Published in final edited form as:

Gastroenterology. 2010 February ; 138(2): 419–422. doi:10.1053/j.gastro.2009.12.014.

Optical Molecular Imaging Approaches in Colorectal Cancer

Umar Mahmood, MD, PhD [Co-Director]

Nuclear Medicine & Molecular Imaging Massachusetts General Hospital 55 Fruit Street, White 427
Boston, MA 02114 Office 617-726-6477 Fax 617-726-6165 umahmood@mgh.harvard.edu

Major clinical applications for molecular imaging are improvement of disease detection through increased image contrast between normal and diseased tissue, in situ characterization of lesions for improved patient stratification, and generation of an early measurement of the likelihood of successful response to specific therapies. Imaging concentrations of biological targets such as cell surface receptors, proteins in the extracellular matrix, levels of metabolites and their utilization rates, and levels of specific cell types in lesions can help achieve the broad clinical goals of detection, characterization, and therapy response assessment sooner and more accurately than we can currently achieve with standard-of-care imaging techniques that rely largely on anatomic appearance for such evaluation.

Independent advances in optical devices, molecular therapeutics using biologically derived material, fluorochrome design, and conjugation methods have converged to advance optical molecular imaging closer to practical clinical use. In this issue of *Gastroenterology*, Goetz, et. al.¹ have fluorescently labeled a whole antibody targeting EGFR, and tested the targeting of this antibody conjugate against cell lines with either low or high EGFR expression, grown subcutaneously in mice, and further tested the imaging agent on ex vivo human samples. Imaging was performed using confocal laser endomicroscopy that excites samples at 488 nm and records high-resolution images from resultant fluorescence in the 505-585 nm range. This work, which builds on fluorescent antibody imaging, helps further the road towards clinical translation.

A broad array of imaging modalities have been used in the preclinical or clinical setting to evaluate molecular targets in vivo. Many of these studies have been performed in rodent models of disease to highlight translational approaches. The large majority of human molecular imaging to date has used positron emission tomography (PET) or single photon emission computed tomography (SPECT) labels for evaluating molecular processes in people. However, many different modalities have been employed at least initially for human molecular imaging, and active work continues to increase the role these methods, especially optical imaging, will have at non-invasively and minimally invasively reporting on molecular processes clinically. Table 1 compares some of the advantages and disadvantages that the different imaging modalities have for evaluating molecular processes in vivo. In particular, it is useful to separate fluorescent imaging into three spatial realms. Microscopic fluorescent imaging has made rapid advances over the last several years, and clinically approved systems are available in two different useful wavelengths: a range that detects green fluorescence such as emitted by clinically-approved fluorescein, and a range that detects far red and near infrared fluorescence. These devices yield extremely high spatial resolution, allowing visualization of individual cells.^{2, 3} The limited depth penetration and field of view of such systems currently necessitates the evaluation of focal mucosal regions that are of interest, rather than a survey of the entire colon. As an example of human molecular optical imaging, such systems have been used recently to image colonic dysplasia in humans by employing a targeting peptide that was

selected by phage display technology.⁴ This peptide was shown to bind premalignant tissue; fluorescein-conjugation resulting in a molecularly targeted imaging agent that could be applied topically.

Macroscopic fluorescence imaging systems typically provide resolution identical to standard white light colonoscopy. In some cases they require a separate pass of the endoscope to image fluorescence, especially when the fluorescence wavelengths fall in the visible spectrum.⁵ In cases in which the fluorescent reporter molecules emit light in the near infrared, the fluorescent and the full color white light images may be obtained simultaneously, quantitatively, and in real time.⁶ The optical imaging systems with the deepest light penetration, albeit with low spatial resolution, are the near infrared tomographic systems. These devices have human application in imaging breast pathology and in evaluation of the neonatal brain. However, their use in the human abdomen is precluded due to the depth of penetration of light needed and the multiple air-tissue interfaces present.

