to take advantages of resources and strengths offered by each. A recent NCI Network for Translational Research in Optical Imaging workshop on imaging and drug development included a session on drug discovery and preclinical development that addressed the current status of the field including perspectives from both academia and industry. It is clear that significant progress is being made within academia to develop screening assays that rely on imaging, as well as animal models that rely on noninvasive imaging, for drug testing and development. Nonetheless, within academia progress has been slow, in part because of limited resources, limited infrastructure, limited tools/models, and the general complexity of cancer. Pharmaceutical companies are using small animal imaging for drug development, although interaction and collaboration with academia has been limited. From industry there is greater emphasis on decision-relevant measurements, and there is a point of view that the impact of imaging, particularly optical imaging, is not yet clear. Even within industry, resources have limited development to a few therapeutic targets due to the high cost associated with performing large-scale clinical trials.

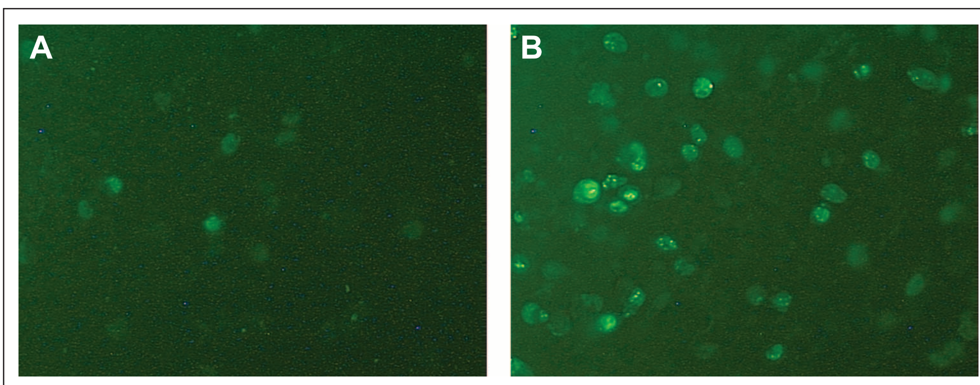

Fig 9. Imaging gene expression changes in response to chemotherapy exposure of human cancer cells. A p21-specific molecular beacon emitted fluorescence (B) after 8 hours of adriamycin treatment of lung cancer cells, as compared to untreated cells (A). The goal of this approach is to develop a cocktail of beacons that can predict response to therapy based on molecular events occurring in cells prior to death. Results are from reference 90.

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Some of the barriers to clinical translation include a diversity of imaging platforms with little standardization across testing sites or even among the different platforms at a given site. Probe development is a major issue with respect to clinical translation and acceleration of the drug approval process. Although it is acknowledged that imaging could be extremely helpful in accelerating the process of drug approval, for example by assisting with target validation, safety, and efficacy studies, there is a need for demonstration projects to document use of imaging at the various stages of drug development. It is also clear that more informed design of clinical trials could save billions of dollars that could be used to develop additional targets by better selection of patients who are more likely to benefit from specific therapies. For example, if only the 10% of patients with NSCLC who carry *EGFR* mutations are tested in clinical trials with EGFR kinase inhibitors, 90% of the patients who do not respond could be spared the time lost and the costs associated with ineffective therapy.

Investigators can access a number of resources for assistance with drug discovery and development research, including development and use of imaging approaches. The NCI has a number of grant programs that can assist with several aspects of drug discovery up to clinical testing, including Molecular Libraries Screening Networks, Network for Translational Research in Optical Imaging, In Vivo Cellular and Molecular Imaging Centers, and the Small Animal Imaging Research Program. The Developmental Therapeutics Program at the NCI has several hundred thousand chemical compounds that are available to investigators interested in screening to identify small molecules that can modulate molecular targets in cancer for therapy. There are existing databases that include toxicity profiles against the panel of 60 tumor cell lines from the NCI, gene expression profiles, and information about toxicity in mice. The Rapid Access to NCI Discovery Resources program provides assistance with discovery through assay development, computer modeling, protein production, chemical analog generation, and toxicity information, and the Development of Clinical Imaging Drug Enhancers program provides similar resources for development of molecular imaging probes and contrast agents. The NCI Rapid Access to Intervention Development programs assist with translation to the clinic, including providing materials synthesized using Good Manufacturing Processes, formulation research, pharmacologic methods, and investigational new drug–directed toxicology.

With regard to development of imaging approaches through multidisciplinary interactions to facilitate translational research, the NCI and US Food and Drug Administration have been working together in the Interagency Oncology Task Force and more recently on the Oncology Biomarker Qualification Initiative. In addition, the NCI in 2005 formed an Imaging and Drug Development Working Group to address the barriers to using imaging in drug development research from discovery to clinical use. These activities place integration of imaging approaches as a high priority, and ways to optimize use of imaging in translational research on cancer are being developed. This includes strategies to increase support for drug development and imaging research, as well as involvement of professional societies such as the American Society of Clinical Oncology, American Association for Cancer Research, and other stakeholders including academia, pharmaceutical companies, device industry, and government agencies (US Food and Drug Administration, as well as NCI and its various programs including the Cancer Imaging, Specialized Program of Research Excellence, Developmental Therapeutics, and Cancer Therapy Evaluation Programs), all of which represent important resources to achieve these goals.

In summary, molecular imaging offers great potential to accelerate drug discovery and development for the benefit of cancer patients. This includes the development of numerous cell-based assays for molecularly targeted therapy and their use in high-throughput screens that rely on noninvasive optical imaging. This also includes the development of molecular probes for imaging cancer drug targets; imaging drug-binding to its target(s) in vivo; imaging molecular determinants of therapeutic response after administration of therapy; and imaging activation of specific pathways, such as those associated with cell proliferation and cell survival, or physiologic changes associated with response, such as decreased vascular supply. A number of obstacles to progress in these areas, including the rapid and efficient integration of imaging technologies within all aspects of the drug discovery and development process, have been identified and efforts are underway to address the obstacles to facilitate translational drug development research on cancer. It is clear that in the future imaging will play a greater role in the drug approval process, given that target imaging and validation are becoming increasingly important at earlier stages of efficient drug development and clinical translation.

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The authors indicated no potential conflicts of interest.

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