Development of New Imaging Probes for CVD: Challenges and Possible Benefits

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CVD continues to be the leading cause of death and disability in the US. In this talk I will describe current approaches, limitations, and areas where imaging probe and/or platform development might make a clinical impact in identifying patients at risk for acute myocardial infarction (AMI) (coronary bed), strokes (carotid disease), and limb ischemia (peripheral arterial disease) and heart failure (myocardium).

Identifying patients at risk for AMI before the event is still an unmet need. AMI can present without warning symptoms and/or as sudden cardiac death. Successfully identifying patients with vulnerable plaque from all presenting with chest pain (cp) currently involves 24 hr hospital admission for troponins and either stress nuclear or coronary CT which is time consuming. Developing perfusion imaging probes with higher myocardial extraction and shorter retention times than current agents would shorten the time. Screening asymptomatic patients with high risk factor profiles or mildly symptomatic patients is even more challenging. As better methods such as new biomarkers or GWAS are developed a group of "vulnerable" patients may be identified but localizing vulnerable lesions to plan interventions may benefit from an imaging test that is simple, fast, and inexpensive. Molecular biology has identified many possible targets for plaque imaging and over 500 preclinical papers over the past 30 years have described probes for nuclear, MR, US, and optical platforms generally falling into these categories: uptake of oxidized LDL, plaque inflammation, apoptosis, ECM growth, and neoangiogenesis. A drawback to the practical implementation or these approaches as screening is perceived need for hybrid imaging to localize uptake to vessels. Single platform approaches include tissue characterization of non-contrast MRI scans of the carotids, and vessel remodeling from coronary CTA. Nuclear approaches to image vulnerable coronary plaque is challenging but perhaps not impossible with a probe with high S/N and short imaging protocols. Shallower depth targets including carotids or PAD might be approached with optical imaging.

PAD is a neglected disease and possible unmet need for new probes. Clinical testing has not changed for years and includes non-invasive flow studies (NIFs) and CTA or MRI for anatomy. The only probe targeted imaging reported is F-18 sodium fluoride for calcium in arterial medial necrosis, and analysis of CT scans of legs to measure muscle atrophy. Heart failure is a growing problem with aging of the population. There is an important unmet need to develop better approaches to access efficacy of new treatments. Current approaches include the 6 min walk, cardiopulmonary testing, EF or regional wall motion by echo or MRI. Nuclear imaging has focused on select patient groups with PET metabolic imaging or applying clinically available probes to select groups of patients including FDG for inflammation (sarcoid, RA), Tc-99m PYP for senile amyloid, MIBG for presynaptic SNS, and annexin for AMI. A potential unmet need would be development of probes that target other pathways involved in oxidative stress, inflammation, and/or cell death and consequent adverse remodeling and development of fibrosis. Current approaches to quantify myocardial fibrosis include 3D ECV by cardiac CT and cardiac MR T1 mapping but no there is no probe targeting collagen.

