Guidance for Industry

Developing Medical Imaging Drug and Biological Products

Part 3: Design, Analysis, and Interpretation of Clinical Studies

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)

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Clinical Medical

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This guidance represents the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. An alternative approach may be used if such approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the FDA staff responsible for implementing this guidance. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.

I. INTRODUCTION

This guidance is one of three guidances intended to assist developers of medical imaging drug and biological products (*medical imaging agents*) in planning and coordinating their clinical investigations and preparing and submitting investigational new drug applications (INDs), new drug applications (NDAs), biologics license applications (BLAs), abbreviated NDAs (ANDAs), and supplements to NDAs or BLAs. The three guidances are*: Part 1: Conducting Safety Assessments*; *Part 2: Clinical Indications*; and *Part 3: Design, Analysis, and Interpretation of Clinical Studies*.

Medical imaging agents generally are governed by the same regulations as other drug and biological products. However, because medical imaging agents are used solely to diagnose and monitor diseases or conditions as opposed to treat them, development programs for medical imaging agents can be tailored to reflect these particular uses. Specifically, this guidance discusses our recommendations on how to design a clinical development program for a medical imaging agent including selecting subjects and acquiring, analyzing, and interpreting medical imaging data.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are

[1] This guidance has been prepared by the Division of Medical Imaging and Radiopharmaceutical Drug Products and the Office of Therapeutics Research and Review in the Center for Drug Evaluation and Research (CDER) at the Food and Drug Administration.

cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.

A glossary of common terms used in diagnostic medical imaging is provided at the end of this document.

II. SCOPE — TYPES OF MEDICAL IMAGING AGENTS

This guidance discusses medical imaging agents that are administered in vivo and are used for diagnosis or monitoring with a variety of modalities, such as radiography, computed tomography (CT), ultrasonography, magnetic resonance imaging (MRI), and radionuclide imaging. The guidance is not intended to apply to the development of in vitro diagnostic or therapeutic uses of these agents.[2]

Medical imaging agents can be classified into at least two general categories:

A. Contrast Agents

As used in this guidance, a contrast agent is a medical imaging agent used to improve the visualization of tissues, organs, and physiologic processes by increasing the relative difference of imaging signal intensities in adjacent regions of the body. Types of contrast agents include (1) iodinated compounds used in radiography and CT; (2) paramagnetic metallic ions (such as ions of gadolinium, iron, and manganese) linked to a variety of molecules and microparticles (such as superparamagnetic iron oxide) used in MRI; and (3) microbubbles, microaerosomes, and related microparticles used in diagnostic ultrasonography.

B. Diagnostic Radiopharmaceuticals

As used in this guidance, a *diagnostic radiopharmaceutical* is (1) an article intended for use in the diagnosis or monitoring of a disease or a manifestation in humans and that exhibits spontaneous disintegration of unstable nuclei with the emission of nuclear particles or photons or

[2] The guidance is not intended to apply to the development of research drugs that do not provide direct patient benefit with respect to diagnosis, therapy, prevention, or prognosis, or other clinically useful information. These include radioactive drugs for research that are used in accordance with 21 CFR 361.1. Section 361.1 states that radioactive drugs (defined in 21 CFR 310.3(n)) are generally recognized as safe and effective when administered under specified conditions to human research subjects in the course of a project intended to obtain basic information about the metabolism of a radioactively labeled drug or about human physiology, pathophysiology, or biochemistry. However, if a radioactive drug is used for immediate therapeutic, diagnostic, or similar purpose or to determine the safety and effectiveness of the drug in humans, or if the radioactive drug has a pharmacological effect in the body, an IND is required. FDA is developing a guidance on determining when research with radioactive drugs may be conducted under § 361.1.

The Agency recognizes the potential of imaging agents as research tools for aiding the development of therapeutic drugs, and some of the principles of the guidance may be applicable to such research.. Sponsors of such imaging research agents are urged to contact the Division of Medical Imaging and Radiopharmaceutical Drug Products for advice on development of the imaging research agent.

(2) any nonradioactive reagent kit or nuclide generator that is intended to be used in the preparation of such an article.[3] As stated in the preamble to FDA's proposed rule on Regulations for In Vivo Radiopharmaceuticals Used for Diagnosis and Monitoring, the Agency interprets this definition to include articles that exhibit spontaneous disintegration leading to the reconstruction of unstable nuclei and the subsequent emission of nuclear particles or photons (63 FR 28301 at 28303; May 22, 1998).

