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Advancing the translation of optical imaging agents for clinical imaging

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Abstract: Despite the development of a large number of promising candidates, few contrast agents for established medical imaging modalities have successfully been translated over the past decade. The emergence of new imaging contrast agents that employ biomedical optics is further complicated by the relative infancy of the field and the lack of approved imaging devices compared to more established clinical modalities such as nuclear medicine. Herein, we propose a navigational approach (as opposed to a fixed "roadmap") for translation of optical imaging agents that is (i) proposed through consensus by four academic research programs that are part of the cooperative U54 NCI Network for Translational Research, (ii) developed through early experiences for translating optical imaging agents in order to meet distinctly varied needs in cancer diagnostics, and (iii) adaptable to the rapidly changing environment of academic medicine. We describe the pathways by which optical imaging agents are synthesized, qualified, and validated for preclinical testing, and ultimately translated for "first-in-humans" studies using investigational optical imaging devices. By identifying and adopting consensus approaches for seemingly disparate optical imaging modalities and clinical indications, we seek to establish a systematic method for navigating the ever-changing "roadmap" to most efficiently arrive at the destination of clinical adoption and improved outcome and survivorship for cancer patients.

OCIS codes: (170.0170) Medical optics and biotechnology; (170.3880) Medical and biological imaging; (170.3890) Medical optics instrumentation.

References and links

1. J. T. Liu, N. O. Loewke, M. J. Mandella, R. M. Levenson, J. M. Crawford, and C. H. Contag, "Point-of-care pathology with miniature microscopes," Anal Cell Pathol (Amst) **34**(3), 81–98 (2011).
2. W. Piyawattanametha, H. Ra, Z. Qiu, S. Friedland, J. T. Liu, K. Loewke, G. S. Kino, O. Solgaard, T. D. Wang, M. J. Mandella, and C. H. Contag, "In vivo near-infrared dual-axis confocal microendoscopy in the human lower gastrointestinal tract," J. Biomed. Opt. **17**(2), 021102 (2012).
3. M. J. Uddin, B. C. Crews, A. L. Blobaum, P. J. Kingsley, D. L. Gorden, J. O. McIntyre, L. M. Matrisian, K. Subbaramaiah, A. J. Dannenberg, D. W. Piston, and L. J. Marnett, "Selective visualization of cyclooxygenase-2 in inflammation and cancer by targeted fluorescent imaging agents," Cancer Res. **70**(9), 3618–3627 (2010).
4. M. Goetz and T. D. Wang, "Molecular imaging in gastrointestinal endoscopy," Gastroenterology **138**(3), 828–833.e1 (2010).