PET and SPECT imaging approaches have been used for human molecular imaging over many decades. The imaging-reporter detection threshold of radionuclide imaging is much less than that of other clinical modalities, allowing evaluation of rare molecular events. Hundreds of single photon and positron emitting compounds have been used for molecular and physiological imaging in people.⁷ In addition to assessment of primary cancers of the gastrointestinal tract, such techniques are also ideally suited for molecular characterization of metastatic foci. PET imaging readily provides non-invasive, quantitative, tomographic evaluation of tracer uptake though the entire body and can be especially useful for highlighting differences among metastatic foci and for prediction of differences in response to therapy. An advantage of optical molecular imaging over PET molecular imaging is the ability to intervene in the colon at the time of lesion detection, since the fluorescent imaging devices are coupled to endoscopes, provide real-time image information, and can thus be more easily used for molecularly-targeted procedure guidance.

In addition to the type of imaging device and the associated trade offs in spatial resolution, depth of penetration, and temporal resolution, there are four fundamental choices for optical molecular imaging of the colon: 1) the molecular target or pathway to be imaged, 2) route of administration of the exogenous imaging agent, 3) the optical reporter and its excitation and emission wavelengths, and 4) the targeting moiety that is conjugated to the optical reporter.

The choice of which molecular target to image relates to the goals of the imaging procedure: the target selection criteria is quite different if the goals are improved polyp and adenocarcinoma detection in a screening setting; in situ lesion characterization to determine if a finding is clinically significant and needs intervention or is a benign finding; or assessment of the value of a potential therapeutic intervention. Improved dysplasia/cancer detection (sensitivity) and improved differentiation between neoplastic and non-cancerous lesions (specificity) can be competing constraints in imaging target selection. However, an absolute differentiation is not required; population enrichment by finding adenomas or adenocarcinomas that would otherwise be missed or excluding benign lesions prior to biopsy can have marked implications in associated procedure value.

Assessment of potential overexpression of a target to help select patients who may benefit from molecularly specific therapy is best utilized in settings where the lesion is not removed entirely, such as when neoadjuvant therapy is administered prior to resection. If a lesion is to be removed before therapy, then assessment may be more readily quantified on the excised sample. Evaluation of the expression level of a target provides greater benefit if the expression correlates with response to an associated specific therapy. As pointed out by Goetz, et. al.¹, there is a lack of a definitive relationship between response to cetuximab therapy and EGFR

expression. An interesting approach to attempt to overcome such discordance was recently evaluated in a preclinical lung cancer model. An ^{11}C -labeled imaging analog to erlotinib, a commonly prescribed tyrosine kinase inhibitor, was used in combination with PET imaging to predict which tumors would be responsive to erlotinib itself.⁸

The two primary choices for route of agent delivery for optical molecular imaging of the colon are topical spray and intravenous injection. Each has specific advantages. Topical administration results in a much lower systemic concentration of the imaging agent, decreasing safety concerns and the regulatory hurdles to human translation. To date human molecular optical imaging of the colon^{4, 5} has relied on topical administration. Timing of the administration of the agent is also favorable for topical application, since it is typically applied during or immediately before the imaging procedure. Advantages of intravenous administration include a much more homogenous delivery of the imaging agent and a greater repeatability of agent concentration for serial studies. Additionally, there are fewer physical barriers to imaging agent-target interaction, decreasing the variability in binding. Finally, IV administration allows for an assessment of sub-surface components of a lesion, not just the lumenally-exposed layers.

The choice of which optical reporter to use centers on the safety, size, and spectral properties of the fluorochrome. Fluorescein, which emits a green fluorescence, and indocyanine green (ICG), which emits a near infrared fluorescence, are both approved for human use. ICG is difficult to chemically modify with a targeting moiety, whereas fluorescein derivatives are readily attached to peptides and proteins. While many other fluorochromes are likely safe for human use, routine human use of fluorescein and the relative ease of attachment of fluorescein derivatives makes this an attractive option in terms of fluorochrome safety. Fluorescence emission wavelengths in the near infrared, approximately in the range of 700-1000 nm, have several relative advantages over visible fluorescence emission in terms of optical molecular imaging. There is less autofluorescence in the NIR relative to visible wavelengths, which results in a decrease in the non-specific background signal. Additionally, the NIR emission allows full color imaging of the tissue for anatomic analysis, while providing a simultaneous molecularly selective signal. A third general advantage, increased depth penetration of NIR light compared to visible light, is of decreased relevance for colonic imaging, since the lesions of interest are in the superficial layers.