Clinical Needs for Molecular Imaging in Diabetes and Metabolic Diseases

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Diabetes and obesity have developed into a health crisis in much of the world, and our ability to treat these chronic metabolic diseases has depended on an understanding of the natural history and mechanisms derived from imperfect animal models. Although there are many aspects of human metabolic diseases that remain to be elucidated, four specific areas will be discussed in which visualization of the biological or disease process would be transformative. 1) Diabetes results from the loss of function of the pancreatic beta cell, which has until recently been understood to be accompanied by cell death. New data from cadaveric pancreases and animal models imply instead that beta cells may persist for years following disease onset and that waning of beta cell function may sometimes be due to changes in cell phenotype (de- or re-differentiation). These sorts of data have called into question everything that was presumed known about the natural history of the beta cell in diabetes. Badly needed are means to noninvasively visualize beta cell mass and function in order to monitor disease onset and progression. This information would allow us to design and test prevention strategies and therapies in people, to aid in differential diagnosis of diabetes, and to monitor an individual's response to therapy. 2) Heat generating brown (BAT) and beige adipose tissue, studied for years in rodents, is now known to exist in adult human beings. Animal studies imply that metabolic activity in this tissue can be protective against diet-induced obesity, and researchers and pharmaceutical companies are interested to see if this applies to people as well. Noninvasive measures of BAT mass and metabolic activity are needed to establish the incidence of BAT in the human population and to monitor its effects on metabolic health. 3) Non-alcoholic fatty liver disease (NAFLD) is currently thought to affect about 30% of the population and a much larger fraction of the obese. NAFLD can progress in a small fraction of people to cirrhosis and liver cancer. Non-invasive imaging approaches to detect early inflammation and fibrosis are needed to stage disease and to identify people at elevated risk of progression so they can be more effectively treated. 4) Chronic metabolic diseases upset energy homeostasis in the body, and it has become clear that the brain is both involved in disease onset and altered by disease state. Functional MRI and PET approaches are helping to elucidate these processes, but much more work is needed. Specific molecular imaging approaches will help tease out the neural pathways involved, and technological advances are needed in order to monitor the activity of specific neurons particularly in the deep structures (hypothalamus and brainstem) involved in energy homeostasis.

Molecular Imaging Needs in Translational and Clinical Neuroscience

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Disorders of the CNS are among the most common causes of death and disability. The most common conditions, dementia and parkinsonism, consist of multiple syndromes with limited ability to distinguish among differing pathologies in life. Many of these syndromes have only limited symptomatic therapy (e.g. Alzheimer disease, dementia with Lewy bodies, progressive supranuclear palsy, frontotemporal dementias), and virtually none of these syndromes have effective disease-modifying therapies available. An important unmet goal in molecular neuroimaging is the accurate identification of neuropathologic processes that serve to distinguish subpopulations of these syndromes. The recent advent of imaging probes selective for Aß-amyloid deposition is one example of molecular classification of cognitive decline and dementia patients. However, there remain many other molecular targets that could serve to distinguish subpopulations of dementia and movement disorders patients with similar specificity. Some of these molecular targets include: Tau protein aggregates (of multiple types) and α-synuclein aggregates.

Other important processes in neurodegenerations include losses of vulnerable neurons and synapses and presence of inflammation. Both neuronal/synaptic numbers and integrity and inflammation are amenable to molecular imaging approaches. Notably absent from the available probes are approaches to quantification of glutamate neurons and GABA neurons. Similarly, while there is considerable emphasis on TSPO ligands for depiction of inflammation, this is but one of many aspects of the inflammatory cascade, and complimentary molecular approaches are needed.

Finally, many neurobehavioral syndromes, including affective disorders and addictions, are not associated with overt structural neuropathology and are most likely related to altered synaptic function and regulation. In these instances, molecular measures of synaptic activity, particularly neurotransmitter release and synaptic levels, has the potential to advance mechanistic understanding and treatment effects. At present, there are established tracers of synaptic dopamine and opiate peptide levels, but not for other monoamines, acetylcholine or acidic amino acid transmitters.

Improved tracers for the above targets and indications will be critical to the mechanistic understanding of CNS disorders and ultimately to the development of new and effective therapeutics.