Diagnostic radiopharmaceuticals are generally radioactive drugs or biological products that contain a radionuclide that typically is linked to a ligand or carrier.[4] These products are used in planar imaging, single photon emission computed tomography (SPECT), positron emission tomography (PET), or with other radiation detection probes.

Diagnostic radiopharmaceuticals used for imaging typically have two distinct components.

- A radionuclide that can be detected in vivo (e.g., technetium-99m, iodine-123, indium-111).

  The radionuclide typically is a radioactive atom with a relatively short physical half-life that emits radioactive decay photons having sufficient energy to penetrate the tissue mass of the patient. These photons can then be detected with imaging devices or other detectors.

- A nonradioactive component to which the radionuclide is bound that delivers the radionuclide to specific areas within the body.

  This nonradionuclidic portion of the diagnostic radiopharmaceutical often is an organic molecule such as a carbohydrate, lipid, nucleic acid, peptide, small protein, or antibody.

As technology advances, new products may emerge that do not fit into these traditional categories (e.g., agents for optical imaging, magnetic resonance spectroscopy, combined contrast and functional imaging). It is anticipated, however, that the general principles discussed here could apply to these new diagnostic products. Developers of these products are encouraged to contact the appropriate reviewing division for advice on product development.

[3] 21 CFR 315.2 and 601.31.

[4] In this guidance, the terms *ligand* and *carrier* refer to the entire nonradionuclidic portion of the diagnostic radiopharmaceutical.

III. GENERAL CONSIDERATIONS IN THE CLINICAL EVALUATION OF MEDICAL IMAGING AGENTS

A. Phase 1 Studies

The general goal of phase 1 studies[5] of medical imaging agents is to obtain pharmacokinetic and human safety assessments of a single mass dose and increasing mass doses of a drug or biological product. We recommend that evaluation of a medical imaging agent that targets a specific metabolic process or receptor include assessments of its potential effects on these processes or receptors.

We recommend that, for diagnostic radiopharmaceuticals, organ and tissue distribution data over time be collected to optimize subsequent imaging protocols and calculate radiation dosimetry (see Part I, section IV.D). We also recommend that, as appropriate, pharmacokinetic and pharmacodynamic evaluations be made of the intact diagnostic radiopharmaceutical, the carrier or ligand, and other vial contents, especially when large amounts of cold components are present as determined by absolute measurement or by relative concentration of labeled to unlabeled carrier or ligand. This can be achieved by administering large mass doses of a medical imaging agent with low specific activity, administering the contents of an entire vial of a medical imaging agent (assuming that this approximates a worst-case scenario in clinical practice), or both. Because of potential toxicities, this approach may not be appropriate for some drugs nor for most biological products. In such cases, we recommend you contact the review division.

B. Phase 2 Studies

The general goals of phase 2 studies of medical imaging agents include (1) refining the agent's clinically useful mass dose and radiation dose ranges or dosage regimen (e.g., bolus administration or infusion) in preparation for phase 3 studies, (2) answering outstanding pharmacokinetic and pharmacodynamic questions, (3) providing preliminary evidence of efficacy and expanding the safety database, (4) optimizing the techniques and timing of image acquisition, (5) developing methods and criteria by which images will be evaluated, and (6) evaluating other critical questions about the medical imaging agent. With the accomplishment of these elements, phase 3 development should proceed smoothly.

We recommend that sponsors explore the consequences of both mass dose and radiation dose (or dosage regimen) adjustment on image acquisition and on the safety or effectiveness of the administered product. We recommend that additional exploration include adjusting the following if relevant:

- Character and amount of active and inactive ingredients
- Amount of radioactivity

[5] See also the guidance *Content and Format of Investigational New Drug Applications (INDs) for Phase-1 Studies of Drugs, Including Well-Characterized, Therapeutic, Biotechnology-Derived Products.* This and all other guidances cited in this document are available at FDA's Web site at http://www.fda.gov/cder/guidance/index.htm.