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5. P. L. Hsiung, J. Hardy, S. Friedland, R. Soetikno, C. B. Du, A. P. Wu, P. Sahbaie, J. M. Crawford, A. W. Lowe, C. H. Contag, and T. D. Wang, "Detection of colonic dysplasia in vivo using a targeted heptapeptide and confocal microendoscopy," Nat. Med. **14**(4), 454–458 (2008).
6. Z. Liu, S. J. Miller, B. P. Joshi, and T. D. Wang, "In vivo targeting of colonic dysplasia on fluorescence endoscopy with near-infrared octapeptide," Gut, 17 March 2012, http://gut.bmj.com/content/early/2012/03/16/gutjnl-2011-301913.abstract.
7. L. V. Wang and S. Hu, "Photoacoustic tomography: in vivo imaging from organelles to organs," Science **335**(6075), 1458–1462 (2012).
8. C. Kim, T. N. Erpelding, L. Jankovic, M. D. Pashley, and L. V. Wang, "Deeply penetrating in vivo photoacoustic imaging using a clinical ultrasound array system," Biomed. Opt. Express **1**(1), 278–284 (2010).
9. W. J. Akers, W. B. Edwards, C. Kim, B. Xu, T. N. Erpelding, L. V. Wang, and S. Achilefu, "Multimodal sentinel lymph node mapping with single-photon emission computed tomography (SPECT)/computed tomography (CT) and photoacoustic tomography," Transl. Res. **159**(3), 175–181 (2012).
10. D. Pan, X. Cai, C. Yalaz, A. Senpan, K. Omanakuttan, S. A. Wickline, L. V. Wang, and G. M. Lanza, "Photoacoustic sentinel lymph node imaging with self-assembled copper neodecanoate nanoparticles," ACS Nano **6**(2), 1260–1267 (2012).
11. D. Pan, M. Pramanik, A. Senpan, J. S. Allen, H. Zhang, S. A. Wickline, L. V. Wang, and G. M. Lanza, "Molecular photoacoustic imaging of angiogenesis with integrin-targeted gold nanobeacons," FASEB J. **25**(3), 875–882 (2011).
12. W. J. Akers, C. Kim, M. Berezin, K. Guo, R. Fuhrhop, G. M. Lanza, G. M. Fischer, E. Daltrozzo, A. Zumbusch, X. Cai, L. V. Wang, and S. Achilefu, "Noninvasive photoacoustic and fluorescence sentinel lymph node identification using dye-loaded perfluorocarbon nanoparticles," ACS Nano **5**(1), 173–182 (2011).
13. I. C. Tan, E. A. Maus, J. C. Rasmussen, M. V. Marshall, K. E. Adams, C. E. Fife, L. A. Smith, W. Chan, and E. M. Sevick-Muraca, "Assessment of lymphatic contractile function after manual lymphatic drainage using near-infrared fluorescence imaging," Arch. Phys. Med. Rehabil. **92**(5), 756–764.e1 (2011).
14. K. E. Adams, J. C. Rasmussen, C. Darne, I. C. Tan, M. B. Aldrich, M. V. Marshall, C. E. Fife, E. A. Maus, L. A. Smith, R. Guilloid, S. Hoy, and E. M. Sevick-Muraca, "Direct evidence of lymphatic function improvement after advanced pneumatic compression device treatment of lymphedema," Biomed. Opt. Express **1**(1), 114–125 (2010).
15. M. A. Hall, S. Kwon, H. Robinson, P. A. Lachance, A. Azhdarinia, R. Ranganathan, R. E. Price, W. Chan, and E. M. Sevick-Muraca, "Imaging prostate cancer lymph node metastases with a multimodality contrast agent," Prostate **72**(2), 129–146 (2012).
16. M. A. Hall, K. L. Pinkston, N. Wilganowski, H. Robinson, P. Ghosh, A. Azhdarinia, K. Vazquez-Arreguin, A. M. Kolonin, B. R. Harvey, and E. M. Sevick-Muraca, "Comparison of mAbs targeting epithelial cell adhesion molecule for the detection of prostate cancer lymph node metastases with multimodal contrast agents: quantitative small-animal PET/CT and NIRF," J. Nucl. Med. **53**(9), 1427–1437 (2012).
17. D. A. Ostrov and C. H. Contag, "Molecular imaging of inflammation and carcinogenesis," Cancer Prev. Res. (Phila.) **4**(10), 1523–1526 (2011).
18. L. Sampath, S. Kwon, M. A. Hall, R. E. Price, and E. M. Sevick-Muraca, "Detection of cancer metastases with a dual-labeled near-infrared/positron emission tomography imaging agent," Transl Oncol **3**(5), 307–217 (2010).
19. J. P. Houston, S. Ke, W. Wang, C. Li, and E. M. Sevick-Muraca, "Quality analysis of in vivo near-infrared fluorescence and conventional gamma images acquired using a dual-labeled tumor-targeting probe," J. Biomed. Opt. **10**(5), 054010 (2005).
20. Y. Zhang, H. Hong, J. W. Engle, Y. Yang, C. P. Theuer, T. E. Barnhart, and W. Cai, "Positron emission tomography and optical imaging of tumor CD105 expression with a dual-labeled monoclonal antibody," Mol. Pharm. **9**(3), 645–653 (2012).
21. H. Lee, W. J. Akers, P. P. Cheney, W. B. Edwards, K. Liang, J. P. Culver, and S. Achilefu, "Complementary optical and nuclear imaging of caspase-3 activity using combined activatable and radio-labeled multimodality molecular probe," J. Biomed. Opt. **14**(4), 040507 (2009).
22. W. B. Edwards, W. J. Akers, Y. Ye, P. P. Cheney, S. Bloch, B. Xu, R. Laforest, and S. Achilefu, "Multimodal imaging of integrin receptor-positive tumors by bioluminescence, fluorescence, gamma scintigraphy, and single-photon emission computed tomography using a cyclic RGD peptide labeled with a near-infrared fluorescent dye and a radionuclide," Mol. Imaging **8**(2), 101–110 (2009).
23. J. Culver, W. Akers, and S. Achilefu, "Multimodality molecular imaging with combined optical and SPECT/PET modalities," J. Nucl. Med. **49**(2), 169–172 (2008).
24. J. Kuil, A. H. Velders, and F. W. van Leeuwen, "Multimodal tumor-targeting peptides functionalized with both a radio- and a fluorescent label," Bioconjug. Chem. **21**(10), 1709–1719 (2010).
25. H. G. van der Poel, T. Buckle, O. R. Brouwer, R. A. Valdés Olmos, and F. W. van Leeuwen, "Intraoperative laparoscopic fluorescence guidance to the sentinel lymph node in prostate cancer patients: clinical proof of concept of an integrated functional imaging approach using a multimodal tracer," Eur. Urol. **60**(4), 826–833 (2011).
26. A. Azhdarinia, P. Ghosh, S. Ghosh, N. Wilganowski, and E. M. Sevick-Muraca, "Dual-labeling strategies for nuclear and fluorescence molecular imaging: a review and analysis," Mol. Imaging Biol. **14**(3), 261–276 (2012).
27. M. B. Aldrich, M. V. Marshall, E. M. Sevick-Muraca, G. Lanza, J. Kotyk, J. Culver, L. V. Wang, J. Uddin, B. C. Crews, L. J. Marnett, J. C. Liao, C. Contag, J. M. Crawford, K. Wang, B. Reisdorph, H. Appelman, D. K.