Imaging Chemokine Receptors: The Case of CXCR4

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NIAID/NIH

The chemokine system in humans contains more than forty chemotactic proteins and nineteen seven-transmembrane domain receptors. Chemokines and their receptors have as their chief physiological role the control of leukocyte trafficking, which is essential for leukocyte development, establishing the anatomy of the immune system, and immune and inflammatory responses in lymphoid organs and peripheral tissues. The receptor CXCR4 and its chemokine ligand, CXCL12, are, however, unusual in that they play fundamental roles not only within, but also outside the hematopoietic system. Within the hematopoietic system, CXCR4 is important in stem cell homing to bone marrow, which forms the basis for the clinical use of plerixafor, a CXCR4 antagonist. Plerixafor is approved in combination with G-CSF for the mobilization of hematopoietic stem cells into blood in order to enable autologous transplantation as a component of therapy for multiple myeloma and some lymphomas. CXCR4 itself is expressed on many human cancers, and its expression has been implicated in metastasis and poor prognosis. We have produced plerixafor loaded with the positron-emitter, 64Cu, and shown that it can visualize CXCR4-expressing tumors in mice in a receptor-specific fashion using PET. In patients with cancer, detection and quantification of CXCR4 expression on primary and metastatic tumors over time might provide information regarding the tumors' behaviors and responses to therapies, including therapies targeting CXCR4. Our data suggest that in addition to 64Cu-plerixafor, antagonists of other chemokine receptors might be radiolabeled for use in PET. Such probes could allow for the visualization of specific leukocytes subsets in tissue, providing information about the cellular components of infiltrates that could be of value in studying, diagnosing, and treating infectious and inflammatory diseases.

Recent Advances in MR Probes: Are We There Yet?

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Not long ago the only tools available to probe cellular and physiological processes at any level were the senses. Morphology of the specimen was the determining factor in the investigation of mechanisms of biological and clinical diagnostic questions. The quest to understand fundamental biological questions has driven technological advances in an emerging, and rather fast changing area of research known as molecular imaging. One technique that has been a powerful tool in clinical and biological settings is magnetic resonance imaging (MRI). It is non-invasive and yields a true volume rendering of the subject with cellular resolution (10 microns).

To permit the direct observation of ongoing developmental events in living embryos, the descendants of individual precursors in an intact embryo are labeled by microinjection of a stable, nontoxic, membrane impermeable MRI lineage tracers. Since a complete time-series of high-resolution three-dimensional MR images can be analyzed forward or backward in time, one can fully reconstruct the cell divisions and cell movements responsible for any particular descendant(s). Unlike previous methods, where labeled cells are identified at the termination of the experiment, MR imaging allows the entire kinship relationships of a clone to be determined.

We have been investigating the development of molecular MR probes that are biochemically activated (in vivo) and report this information in the form of an acquired 3D MR image. The agents modulate fast water exchange with the paramagnetic center, yielding distinct “strong” and “weak” relaxivity states. The modualtion is triggered by two types of biological events: 1) enzymatic processing of the contrast agent, and 2) the reversible binding of an intracelluar messenger. These agents represent the first examples of direct, three-dimensional visualization of gene expression and intracellular second messenger concentration in the form of a 3D MR image.

Unmet Needs in PET Imaging of Neurodegenerative Diseases

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Two unmet needs in PET imaging of neurodegenerative diseases include the development of selective alpha-synuclein PET radioligands and a sensitive and specific marker of neuroinflammation.

Analogous to successful work accomplished with amyloid-beta and tau imaging agents in Alzheimer's disease, the ability to visualize alpha-synuclein in the brain would provide a useful biomarker of the presence of disease and disease progression and a tool for drug development. The accumulation of alpha-synuclein in the brain of Parkinson's disease subjects is a pathological hallmark of the disease, and drugs that target alpha-synuclein build up in brain are under development. A non-invasive method to assess the efficacy of anti-alpha-synuclein drugs in removing excess aggregated alpha-synuclein from the brain would be very useful as a tool to assist drug development efforts.

Increasing evidence suggests that inflammation in the CNS can augment neuronal damage in many neurological disorders. Over the last three decades, efforts to develop and apply a non-invasive imaging agent indexing CNS inflammation have focused on a single molecular target, TSPO. Recently, several new classes of TSPO-specific radioligands have been identified that possess more favorable characteristics for PET imaging compared to [11C]PK-11195. Despite these successes, several problems remain and call into question the usefulness of TSPO as a target for continued probe development efforts. Among these problems are human gene polymorphism in the region encoding TSPO that complicates the interpretation of the binding of TSPO radioligands, as well as issues of cell selectivity and low sensitivity for more subtle inflammatory pathology characteristic of many CNS diseases. The development of new probes aimed at identifying other targets of CNS inflammation are needed to move the field forward.