- Amount of nonradioactive ligand or carrier
- Specific activity
- Radionuclide that is used

We recommend that methods used to determine the comparability, superiority, or inferiority of different mass and radiation doses or regimens be discussed with the Agency. To the extent possible, the formulation that will be used for marketing should be used during phase 2 studies. When a different formulation is used, we recommend that bioequivalence and/or other bridging studies be used to document the relevance of data collected with the original formulation.

We recommend that phase 2 studies be designed to define the appropriate patient populations and clinical settings for phase 3 studies. To gather preliminary evidence of efficacy, however, both subjects with known disease (or patients with known structural or functional abnormalities) and subjects known to be normal for these conditions may be included in clinical studies. However, for products that are immunogenic or exhibit other toxicities, use of healthy subjects may not be appropriate. We recommend that methods, endpoints, and items on the case report form (CRF) that will be used in critical phase 3 studies be tested and refined.

C. Phase 3 Studies

The general goals of phase 3 efficacy studies for medical imaging agents include confirming the principal hypotheses developed in earlier studies, demonstrating the efficacy and continued safety of the medical imaging agent, and validating instructions for use and for imaging in the population for which the agent is intended. We recommend that the design of phase 3 studies (e.g., dosage, imaging techniques and times, patient population, and endpoints) be based on the findings in phase 2 studies. We recommend that the formulation intended for marketing be used, or bridging studies be performed.

When multiple efficacy studies are performed, the studies can be of different designs.[6] To increase the extent to which the results can be generalized, we recommend the studies be independent of one another and use different investigators, clinical centers, and readers that perform the blinded image evaluations (see section IV.B).

IV. ADDITIONAL CONSIDERATIONS IN THE CLINICAL EVALUATION OF EFFICACY

The following sections describe special considerations for the evaluation of efficacy in clinical trials for medical imaging agents (see *Part 2: Clinical Indications*, section IV, for recommendations on general considerations for establishing effectiveness, clinical usefulness, and clinical setting).

[6] See the guidance *Providing Clinical Evidence of Effectiveness for Human Drug and Biological Products.*

A. Selecting Subjects

We recommend that subjects included in phase 3 clinical efficacy studies be representative of the population in which the medical imaging agent is intended to be used. We also recommend that the protocol and study reports specify the method by which patients were selected for participation in the study (e.g., consecutive subjects enrolled, random selection) to facilitate assessments of potential selection bias (e.g., using a comparator test result to pre-select subjects most likely to have the desired image finding).[7]

B. Imaging Conditions and Image Evaluations

The following guidance may be customized to the specific medical imaging drug, biological product, or imaging modality under development. (The term *images* is nonspecific and may refer to an individual image or to a set of images acquired from different views, different sequences and timing.)

1. Imaging Conditions

We recommend that the effects of changes in relevant imaging conditions (e.g., timing of imaging after product administration, views, instrument settings, patient positioning) on image quality and reproducibility, including any limitations imposed by changes in such conditions, be evaluated in early product development. We recommend that subsequent, phase 3 efficacy trials substantiate and possibly refine these conditions for use. Appropriate imaging conditions, including limitations, can be described in the product labeling.

2. Methods and Considerations for Image Evaluation

We recommend that methods and criteria for image evaluation (including criteria for image interpretation) be evaluated in early product development. Subsequently, we recommend that the methods and criteria that are anticipated for clinical use be employed and substantiated in the phase 3 efficacy trials. For example, early clinical trials might compare ways in which regions of interest on images are selected or ways in which an organ will be subdivided on images for purposes of analysis. Similarly, early clinical trials might evaluate which objective image features (e.g., lesion conspicuity, relative count rate density) appear to be most affected by the medical imaging agent and which of

[7] To aid in the subsequent use of this information in clinical trial design, the pretest odds or pretest probabilities of disease can be used as part of the selection criteria as a method of ensuring enrollment of the population of intended use and/or as part of the patient stratification or subsetting criteria for analysis. We recommend that the range of pretest probabilities enrolled be determined by the type of clinical setting that will support the labeling (e.g., a screening setting, a case finding setting, a pivotal decision setting). We recommend that the pretest odds or probabilities be estimated for all subjects after enrollment, but before any trial results are made available. We also recommend that these odds and probabilities be derived from prespecified criteria for disease (e.g., history, physical findings, results of other diagnostic evaluations) according to prespecified algorithms. We recommend that the estimated pretest odds and probabilities of disease should be compared with the pretest odds and probabilities actually observed in the studies. (See the glossary for the definition of terms relating to pretest odds and probabilities for study analysis.)

these are most useful in image interpretation, such as making a determination of whether a mass is benign or malignant (see section IV.B.3).