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1. Introduction

Biomedical optical imaging technologies have substantive potential for revolutionizing cancer diagnosis, staging, treatment and survivorship, but translation of these technologies from the academic research laboratories into academic medicine is stymied by the lack of a "model" or "roadmap". Since there have been few targeted optical imaging agents that have been successfully translated, there is a need to chart the translational pathway. "First-in-humans" studies of optical imaging agents are needed for initial demonstration of potential clinical utility, but this uncharted territory is further complicated by the lack of appropriate imaging instruments that are approved for clinical use. Regulatory pathways that require combinational drug (contrast agent) approval from Center for Drug Evaluation and Research (CDER) or Center for Biologics Evaluation and Research (CBER), and device approval (imaging instrument) from Center for Devices and Radiological Health (CDRH), comprise one of the requisite steps in translation that can be more substantial if the synthesis, validation, and qualification of an imaging agent is conducted independently from its respective investigational imaging device. The Chemistry Probes and Guided Therapies Core working group of the NCI Network of Translational Research (NTR) is charged with describing the development of optical imaging agents within the context of the diverse translational optical imaging projects in each of the NTR Centers (comprised of Stanford University (SU), University of Michigan (UM), Washington University at St. Louis (WUSTL), and the University of Texas Health Science Center (UTHSC)), and sharing common experiences to accelerate the translation of optical imaging agents. The NTR is the second of two consecutive programs in the NCI Cancer Imaging portfolio that seeks to translate imaging technologies. In its first NCI U54 program (2003-2008), the Network for Translational Optical Imaging (NTROI) focused upon translating optical devices while the current NTR (2008-2013) focuses upon translating optical imaging devices and imaging agents together with a conventional imaging modality to provide embedded validation.

2. NTR translational projects

As a means of introduction, Table 1 provides a listing of the device platform/modality, proposed imaging agent, and unmet clinical need addressed in each of the NTR projects. Not all biomedical optical imaging modalities are presented within the NTR, but all possess the translation of a combinational drug/device in some type of hybrid imaging approach that could lend further validation through comparative efficacy studies. Initially, each team is advancing combinations of contrast agents and instruments for very specific unmet clinical needs, however, each set of technologies is designed to have broader clinical utility thereafter and are described briefly below.

SU employs next generation, multi-wavelength, fiber-optic fluorescent micro-endoscopes for gastrointestinal screening of high-risk populations. Their technology development targets a high-risk population with hereditary diffuse gastric cancer (HDGC), which is an autosomal dominant cancer susceptibility syndrome caused by germline mutations in E-cadherin (CDH1). Without standard-of-care, prophylactic gastrectomy, subjects with HDGC experience an 80% lifetime risk of developing, and dying from, gastric cancer. The translational paradigm of SU focuses upon conducting esophagogastroduodenoscopy in these patients using wide-field fluorescence endoscopes (macroscopic) and miniaturized dual-axis confocal microscopes (microscopic) [1,2] for interrogation of fluorescence following intravenous (i.v.) administration of a "first-in-humans" imaging agent, fluorocoxib, that was developed in collaboration with Vanderbilt University (VU) to target cyclooxygenase-2 (COX-2) [3]. The off-label use of indocyanine green (ICG) comprises a first step toward translation enabling validation of the microendoscope prior to deploying the COX-2 agent.

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Table 1. Overview of NTR teams and research strategies

Research Team	Program Title	Technology development strategy	Disease models investigated	Clinical Need/Relevance
SU	Multimodal Imaging of GI Cancers for Diagnosis and Directed Therapy	• Endoscopy • Fluorescently labeled COX-2 targeting probes	Gastrointestinal tract cancers	• Molecularly guided detection of neoplasia in the GI is non-existent • Optical imaging at time of visual endoscopy will improve sensitivity of endoscopic detection in high risk cases
UM	*In Vivo* Detection of Neoplasia in the Digestive Tract	• Multi-spectral endoscopy • Fluorescently labeled peptides	Colonic neoplasia	• Molecularly guided detection of neoplasia in the colon is non-existent • Fluorescence molecular imaging at time of visual endoscopy will improve sensitivity of endoscopic detection in high risk cases
WUSTL	Photoacoustic/ Optical/ Ultrasonic Imaging of Sentinel Lymph Nodes and Metastases	• Photoacoustic and diffuse optical tomography • Dual-labeled imaging probes	LN mapping in breast cancer	• PAT techniques offer a high resolution, non-ionizing method for detecting SLNs • Ability to combine the sensitivity/specificity of optical imaging with depth and resolution of ultrasound
UTHSC	Diagnostic Nodal Staging with Nuclear and NIR Molecular Optical Imaging	• NIRF imaging • Dual-labeled imaging probes	Lymphatic imaging and LN detection in cancer	•NIR fluorescence provides a rapid method for visualizing the lymphatic function architecture not possible with lymphoscintigraphy •Dual-labeled imaging agents targeting cancer in LNs lymph nodes provide non-invasive TNM staging through whole body and intraoperative imaging.

The translational paradigm at UM involves combination instruments for fluorescent colonoscopy in high-risk individuals diagnosed with familial adenomatous polyposis (FAP) and hereditary nonpolyposis colorectal cancer (HNCPP, Lynch Syndrome). In their proposed "first-in-humans" studies, peptides discovered through phage display technologies are labeled with fluorescein isothiocyanate (FITC), a derivative of the green fluorophore, fluorescein, which is already used in humans. These are designed for topical administration and to bind to luminal intestinal surfaces of dysplastic mucosa to enable early detection and screening in high-risk populations [4–6].

