Refinements are being made to apply this system to animal models. First, photoactivation furnishes only transient protein expression, thereby limiting the applicability of this approach to short-term biological phenomena. However, it should be possible to permanently alter gene expression profiles with a single treatment of light by coupling the light-inducible ecdysteroid strategy with the well established *Cre/loxP* recombinase system. Second, the *ortho*-nitrobenzyl moiety is removed with relatively short-wavelength (300–360 nm) light. The poor tissue penetrating properties of the latter precludes ready access to deeply interred tissue microenvironments. By contrast, multiphoton technology furnishes significantly deeper tissue penetration. A photolabile-protecting group containing a large, 2-photon absorbance cross-section has been introduced as a caging agent (*19*). These studies are currently in progress in the context of animal models.

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Rational Design for Peptide Drugs

The evolution of targeted macromolecules for molecular imaging with radiolabels transitioned from the highly specific but biologically long-lived monoclonal antibodies (mAbs) in blood resulting in images with low signal-to-noise ratio (SNR). The large size of mAbs (~145 kDa for IgG) (*1*) slows their diffusion into tissue, thus hindering their utility as imaging probes—at least for nuclear imaging, because of the hepatic metabolism of mAbs. The imaging requirement for low nonspecific signal from background necessitates rapid clearance and excretion of the labeled macromolecule probe from the blood to urine via the kidneys. The targeting macromolecule and labeling approach with radioactivity, fluorophores, MR-active metals, radioopaque atoms for CT, chelators, and linkers all may affect the biodistribution and excretory routes of a macromolecular probe.

Two possible alternatives to labeled mAbs using macromolecules with optimal biodistribution and excretory routes include (a) encapsulating the label into a particle, and (b) attaching multiple labels onto a macromolecular scaffold (*2*). In the first instance, a number of different macromolecular platforms, including polymers, proteins, dendrimers, liposomes, and ultrasmall superparamagnetic iron oxide particles, have been studied as carriers for imaging labels. In the second instance, multiple labels are attached to the backbone of a macromolecule (*3,4*). Preclinical studies of macromolecular carriers have established trends in routes and rates clearance with respect to agent size and charge, which depend on glomerular filtration, particularly over the ~30–50 kDa range (*5,6*).

The current discussion focuses on peptides targeting receptors for optical, PET, SPECT, MR, and CT imaging,

where it is essential that the amount of label (or degree of labeling for optical probes or specific activity for radiopharmaceuticals) be sufficient per probe per receptor for optimal SNR. Indeed, low-density receptors can be imaged in vivo with high-specific-activity radiotracers for PET and SPECT in conjunction with highly sensitive scanners. The development of CT and MR probes for receptor imaging, however, has been difficult because of the relatively low sensitivity of these imaging modalities. Thus, the broad challenge with designing optimal peptide probes for a single modality or cross-modality requires considerations of binding affinity, specificity, molecular weight, size, clearance rates, excretion routes, and toxicity.

Current State of Peptide Probes

The challenges with achieving regulatory approval of optical, PET, SPECT, MR, and CT macromolecular probes generally stem from a peptide probe's poor pharmacokinetics and excretion from the body. The unrealized promise of gadolinium-labeled macromolecules for receptor imaging with MR and CT is the result of long plasma half-lives, long-term tissue retention, and immunogenicity (*7,8*).

Modality-specific challenges also hinder clinical translation of visible fluorescence imaging, which suffers from high autofluorescent background, scatter, limited penetration, and absorption. Radiolabeled macromolecules (e.g., >30 kDa) require relatively long integration times for imaging, resulting in excessive exposure of patients and caregivers to ionizing radiation.

These hurdles to reaching ideal targeted peptide-imaging probes for receptor imaging slow clinical adoption of the broad imaging platform based on peptides. Yet the advances in imaging hardware enable even higher sensitivity PET, SPECT, and optical imaging. Furthermore, it appears that instrument performance is not a bottleneck to translating peptide probes to the clinic, where faster CT scanners have higher resolution and new technologies are pushing MR sensitivities toward those found in nuclear imaging. Better quantitation of signal in all of these imaging modalities enable tracer and pharmacokinetic modeling of peptide probes for improved diagnostic and prognostic accuracy. Thus, imaging hardware and software are available and could be used to optimize pharmacokinetic characteristics of a family of lead peptide probes to generate a subclass of candidate peptides for further development to target receptors.