We recommend that the most appropriate of these methods and criteria for image evaluation be incorporated into the protocols of the phase 3 efficacy trials.

A description of the appropriate methods and criteria for image evaluation, including limitations, should be described in the product labeling.

We recommend that sponsors seek FDA comment on the designs and analysis plans for the principal efficacy trials before they are finalized. In some cases, special protocol assessments may be appropriate (see guidance for industry *Special Protocol Assessment*). In addition, we recommend that the following elements be completed and submitted to the IND before the phase 3 efficacy studies enroll subjects:

- Proposed indications for use
- Protocols for the phase 3 efficacy trials
- Investigators' brochure
- CRFs to be used by on-site investigators
- Plan for blinded image evaluations[8]
- CRFs to be used by the blinded readers
- Statistical analysis plan
- Plan for on-site image evaluation and intended use of such evaluation in patient management, if any

We recommend that sponsors submit a single comprehensive statistical analysis plan for each principal efficacy study. We recommend that this statistical analysis plan be part of the study protocol, include the plan for blinded image evaluations, and be submitted to the protocol before images have been collected.

3. Steps in Image Evaluation

The evaluation of medical images generally consists of two distinct steps: assessing objective image features and interpreting findings on the image.

a. Assessing objective image features

As used in this guidance, *objective image features* are attributes on the image that are either visually perceptible or that can be detected with instrumentation. Examples of objective image features include signal-to-noise ratios; degree of delineation; extent of opacification; and the size, number, or density of lesions.

[8] *Blinded* image evaluations may also be referred to as *masked* or as *uninformed* image evaluations.

Objective image features can be captured on scales that are continuous (e.g., the diameter of a mass), ordinal (e.g., a feature can be classified as definitely increased, probably increased, neither increased nor decreased, probably decreased, definitely decreased), or dichotomous (e.g., a feature can be classified as present or absent).

Medical imaging agents have their intended effects by altering objective image features. We recommend that both the nature and location of such changes on the image be documented fully during image evaluations in clinical trials intended to demonstrate efficacy. We also recommend that such documentation also include changes that are unintended or undesirable. For example, a diagnostic radiopharmaceutical intended for cardiac imaging also might localize in the liver, thereby obscuring visualization of parts of the heart.

When possible, it is often desirable to perform both a qualitative visual evaluation of images as well as a quantitative analysis of images with instrumentation. However, a quantitative image analysis with instrumentation by itself may not be sufficient to establish efficacy of the medical imaging agent, such as in cases where images are not intended (or not likely) to be evaluated quantitatively with instrumentation in clinical practice.

b. Image interpretation

As used in this guidance, an *image interpretation* is the explanation or meaning that is attributed to objective image features. We recommend that interpretations of image features be supported by objective, quantitative, and/or qualitative information derived from the images. For example, the interpretation that cardiac tissue seen on an image is infarcted, ischemic, or normal might be supported by objective image features such as the extent and distribution of localization of the medical imaging agent in the heart (e.g., increased, normal, decreased, or absent), the time course of such localization, and how these features are affected by exercise or pharmacologic stress.

4. Endpoints in Trials

Medical imaging agents could be developed for structural delineation; functional, physiological, or biochemical assessment; disease or pathology detection or assessment; diagnostic or therapeutic patient management; or multiple or other indications. The primary endpoints (response variables) relate to the indication's clinical usefulness (see Part 2: Clinical Indications, section IV.B).

a. Image interpretations as endpoints

Image interpretations that are clinically useful can be incorporated into the primary endpoint in phase 3 clinical trials. For example, the primary analysis endpoints of a trial for a medical imaging agent intended for the indication

disease or pathology detection or assessment might be the proportions of subjects with and without the disease who are properly classified against an appropriate truth standard. In this example, the interpretation that a pulmonary lesion seen on an image is benign or malignant has direct clinical meaning and can be incorporated into the primary endpoint.

b. Objective image features as endpoints

When the clinical usefulness of particular objective image features is obvious and apparent, the objective imaging features can be incorporated into the primary endpoint. For example, in a study of a medical imaging agent intended for brain imaging, the ability to delineate anatomy that indicates the presence or absence of cranial masses on images has direct clinical usefulness. The primary endpoint (e.g., cranial mass detection) serves as the primary basis for the indication for the product (e.g., the medical imaging agent is indicated for detecting cranial masses in patients in a particular defined clinical setting).