WUSTL has focused on translation of photoacoustic tomography (PAT) which significantly increases the effective depth of optical contrast detection [7]. Pre-operative PAT detects uptake of off-label intradermal administration methylene blue (MB) and ICG in order to perioperatively identify sentinel lymph nodes (SLNs) in breast cancer patients [8]. Off-label use of approved agents accelerates the deployment of the PAT imaging device in human studies. Novel multimodal agents are also under development to enable hybrid imaging using PAT/fluorescence/nuclear imaging and include (i) a derivative of ICG that can be conjugated to a chelating agent for radiometal sequestration, (ii) MB with isotopic Iodine-125 labeling [9], and (iii) functionalized nanoparticles for targeted, molecular imaging using PAT, nuclear, and fluorescence imaging approaches [10–12]. This multimodal approach provides means for

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validation of PAT with standard-of-care gamma scintigraphy or Single-Photon Emission Computed Tomography (SPECT) for SLN mapping, as well as future molecular imaging applications.

Finally, the UTHSC primary project focuses upon the development of multimodal "first-in-humans" imaging agents possessing a radiolabel and a near-infrared (NIR) fluorophore to permit non-invasive imaging of lymph node (LN) status, and intraoperative imaging of cancer-positive LNs and tumor margins during surgery. In preparation for the clinical evaluation of novel multimodal imaging agents, their team used an off-label, intradermal administration of ICG at microdoses to qualify their investigational, military-grade intensified charge-coupled device (CCD) camera in Phase I/II Investigational New Drug (IND) clinical studies [13,14]. "First-in-humans" targeting vectors under investigation at UTHSC consist of (i) a well-established peptide targeting the somatostatin receptor (SSTR) in neuroendocrine tumors (NETs) and (ii) affinity matured monoclonal antibodies (mAbs) or mAb-based fragments targeting epithelial cell adhesion molecule (EpCAM) [15,16], a protein almost universally overexpressed on the surface of cancer epithelial cells. Dual labeling strategies are based on innovative multimodality chelators which minimally affect the biological and pharmacokinetic (PK) properties of targeting agents.

3. Navigational pathway

Certain members from each of the NTR teams constituted the Chemistry Probes and Guided Therapies Core and have devised logical pathways for target identification (where applicable), design and synthesis of agents, production, batch release, validation, and subsequent deployment in both preclinical and investigational human studies (Fig. 1).

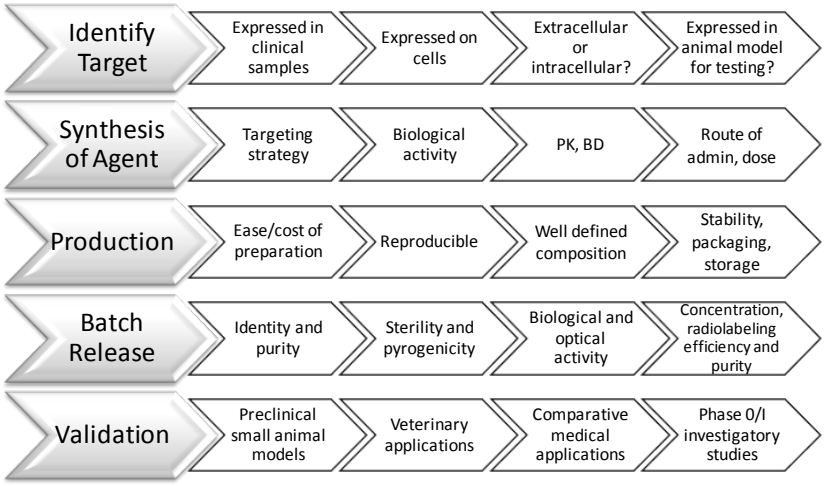

Fig. 1. Pathways for developing and validating a molecular imaging agent (BD = biodistribution).

Once beyond the agent discovery and development stages, the next three steps of synthesis, production, and batch release are defined strictly in a standard operating procedure (SOP). SOPs are critical for the subsequent translational phase wherein production steps and analytical assays for batch release are replicated, validated, and conducted under good laboratory practice (GLP) and typically include certificates of analysis for all reagents, calibration and maintenance logs for all equipment used, audit trails, and defined packaging and storage conditions that ensure validated stability. Examples of navigational pathways for the four NTR Centers are represented herein as agent development flowcharts.

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3.1. SU/VU agent development approach

At SU, the focus has been on a single molecular probe, fluorocoxib (Fig. 2). The rationale for probe selection was to select a high-value imaging target that would have the greatest impact on early detection, guided resection, and prognosis, and to provide an outcome measure for a range of malignancies using a single molecule [17]. Based on the association of chronic inflammation with neoplasia and increased activity of the intracellular enzyme COX-2, the SU/VU collaboration identified several well-characterized COX-2 inhibitors as candidates for a molecularly target optical imaging agent. By employing various combinations of linkers and fluorophores during the agent synthesis process, they were able to generate a library of agents for subsequent evaluation. Since commercially available reagents and dyes may not always address specific needs for given molecule, the SU/VU collaboration provided the know-how to perform custom syntheses in-house and greatly enhanced the flexibility of agent design strategies. Ultimately, fluorocoxib was prepared by fluorescently labeling the COX-2 inhibitor, indomethacin, and validated preclinically *in vitro* and in tumor-bearing animals. The *in vivo* imaging studies with fluorocoxib also allowed the investigators to define detection limits of the instrument as an added characterization step. By successfully demonstrating efficacy for tumor targeting with acceptable PK, the good manufacturing practice (GMP) synthesis, optimization of formulation, and toxicological testing were outsourced to a contract facility to complete the required preclinical characterization steps and initiate preparation of the IND application.