The choice of targeting ligand in the design of optical molecular imaging agents has an impact on the type of targets one can image, the optimal timing of imaging, and the specificity of the imaging signal. Table 2 overviews the general classes of targeting ligands that are employed across all molecular imaging modalities. In general, labeling of whole antibodies is among the most direct approaches for molecular imaging in terms of agent synthesis, and dates back more than 30 years for in vivo preclinical imaging using radiolabels⁹ and more than 15 years for in vivo preclinical imaging using fluorescent labels.¹⁰ Antibodies have the advantage of very high binding affinity, and numerous antibodies have been developed as therapeutics. The long circulation times of antibodies and the resultant dosing intervals are an advantage when used for treatment. However, there are several disadvantages when whole antibodies are used for imaging. The long blood half-life decreases the specificity of signal due to increased non-specific background from the labeled antibodies that remain in the blood. Additionally, the enhanced permeability and retention (EPR) effect of non-specific macromolecule accumulation in tumors due to increased vascular permeability often results in a high tumor background signal for antibodies and other targeted macromolecules that does not reflect levels of biological target expression. Such non-specific signal in tumors may result in false positive findings. Given their relatively large size, labeled antibodies are best suited for imaging applications that focus on extracellular targets or cell surface receptors.

There are several approaches that overcome the limitations of whole antibody imaging. Chemical modifications to generate antibody fragments have long been used to decrease the ligand size. More recently, genetically engineered antibody fragments, including minibodies and diabodies, have increased in popularity for imaging applications due to improved clearance times while retaining high target affinity.¹¹ As part of this continuum, fluorescently labeled peptides offer even shorter blood clearance times; in some cases modification is required to allow sufficient time for the agents to interact with the tumor tissue. For topical colonic administration of imaging agents, these size considerations are less important, as vascular extravasation and blood half-life do not modulate local signal intensity.

There are a number of routes to increased translation of optical molecular imaging of the colon. Understanding the advantages of various approaches in imaging devices and technology coupled with the knowledge of imaging agent design constraints will allow new degrees of in situ lesion characterization and better methods to assess targeted therapies. The future of fluorescent imaging of the colon is quite bright.