However, in some cases the clinical usefulness of particular objective image features may not be readily apparent without additional interpretation. In these cases, we recommend that the objective image features serve as secondary imaging endpoints. For example, the finding that a medical imaging agent alters the conspicuity of masses differentially could lead to the interpretation that specific masses are benign or malignant; acute or chronic; inflammatory, neoplastic, or hemorrhagic; or lead to some other clinically useful interpretations. The interpretations can be incorporated into the primary endpoint and can serve as the primary basis for the indication for the product. However, the objective image feature of lesion conspicuity might be designated more appropriately as a secondary imaging endpoint.

c. Subjective image assessments as endpoints

As used in this guidance, *subjective image assessments* are perceptions or inferences made by the reader. Such assessments are tangible and cannot be measured objectively. For example, a conclusion that use of a medical imaging agent alters *diagnostic confidence* is a subjective assessment as is the conclusion that a medical imaging agent provides *more diagnostic information*.

We recommend that subjective image assessments be linked to objective image features so that the objective basis for such assessments can be understood. Subjective image assessments can be difficult to validate and replicate. They may introduce bias as well. Therefore, subjective image assessments should not be used as primary imaging endpoints.

d. Clinical outcomes as endpoints

Clinical outcomes, such as measurement of symptoms, functioning, or survival, are among the most direct ways to measure clinical usefulness. Clinical outcomes can serve as primary endpoints in trials of medical imaging agents. For example, the primary endpoint of a trial of a medical imaging agent intended for the indication *therapeutic patient management* in patients with colon cancer might be a response variable that measures changes in symptoms, functioning, or survival.

5. Case Report Forms

We recommend that case report forms (CRFs) in trials of medical imaging agents prospectively define the types of observations and evaluations for investigators to record. In addition to data that are usually recorded in CRFs (e.g., inclusion/exclusion criteria, safety findings, efficacy findings), we recommend that the onsite investigator's CRF for a medical imaging agent capture the following information:

- The technical performance of the diagnostic radiopharmaceutical used in the study, if any (e.g., specific activity, percent bound, percent free, percent active, percent inactive)

- The technical characteristics and technical performance of the imaging equipment (e.g., background flood, quality control analysis of the imaging device, pulse height analyzer)

- Methods of image acquisition, output processing, display, reconstruction, and archiving of the imaging study

The collection and availability of the data on the CRF may be important for labeling how the imaging agent is intended to be administered and the appropriate device settings for optimal imaging.

6. CRFs for Image Evaluation

We recommend that imaging CRFs be designed to capture imaging endpoints, including objective features of the images as well as the location and interpretation of any findings. We recommend that interpretations of image features be supported by objective quantitative or qualitative information derived from the images. We recommend that image interpretations be recorded as distinct items from the assessments of the objective image features. We also recommend that items on the CRFs for image evaluation be carefully constructed to gather information without introducing a bias that suggests the answer that is being sought. We recommend that the proposed labeled indication be clearly derived from specific items in the CRF and from endpoints and hypotheses that have been prospectively stated in the protocol.

7. Blinded Imaging Evaluations

We recommend that image evaluations be designed to demonstrate that the specific effects of the medical imaging agent, as manifested in the images, provide such information reproducibly and apart from other possible confounding influences or biases. We recommend that blinded image evaluations by multiple independent readers be performed in the phase 3 efficacy studies.

We recommend that either a *fully blinded image evaluation* or an *image evaluation blinded to outcome* by independent readers serve as the principal image evaluation for demonstration of efficacy.[9] Alternatively, both types of image evaluations can be used; if so, the evaluations can be performed through sequential unblinding. Both primary and secondary imaging endpoints should be evaluated in this manner. We recommend that the nature and type of information available to the readers be discussed with FDA before the trials are initiated.

In addition to the items outlined in the sections below, we recommend that plans for blinded image evaluations include the following elements:

- We recommend that the protocol clearly specify the elements to which readers are blinded.