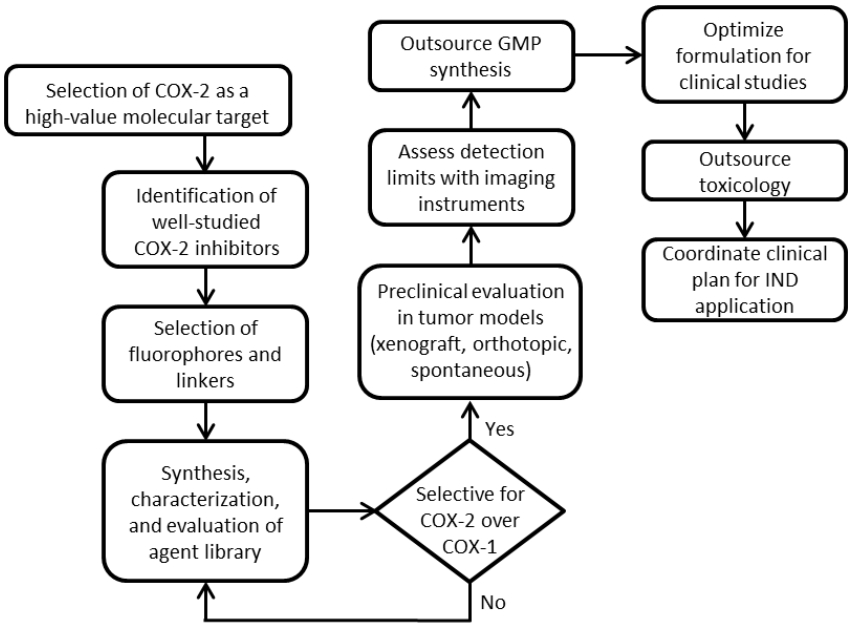

Fig. 2. Screening and development of the COX-2 targeted agent, fluorocoxib by SU/VU.

3.2. UM agent development approach

For UM, the discovery process begins with target identification and peptide selection from screening phage libraries (Fig. 3).

Solid phase synthesis with on-column fluorescent label conjugation enables precise labeling and composition that is characterized by mass spectrometry (MS), high-performance liquid chromatography (HPLC), and optical property measurements prior to being characterized for biological activity by a validated assay. If characterization of composition and biological activity meets a minimal, acceptable, but clearly defined criteria, the agent is

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precisely formulated in its delivery vehicle before undergoing preclinical testing of efficacy with a designated imaging device. If successful, then the manufacturing plan is devised using GMP and the Chemistry, Manufacturing and Controls (CMC) section (which includes production, analytical testing, and batch release criteria) of an IND application is prepared. For a fluorescently labeled peptide such as that under development by UM, batch release

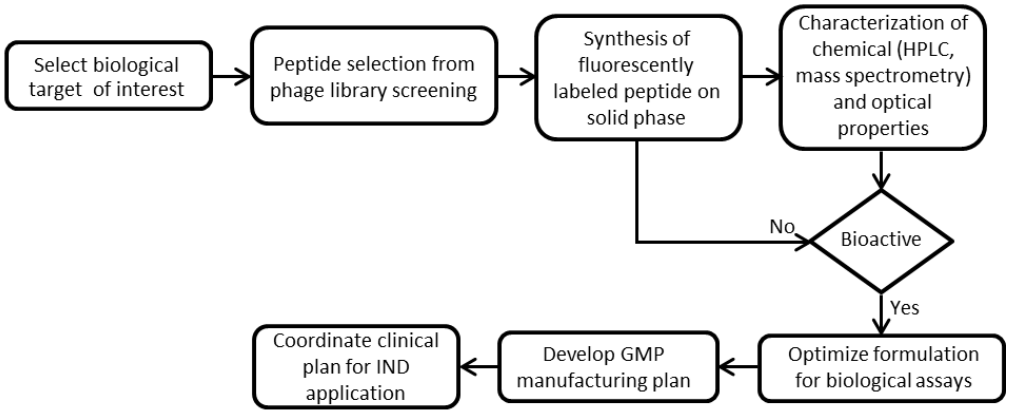

Fig. 3. Flowchart for screening, selection, preparation, and characterization of fluorescent peptide probes at UM.

criteria should include optical property measurements of extinction coefficient, fluorescent yield (quantum efficiency), and lifetime. Efficacy of resulting peptide agents are typically tested using scrambled or non-scrambled peptides *in vitro* as well as *in vivo* to establish potential utility and molecular specificity.