Questions and Controversial Issues

Peptide probes for imaging, unlike those for therapy, should be cleared from the body as rapidly as possible for optimal imaging. Because the requirements for imaging and therapy are at odds, the following questions are meant as primers to determine a specific rationale for optimizing peptide probes for molecular imaging:

(1) Are there general rules that can guide peptide design for molecular imaging? Is there a range of molecular weights and amino acid composition of the peptide backbone that enable optimal delivery to target cells, organs, and tissues as well as optimal plasma clearance?
(2) What are the appropriate metrics of imaging efficacy to identify promising lead candidates of a library of peptide probes?
(3) Do certain labels (e.g., PET or SPECT isotopes, lanthanides for MR, etc.) when free in the body specifically accumulate in organs, posing a threat of organ toxicity or excessive radiation exposure? How can peptide probes be designed to avoid free label toxicities?
(4) What parameters in peptide probe length and composition dictate hepatic and renal clearance?
(5) How do the labels (e.g., lanthanides, radioiostopes, radiometals, fluorophores), chelators, or linkers for imaging affect imaging efficacy and renal/hepatic clearance of peptides (including the effects of charge repulsion and steric hindrance within the probe)?
(6) What minimum specific activity, degree of labeling, or percentage conjugation of paramagnetic metal enables optimal imaging with peptide probes?
(7) What parameters should be considered in peptide design to minimize unwanted macrophage activation, cytokine release, excessive/persistent plasma protein binding, peptide cross-linking, low solubility, and high viscosity issues resulting from administration of peptide probes?
(8) Should peptide design for imaging consider secondary, tertiary, and quaternary structures for optimal targeting in vivo?
(9) What in vitro, in silico, preclinical, or synthetic methods should be included in rational peptide design to reduce the development time required for peptide probes to reach the market?
(10) What considerations are necessary in peptide design to minimize probe degradation in vivo?
(11) How can pharmacokinetic modeling of peptide probe libraries improve rational peptide design?
(12) When designing peptide probes for imaging, how should control peptides be designed to reduce experimental variability longitudinally and between animals?
(13) Should peptide probes for non-PET applications be designed with PET analogs to allow for absolute quantitation of optical and MR images?

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Therapeutic Applications of Antibodies: Background and Current State

Radioimmunotherapy (RIT), using radiolabeled antibodies against the CD20 antigen, is now approved by both the U.S. Food and Drug Administration and the European Agency for the Evaluation of Medicinal Products for the therapy of refractory or relapsed CD20+ lymphoma (*1,2*). Although its efficacy has been reported in first-line therapy (*3*), RIT is currently utilized either as second-line or later therapy. Several factors, including the incorporation of immunotherapy into first-line therapy regimens, as well as the potential immunogenicity of current RIT, make it unlikely that RIT will ever be part of routine first-line treatment. There is an unmet need to initiate RIT as soon as possible after first relapse. There is little doubt, however, that the efficacy of RIT is greater the earlier it is used in the management of patients with B-cell lymphoma.

RIT of solid tumors is a different issue altogether. Immunogenicity of murine antibodies (*4*) demanded the development of less immunogenic chimeric (*5*) and humanized (*6*) antibodies, which are currently in phase 1 or early phase 2 trials. However, measurable responses have been elusive, although survival benefits have been reported anecdotally (*7*). It is clear that the field should utilize end points other than those outlined by the Response Evaluation Criteria in Solid Tumors (RECIST) criteria (*8*). End points of survival (progression-free survival, overall survival) might prove more useful than tumor shrinkage.

Metabolic imaging, particularly using PET, may also be a surrogate end point of value, as was so elegantly demonstrated with gastrointestinal stromal tumors (*9*).

Hematopoietic toxicity is usually dose limiting for RIT. Most RIT has focused on nonmyeloablative regimens. Some groups have studied myeloablative RIT with stem-cell support, where second-organ toxicity is usually pulmonary and reversible. Again, myeloablative RIT has been far more successful in lymphoma (*10*) than in solid tumors (*11*). Myeloablative RIT is unlikely to be applied at centers other than those specialized sites with adequate infrastructure and with special dosimetry expertise.

RIT has been utilized in 2 additional areas, again as novel therapy unlikely to be expanded to the broad clinical setting. The first is systemic RIT with novel radionuclides, primarily α emitters. This has been studied most extensively in myelogenous leukemia (*12*). Another is intracavitary RIT, shown to have great promise in intracranial neoplasms (*13*). Intraperitoneal RIT has also shown promise (*14*), but several constraints, not least those in trial design and implementation, have forestalled development.

Most promising is RIT using multistep approaches, with the potential for delivery of significantly higher tumor radiation absorbed dose (relative to marrow, the critical organ). These approaches have been tested in therapeutic clinical trials (*15*) that have not progressed for a variety of reasons. Newer approaches may have greater promise (*16*).

In summary, RIT is approved for CD20+ lymphoma, where it should be pursued with more vigor and is being pursued in solid tumors with less success. Multistep targeting methods may overcome the obstacle of adequate tumor dose delivery. Awareness of the potential of RIT, especially among oncologists, is woefully inadequate.

Imaging with radiolabeled antibodies has been used for more than 2 decades to determine antibody biodistribution and to arrive at decisions regarding optimal mass amounts of antibody—an amount that would "saturate" normal antigen sites with adequate tumor targeting (*17*). The development of whole-body PET made it more feasible to carry out studies using positron-labeled antibodies, and several groups have explored the feasibility of this methodology to determine antibody residence times in tumor and normal tissue (*18,19*). The feasibility of labeling various positron emitters (^{124}I, ^{86}Y, ^{64}Cu) in a stable fashion with antigen-binding constructs is expected to lead to