Ultrasound-based Molecular Imaging: Advantages, Limitations and Outlook

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Quantitative molecular imaging capable of visualizing biochemical, pharmacological and other processes in vivo and repetitively during stages of various pathologies (e.g., cancer, cardiovascular diseases, etc.) is needed in many fundamental, preclinical and clinical applications. In addition, visualization of molecular and cellular events in context of morphological and physiological properties of the tissue is desired. Recently, several high-resolution, high-sensitivity, depth-resolved ultrasound-based imaging techniques capable of simultaneous visualization of structural, functional and molecular/cellular properties of the tissue at clinically relevant penetration depth were introduced. For example, nanometer scale contrast agents for ultrasound and ultrasound-based imaging were synthesized and tested. Furthermore, several hybrid technologies such as combined ultrasound and photoacoustic (USPA) imaging were introduced. Overall, there is a concerted effort to develop and translate ultrasound-based molecular imaging techniques, augmented with targeted contrast nanoagents, to preclinical research and, ultimately, to clinical practice. Indeed, an imaging system based on clinical ultrasound and capable of simultaneous anatomical, functional, cellular and molecular visualization of tissue will have a significant impact on disease detection, diagnosis, therapy, and monitoring of treatment outcome.

In this presentation, following a brief introduction and overview of ultrasound imaging, approaches in ultrasound-based molecular imaging will be introduced and analyzed. Several ultrasound-based molecular imaging systems will be discussed. Particular attention will be given to the latest preclinical (i.e., mouse model) and clinical endeavors of the developed technologies. Imaging applications ranging from cancer imaging to stem cell tracking to assessment of atherosclerotic plaques will then be used to discuss advantages, limitations and challenges of molecular ultrasound imaging. Finally, the presentation will conclude with the outlook of contrast-agent augmented molecular ultrasound imaging for diagnostic and therapeutic applications.

Multi-modality and Multi-functional Molecular Imaging Agents

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As the field of molecular imaging matures, i.e., equipment of higher spatial resolution and sensitivity is devised, more relevant animal models of disease are generated and new, efficient methods for the synthesis of existing and new classes of imaging agents become more widespread, researchers can begin to address deeper biological questions than previously. Particularly valuable may be multi-modality and multi-functional agents. Such agents may provide information synergistically by leveraging the benefits of two or more imaging modalities concurrently. They may also be more efficient, by allowing serial (or concurrent – depending on the device) multi-modal imaging after administration of only one agent. Likewise multi-functional agents can improve efficiency by enabling detection and treatment in one session. Multi-functional agents can also be used in tandem, to enable high correlation between detection (by imaging, for example) and treatment of the precise lesions detected with the multi-functional agent. Strategies for the development of multi-modality and multi-functional molecular imaging agents will be discussed including their uses, potential for overcoming key biological problems, such as tumor heterogeneity, and practicality for clinical use.

NCI Programs in Translational Molecular Imaging Research

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NCI/NIH

Molecular imaging agents have great promise for investigating biology and for assisting in the development of new therapeutic drugs. However, it can be difficult to obtain funding for the needed mundane preclinical studies needed and to obtain the regulatory approvals to investigate them in the clinical trial setting, particularly when pairing an investigational molecular imaging probe with an investigational therapeutic drug. This presentation will discuss some of the NCI's mechanisms to help bridge the "valley of death" in translational development and discuss our experience in with non-proprietary investigational molecular probes in both imaging only and imaging-therapy trial settings with variety of regulatory strategies.