3.3. WUSTL agent development approach

Investigators at WUSTL have focused on translation of a clinical PAT device with off-label use of darkly colored dyes such as MB and ICG. PAT contrast is based on optical absorption and detection of ultrasound generation rather than fluorescence. Thus PAT enables detection of optical agents several centimeters below the skin surface. As a means of validating clinical PAT devices and pushing the boundaries of optical molecular imaging, WUSTL is also developing an array of agents that generate multiple contrast modes to combine optical imaging (PAT or Diffuse Optical Tomography (DOT)) with nuclear imaging (SPECT or Positron Emission Tomography (PET)). Isotopically labeled Iodine-125 MB may be used for whole-body SPECT/CT to validate SLN identification by PAT. Cypate, a functionalizable analog of ICG, possesses a high extinction coefficient for excellent PAT contrast as well as good quantum yield for fluorescence imaging. Conjugation of tyrosine for radio-iodination or a chelator for sequestration of radiometals enables combinations of PAT and DOT with SPECT (Indium-111) or PET (Copper-64). These agents provide non-specific lymph node mapping while other agents that are conjugated to targeting moieties will enable specificity to cancer.

At the initial optical reporter discovery step depicted in Fig. 4, NIR reporters are sought due to the relative transparency of biological tissues in this light region and are needed to enable greatest tissue penetration and largest detectable PAT signal. Functionalizable NIR contrast agents with high absorption coefficients demonstrate a broad range of possibilities for molecular imaging contrast and potential for clinical applications of PAT.

The development of the PAT dye with a functional group for conjugation to a chelator or targeting moiety is then decided. Whether using isotopic substitution or a chelated radiometal, radiolabeling of the final conjugate requires testing for labeling efficiency, radiochemical purity, and stability of the agent. Assessment of composition and possible alteration of optical

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properties under the sometimes harsh radiolabeling conditions are then evaluated through HPLC, as well as measurement of optical properties. After formulation to the correct dosage, the *in vivo* efficacy of the probe is evaluated to assess the PK for reaching sentinel and subsequent LNs within the draining basin. If the agent provides adequate signal with a reasonable PK profile, it is then considered for further development, including stability testing, GMP production, GLP safety and toxicity testing, and eventually, investigational studies in humans.

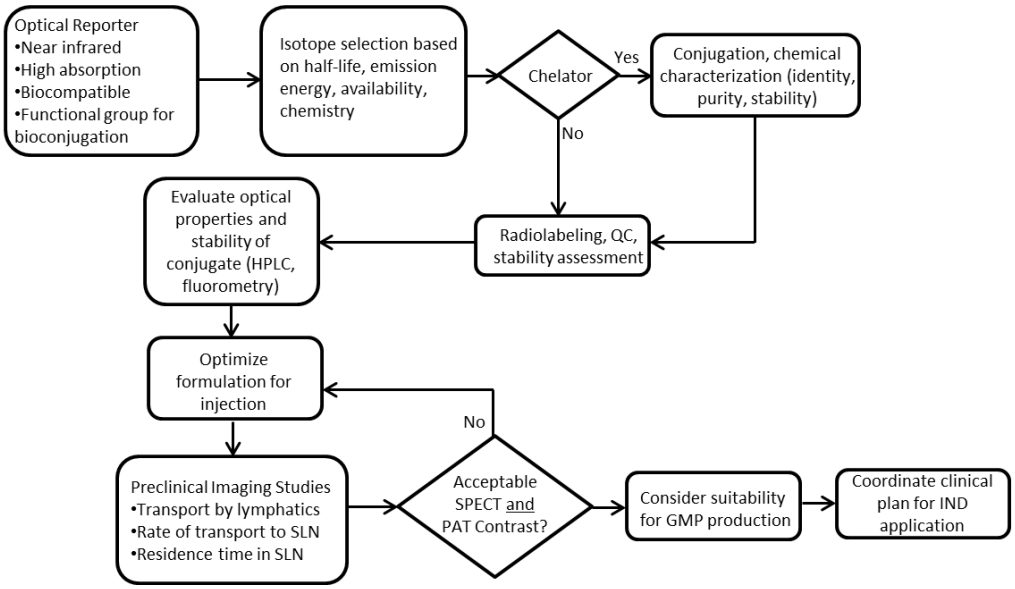

Fig. 4. Flowchart for synthesis and characterization multimodal agents for SLN mapping by WUSTL.

3.4. UTHSC agent development approach

The translational paradigm at UTHSC is twofold. First, the team engineers unique mAbs and mAb fragments with maximal affinity which contain chemical modifications to modulate PK and reactive groups introduced away from binding domain for site specific conjugation of a chelator, NIR fluorophore, or a customized multimodality chelator. In a second complementary approach, they conjugate a selected SSTR-targeting peptide with the multimodality chelator. As shown in Fig. 5, both cases choose the fluorophore on the basis of fluorescent properties, size, and stability in selected radiolabeling conditions, whether with Gallium-68 ($t_{1/2}$ = 68 min) for rapidly clearing agents (e.g., peptides or mAb fragments), or Copper-64 ($t_{1/2}$ = 12.7 h) for agents with longer circulation time (e.g., full-length mAbs).