Acknowledgments

Support: NIH P50CA127003 and U01CA143056

References

- Goetz M, Ziebart A, Foersch S, Vieth M, Waldner MJ, Delaney P, Galle PR, Neurath MF, Kiesslich R. In vivo molecular imaging of colorectal cancer with confocal endomicroscopy of epidermal growth factor receptor. *Gastroenterology*. 2009
- Kiesslich R, Burg J, Vieth M, Gnaendiger J, Enders M, Delaney P, Polglase A, McLaren W, Janell D, Thomas S, Nafe B, Galle PR, Neurath MF. Confocal laser endoscopy for diagnosing intraepithelial neoplasias and colorectal cancer in vivo. *Gastroenterology* 2004;127:706–13. [PubMed: 15362025]
- Wallace MB, Fockens P. Probe-based confocal laser endomicroscopy. *Gastroenterology* 2009;136:1509–13. [PubMed: 19328799]
- Hsiung PL, Hardy J, Friedland S, Soetikno R, Du CB, Wu AP, Sahbaie P, Crawford JM, Lowe AW, Contag CH, Wang TD. Detection of colonic dysplasia in vivo using a targeted heptapeptide and confocal microendoscopy. *Nat Med* 2008;14:454–8. [PubMed: 18345013]
- Maying B, Neumann F, Kastner C, Degitz K, Hahn EG, Schwab D. Early detection of premalignant conditions in the colon by fluorescence endoscopy using local sensitization with hexaminolevulinate. *Endoscopy* 2008;40:106–9. [PubMed: 18197583]
- Upadhyay R, Sheth RA, Weissleder R, Mahmood U. Quantitative real-time catheter-based fluorescence molecular imaging in mice. *Radiology* 2007;245:523–31. [PubMed: 17940307]
- Silberstein EB. Radionuclides and Radiopharmaceuticals for 2005. *J Nucl Med* 2005;46:13N–14N.
- Memon AA, Jakobsen S, Dagnaes-Hansen F, Sorensen BS, Keiding S, Nexø E. Positron emission tomography (PET) imaging with [¹¹C]-labeled erlotinib: a micro-PET study on mice with lung tumor xenografts. *Cancer Res* 2009;69:873–8. [PubMed: 19155297]
- Khaw BA, Beller GA, Haber E, Smith TW. Localization of cardiac myosin-specific antibody in myocardial infarction. *J Clin Invest* 1976;58:439–46. [PubMed: 956377]
- Folli S, Westermann P, Braichotte D, Pelegri A, Wagnieres G, van den Bergh H, Mach JP. Antibody-indocyanin conjugates for immunophotodetection of human squamous cell carcinoma in nude mice. *Cancer Res* 1994;54:2643–9. [PubMed: 8168092]
- Wu AM, Olafsen T. Antibodies for molecular imaging of cancer. *Cancer J* 2008;14:191–7. [PubMed: 18536559]
- Spinelli L, Torricelli A, Pifferi A, Taroni P, Danesini GM, Cubeddu R. Bulk optical properties and tissue components in the female breast from multiwavelength time-resolved optical mammography. *J Biomed Opt* 2004;9:1137–42. [PubMed: 15568933]

Table 1

Modalities for Clinical Molecular Imaging

Imaging Modality	Advantages	Disadvantages
Fluorescence Imaging	High sensitivity for ligand detection. Easiest to combine with interventional procedures.	Poor depth penetration makes imaging of some body parts inaccessible
- Microscopic/confocal	Extremely high spatial resolution allows visualization of subtle anatomic abnormalities	Very limited depth penetration, difficult to examine large surface areas
- Macroscopic	High spatial resolution, quantitative, and ability to screen entire colon	Limited depth penetration best suited for mucosal or submucosal targets
- Tomographic	Specialized equipment can evaluate entire human organs (such as breast) ¹²	Low spatial resolution. Multiple tissue interfaces preclude routine imaging of human colon
Positron Emission Tomography (PET) / Single Photon Emission Computed Tomography (SPECT)	Very high sensitivity for ligand detection, hundreds of agents already tested in people, and many agents approved for human use. Can be used to evaluate metastatic lesions.	Radiation dose somewhat decreases utility in low risk screening applications. Low spatial resolution.
Magnetic Resonance Imaging (MRI)	High spatial resolution and intrinsic simultaneous anatomic correlation	Low sensitivity for ligand detection
Ultrasound	Inexpensive and easy to combine with interventional techniques	Very difficult to image extra-vascular molecular targets
Computed Tomography (CT)	Very high spatial resolution can provide anatomic map for multi-modal imaging applications	Very poor sensitivity for ligand detection

Table 2

Ligands for optical labeling and imaging

Detection ligand	Advantages	Disadvantages
Whole antibody	Easiest to formulate. Many clinically approved antibodies available for labeling. Long history of optical- and radio-labeling of antibodies.	Long blood half-life decreases specificity of signal, especially before blood pool clearance of imaging agent
Engineered or chemically produced antibody fragments	These structures retain high binding affinity. Clearance times well suited for imaging.	Somewhat more complex to formulate compared to whole antibodies
Targeting peptides	High throughput screening methods can yield high-specificity agents with rapid clearance times	Somewhat more complex to formulate compared to whole antibodies
Small molecules	High specificity, rapid clearance. Additional targets including intracellular targets, available for imaging	Fluorochromes and their comparable size to small molecules may affect pharmacokinetics and biodistribution of the resulting labeled ligands