The Expanding Use of PET Molecular Imaging, the Current Trends and Challenges for Pharmaceutical Companies

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Molecular imaging (MI) plays an expanding role in pharmaceutical development. The trends for positron emission tomography (PET) in MI demonstrate a broadening use, across multiple disciplines, ranging from neuroscience, oncology, cardiovascular, and inflammatory diseases. PET molecular imaging provides direct quantitative measurement of the level of target engagement, potential early diagnosis and staging / monitoring of diseases. The current landscape of MI, specifically regarding PET, often entails all aspects of the process from radiotracer development (or selection) to image processing and analysis, thus requiring a broad range of skill sets and resources for the successful incorporation of PET into the drug development process. This necessitates early strategies around overall feasibility, including preclinical and clinical imaging trials, and the use of internal versus external resources and collaborations. The outsourcing of work has increased dramatically over the past decade, emphasizing the need for a strong network of external collaborations. Simultaneously, the rapidly evolving role of PET molecular imaging often outpaces the available expertise of the research units seeking to employ the techniques, making external education and subject expertise essential to bridge the path forward. Drug candidate and tracer discovery timelines must be closely associated to maximize benefits and minimize costly delays. The study designs are often “fit for purpose” answering only the questions of critical interest, potentially leaving scientifically interesting experiments behind for those more academically oriented. The future of PET molecular imaging is bright as a powerful tool in the competitive landscape of pharmaceutical development; it often saves both time and money in the selection of targets, confirmation of mechanisms, dosage selection, and the overall evaluation of candidate drugs in their development.

Use of Exploratory IND for Early Phase Clinical Trials Using PET or SPECT Imaging

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Recent changes in the US regulatory guidelines for theinvestigational use of imaging tracers in humans significantly impact the approach to developing novel radiopharmaceuticals for positron emission tomography (PET) or single photon emission computed tomography (SPECT). Keying off the fact that most of the molecules synthesized by medicinal chemists fail to become useful tools at one of the steps and the high safety profile afforded by microdosing, these guidelines, published by the US FDA exploratory IND (expIND) provide an efficient mechanism for evaluating investigational radiopharmaceuticals in human, including the specific disease indication inwhich the imaging agent might be used. The radiopharmaceutical development model runs as follows: 1) following compound synthesis, in-vitro binding assays are performed to determine affinity and selectivity for the target; 2) radio-labeling and initial in-vivo studies permits verification of the distribution of the new radiotracer, blood metabolites and if further studies are warranted; 3) selection of the appropriate animal model occurs; and 4) after submission of the expIND preliminary human studies in limited numbers of subjects, including within subject comparison of structurally-related radiopharmaceuticals, displacement/occupancy studies, etc.

During this presentation we will present how as a multidisciplinary team (Chemistry, Clinical, Imaging, Data management and Regulatory) we have been able to very efficiently validate some of new radiotracers for PET and SPECT for brain imaging and to apply those radiotracers in evaluating neurological disease such as Huntington Disease and Alzheimer Disease.

Clinical Translation of Hyperpolarized Carbon-13 MRI Technology: Successes and Challenges