The resulting chelator, fluorophore, or multimodality chelator conjugate is then characterized by MS and HPLC, quantitative flow cytometry (for optical assessment), Lindmo assays (for radioactive assessment), or surface plasmon resonance (for quantifying biological activity on target expressing cells or target proteins themselves). If biological activity is quantitatively retained following conjugation(s), then optical properties, radiolabeling efficiency, and stability are determined before the product is deemed suitable for further evaluation. The product may then be lyophilized, frozen, or stored at 4°C for subsequent experiments. Next, *in vivo* studies are employed to determine if adequate signal is attainable at microdoses using clinically validated NIRF imaging instrumentation and a standardized PET imaging scanner [18]. Once validated in animal models, long-term stability testing, GMP production, GLP safety and toxicity testing are performed, and eventually, investigational human studies are conducted. It should be noted that the preclinical validation for mAbs

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typically employ isotype mAbs and mAb fragments as well as mAb products which cross react with both human and mouse targets.

The pathways taken for each team are different, depending upon the translational objectives, but each focuses upon qualification of the imaging agent on the basis of biological activity and imaging performance based upon the appropriate criteria (radiolabeling efficiency, fluorescent yield, absorption coefficient). Eventually, each approach arrives at a common point which where a GMP compliant manufacturing plan is required in order to gain approval for human studies.

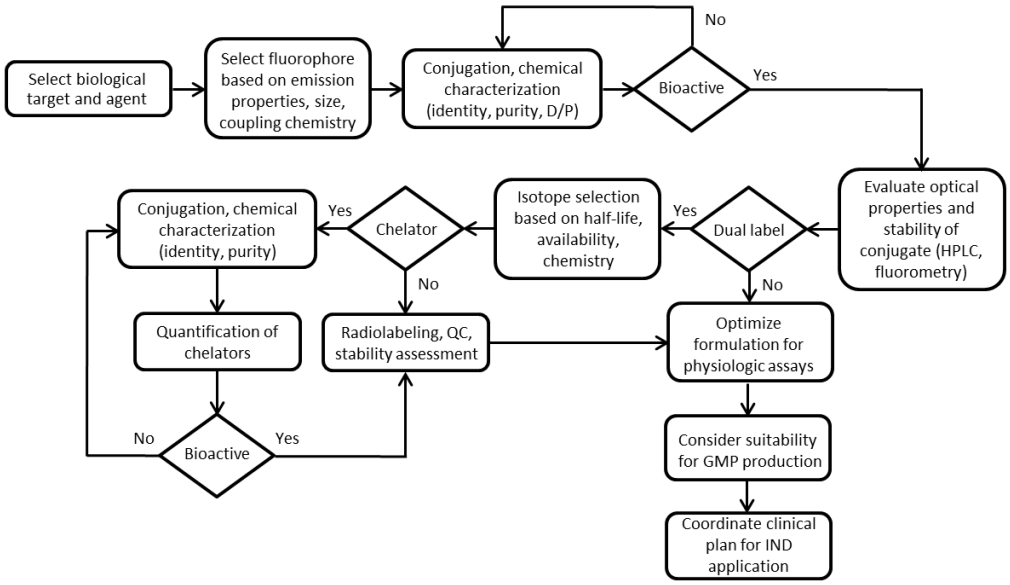

Fig. 5. Flowchart for preparation and characterization of fluorescent and dual-labeled mAbs by UTHSC. (D/P = dye/protein ratio, QC = quality control).

4. Anticipate problems in translational research

While these translational studies are underway, findings of potentially confounding difficulties have been, and will continue to be, encountered as progress is made toward the clinic. The uncertain pathway provides an additional impetus for our community to share common experiences that will in turn enable more efficient navigational routes for shared, effective translation strategies. The first most commonly encountered problem in translation of optical imaging agents is the lack of well-suited instrumentation, including analytical equipment that is operational in relevant wavelengths often in the NIR range. While this deficiency in analytical equipment is lessening with the widespread development and use of NIR fluorophores with low autofluorescent characteristics, common analytical instruments such as confocal microscopes, HPLCs, plate readers, flow cytometers, etc., typically require custom retrofitting with diodes, detectors, filters, and lenses with coatings that enable NIR interrogation. In addition, conventional endoscopy and intraoperative devices are being or have been adapted to image ICG, typically administered i.v. and detected at far larger doses than would be required for the pico- to femto-molar concentrations needed for molecular imaging. However, promising findings from preclinical research have revealed, in some applications, complementary data sets obtained with NIR and nuclear imaging [19–22]. Therefore, dual-labeled agents are rapidly being recognized as emerging clinical opportunities for multimodality validation and development of hybrid agents for diagnostic and intraoperative imaging [23–25]. It should be noted that Azhdarinia and associates showed that radiolabeling can alter the fluorescent properties of NIR fluorophores with varying degrees

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based on the harshness of reaction conditions [26]. As a result, the process of choosing a fluorophore and optimizing radiolabeling conditions must be carefully conducted as indicated in the agent development flow charts above to ensure that the fluorescent optical properties of dual-labeled agents are comparable to a (single) fluorescently labeled counterpart.

Another emerging difficulty relates to some of the companies that produce reagent-grade NIR dyes and have built their business on academic research. Besides the already restrictively high price for reagent-grade dyes, the requirements of increased pricing for GMP-produced dyes and "clinical trial agreements" that include intellectual property rights to optical imaging devices and technologies complicates the translation of NIR conjugates. Moreover, academic and industrial partners are likely unable to financially and legally agree to such an agreement making this practice selectively detrimental to the biomedical optics community. Given that GMP manufacturers of chelators for nuclear imaging do not exhibit the same level of involvement in the translational use of their reagents, the biomedical optics community may face significantly larger obstacles when compared to nuclear imaging. As the value proposition for optical imaging is realized, these relationships may be predicted to evolve and change rapidly. It is noteworthy that an increasing number of companies are manufacturing NIR fluorophores at reasonable costs, but not all provide chemical structures and identity information necessary for incorporation into CMC documentation. As an alternative strategy to overcome this translational barrier, academic centers could engage in the production of NIR fluorophores alone or in consortia of academic/industrial partners. Otherwise, it is never too early to initiate a discussion with the R&D department of a dye manufacturer if one intends to employ its dye in an imaging agent used in translational studies.

Finally, due to the importance of demonstrating innovation in their individual research programs, academicians are constantly employing novel reagents, targeting molecules, chemical compositions, and formulations in their studies. While this accelerates innovation in the field, it reduces commonality and limits the ability to directly compare and cross-validate promising preclinical data which may have translational potential. Therefore, consensus efforts are critical and can add value to the field by identifying general schemes within which current probe development efforts can be grouped, while also proposing a navigational route to improve upon existing translational efforts. Academic researchers are not typically focused on the cumbersome manufacturing process of new imaging agents for clinical trials and may find themselves facing significant obstacles as promising imaging agents advance toward human testing. In particular, the development of reproducible and validated synthetic processes are lacking in academia. As the handoff to a contract manufacturer typically occurs for GMP manufacturing of a probe, the presence of validated SOPs, batch records, and analytical data are critical and can facilitate efficient method development and validation by a contract manufacturer. Accordingly, academic laboratories and consortia would benefit from developing detailed compound characterization checklists which include standard tests such as structural confirmation and purity of the probe, but also more extensive analyses of compound-specific criteria such (i) radiolabeling efficiency of dual-labeled probes, (ii) stability of i.v. vs. topical formulations, (iii) amenability to processing steps such as lyophilization, and (iv) effects of storage conditions. Highly rigorous testing in academic laboratories offers a better likelihood for cross-validation of methods by the outside manufacturer, and ultimately supports more efficient translational development of a probe.

5. Conclusion and summary

Due to the complexities associated with designing and characterizing a molecular imaging probe for human use, it is highly beneficial to the research community to share our experiences in obtaining the necessary know-how and resources to carry the translational process from beginning to end. Building upon the recent NTR-wide publication by Aldrich *et al*. that focused on instrument validation needs of each research team [27], this contribution stresses the importance of creating consensus efforts to bridge gaps that exist in the

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understanding of the translational process specific to imaging agents. Under our model, there is constant interaction between the core and advisors in regulatory agencies as well as industry leaders that are well-versed in translation. Several prominent individuals from imaging-related pharmaceutical companies participate in defining research strategies and milestones for each research program to better obtain alignment with industry standards. The direct input is critical to the success of translating new imaging technologies into the clinic and forms the basis for the recommendations for best practices set forth by the core. Defining plans for probe development from the ground up will provide academicians with a more comprehensive understanding of factors such as milestones, timelines, and expected costs of clinical testing, which substantially improves the likelihood of success for translating new imaging technologies into humans.

As alluded to in Section 4, market segmentation can profoundly influence whether a technology with diverse components can be clinically translated, irrespective of a compelling case for improved patient outcomes. In the current economic climate, collaborations which include these diverse markets are critical for a single, shared and focused mission of technology application for improved patient outcomes. For this case of biomedical optical imaging, the challenges of focusing the instrumentation and reagent industries on a shared mission is best met by roadmap objectives since translation and regulatory strategies will involve not one or the other, but *both* industries. The roadmap for translating biomedical optical imaging (both drug and device) requires assessing the shared translational routes for both instrumentation and reagent industries. Identifying the navigational routes for translating optical imaging agents and (under the charge of the Instrumentation Core working group of the NTR) optical imaging devices, enables a better understanding of how these industry segments can collaborate to realize a new markets to meet unmet clinical needs.

Most importantly, the ability to bring diverse, segmented markets together requires not only a compelling clinical need, but a compelling market as well. The clinical "impact" of research and technology developments to solve unmet needs is judged as a criterion on most research grants. Yet "impact" is not always examined with the consideration of economic or market limitations as are "market assessments." In addition, principles of beneficence require that we engage in clinical research that can benefit populations (whether orphan or not), and by interference, involve technologies that have either an existing or potential market (whether large or small). The navigational routes established for both optical imaging devices and imaging agents enable greater clarity for conducting market assessments that need to be continually updated along the overall route for translating optical imaging technologies. In this NTR Chemistry Probes and Guided Therapies Core working group, market assessments were not part of the general translational roadmap although they were made in varying degrees by each of the participating centers and partners for their entire technology platforms.

Nonetheless, the clinical impact is significant for cancer patients and survivors who need image guidance for improved therapy and real-time risk assessment. As a community, we need to ensure that our discoveries that have clear evidence to fulfill unmet clinical needs actually translate into the clinic. We may need to pose our discoveries in the context of existing or new markets and educate both the clinical end-users and the patient population who can benefit from these advances. Given the number of unmet clinical needs that can be addressed using optical physics, and the advances in optical instrumentation, there is a bright future for biomedical optical imaging agents.

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