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Hyperpolarized carbon-13 MRI using the dissolution dynamic nuclear polarization (DNP) method can provide a >10,000 fold signal enhancement for detecting ^{13}C probes of endogenous, nontoxic, nonradioactive substances such as pyruvate to monitor metabolic fluxes through multiple key biochemical pathways (NIH White Paper; Neoplasia 2011). The hyperpolarization of [1-^{13}C]pyruvate has demonstrated the ability to not only detect pyruvate uptake but also the in vivo enzymatic conversion to ^{13}C-lactate through the enzyme lactate dehydrogenase (LDH), ^{13}C-alanine through the alanine transaminase (ALT) pathway; and $^{13}CO2$ & ^{13}C-bicarbonate through the pyruvate dehydrogenase (PDH) catalyzed metabolic pathway. In addition to basic science and animal studies utilizing HP ^{13}C MRI, we developed new techniques and coils for the first human studies in prostate cancer patients and tested them in preclinical murine prostate cancer and canine models with injections of HP ^{13}C-pyruvate. A proof-of-concept (POC) DNP instrument was constructed through an academic-industry collaboration with GE to operate in a sterile clean room next to a 3T MR scanner under the direction of UCSF Clinical Pharmacy investigators. Specialized C-13 MRI techniques were developed to provide extremely rapid volumetric imaging and serial dynamic acquisitions to monitor temporal metabolic changes in cancer patients following the injection of HP ^{13}C-pyruvate. Led by UCSF Cancer Center investigators, our multidisciplinary research group designed and conducted the world's first clinical trial of hyperpolarized carbon-13 MRI titled, "A Phase 1 ascending-dose study to assess the safety and tolerability and imaging potential of hyperpolarized Pyruvate (^{13}C) Injection in subjects with prostate cancer." This study received FDA-IND approval and 31 patients were studied demonstrating feasibility and safety with no dose-limiting toxicity up to 0.42ml/kg of 250mM HP pyruvate. Increased lactate conversion was observed in prostate cancers up to 70s following injection. Future plans include three new clinical research studies in prostate cancer and brain tumor patients, validation of the new GE commercial dissolution DNP instrument recently purchased through an NIH high-end instrumentation grant, revised IND applications, new acquisition/analysis developments, and future kidney and liver studies.

The Use of Imaging in Clinical Trials: CDER's Perspective

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The use of imaging in a clinical trial intended to support a drug approval can serve different functions. Imaging can provide assurance that study subjects have the condition being studied or are likely to respond to the investigational drug. Imaging may also play a role in the assessment of patient safety or in the assessment of the effectiveness of diagnostic or therapeutic drugs. The quality of the evidence required for drug approval needs to be considered. To obtain marketing approval, drug manufacturers need to demonstrate the effectiveness of their products through the conduct of adequate and well-controlled studies. This means that trials are designed to distinguish the effect of the drug from other influences such as spontaneous change in the disease, placebo effect, or biased observation. The trials are also designed to permit a valid comparison with a control and to provide a reliable assessment of a drug's effect.

Imaging provides information of variable clinical meaningfulness, ranging from indisputably useful information to that of uncertain value. For this reason to establish efficacy in clinical studies of imaging drugs, we recommend that the anatomic, functional, or physiological measurements using an imaging agent be compared with those of a reference product or a procedure of known high validity (i.e., a truth standard). If no standard of truth is available, we recommend that a clinical trial be conducted to determine that the findings are clinically useful. The methods of assessment of an imaging endpoint need to be well defined and reliable. Practice standards used in routine clinical imaging may be sufficient to meet a trial's objective. In other cases clinical trial-specific aspects may require augmented imaging standardization. These augmented standards are intended to ensure that imaging data are obtained in a manner that complies with a trial's protocol, that the quality of imaging data is maintained at study sites, and that there is a verifiable record of the imaging process. Another important objective is to minimize imaging variability to enhance a clinical trial's ability to detect drug treatment effects. When sponsors anticipate an important role for imaging in a clinical trial, they should contact the assigned FDA review division to discuss the clinical meaningfulness of the imaging information to be obtained in the trial.

Medicare Coverage of Molecular Imaging

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Medicare coverage of diagnostic testing is based on the statutory "reasonable and necessary" standard. By rule, a diagnostic test must be ordered by the beneficiary's treating physician who uses the test result in the management of the beneficiary's medical care. When considering coverage for a specific diagnostic test, CMS looks for evidence that allows it to conclude that the use of the test leads to improved patient health outcomes. CMS also considers evidence that speaks to the changes in management that may arise from reasonable application of the test in a diagnostic scheme. With few exceptions as described in statute, Medicare does not cover screening tests in asymptomatic beneficiaries.