- We recommend that meanings of all endpoints be clearly understood for consistency. We recommend that terms to be used in image evaluation and classification be defined explicitly in the image evaluation plan, including such terms as *technically inadequate*, *uninterpretable*, *indeterminate*, or *intermediate*. Blinded readers can be trained in scoring procedures using sample images from phase 1 and phase 2 studies.

- We recommend that images be masked for all patient identifiers.

- We recommend that blinded readers evaluate images in a random sequence. *Randomization* of images refers to merging the images obtained in the study (to the fullest degree that is practical) and then presenting images in this merged set to the readers in a random sequence.

  For example, when images of several diagnostic radiopharmaceuticals read by the same criteria are being compared to establish relative efficacy (e.g., a comparison of a test drug or biological product to an established drug or biological product), we recommend the readers evaluate individual images from the merged set of images in a random sequence.

[9] See section IV.B.8 for a definition of *independent readers*.

a. Fully blinded image evaluation

During a *fully blinded image evaluation*, we recommend that readers not have any knowledge of the following types of information:

- Results of evaluation with the truth standard, of the final diagnosis, or of patient outcome

- Any patient-specific information (e.g., history, physical exam, laboratory results, results of other imaging studies)

We recommend that general inclusion and exclusion criteria for patient enrollment, other details of the protocol, or anatomic orientation to the images not be provided to the readers.

During a *fully blinded image evaluation* in studies where images obtained by different treatments are being evaluated, we recommend that readers not have knowledge of treatment identity, to the greatest extent to which that is possible.[10] For example, in a comparative study of two or more medical imaging agents (or of two or more doses or regimens of a particular medical imaging agent), we suggest the blinded readers not know which agent (or which dose or regimen) was used to obtain a given image.

For contrast agents, we suggest this also can include lack of knowledge about which images were obtained before product administration and which were obtained after product administration, although sometimes this is apparent upon viewing the images.

In cases where the instructions for image evaluation differ according to treatment (e.g., as might be the case when images are obtained using different imaging modalities), blinding the readers to treatment identity may be infeasible.

b. Image evaluation blinded to outcome

As in a *fully blinded image evaluation*, we recommend that readers performing an *image evaluation blinded to outcome* not have any knowledge of the results of evaluation with the truth standard, of the final diagnosis, or of patient outcome.

However, in an *image evaluation blinded to outcome*, the readers might have knowledge of particular elements of patient-specific information (e.g., history, physical exam, laboratory results, or results of other imaging studies). In some cases, the readers also might be aware of general inclusion and exclusion criteria

[10] This is the common meaning of *blinding* in therapeutic clinical trials. See the ICH guidelines *E8 General Considerations for Clinical Trials* and *E9 Statistical Principles for Clinical Trials.*

for patient enrollment, other details of the protocol, or anatomic orientation to the images. We recommend that the particular elements about which the reader will have information be standardized for all patients and defined prospectively in the clinical trial protocol, statistical plan, and the blinded image evaluation plan.

In studies where images obtained by different treatments are being evaluated (including *no treatment*, such as in unenhanced image evaluation of a contrast agent), we recommend that the readers not have knowledge of treatment identity, to the greatest extent to which that is possible (see section IV.B.7.a).

c. Sequential Unblinding

As used in this guidance, *sequential unblinding* is an assessment where readers typically evaluate images with progressively more information (e.g., clinical information) on each read. Sequential unblinding might be used to provide incremental information under a variety of conditions that may occur in routine clinical practice (e.g., when no clinical information is available, when limited clinical information is available, and when a substantial amount of information is available). This can be used to determine when or how the test agent should be used in a diagnostic algorithm. We recommend that a typical s*equential unblinding* image evaluation be a three-step process.

- We recommend that a fully blinded image evaluation be performed. We recommend that this evaluation be recorded and locked in a dataset by methods that can be validated. In a *locked* dataset, we recommend that it not be possible to alter the evaluation later when additional information is available, or if input is received from the clinical investigators, other readers, or the sponsor.
- We recommend that an image evaluation blinded to outcome be performed. We recommend this evaluation be recorded and locked in the dataset.
- To determine diagnostic performance of the imaging agent, we recommend that the result of the above two blinded evaluations be compared to the results of evaluation with the truth standard (or of the final diagnosis, or of patient outcome).

Such sequential unblinding can be expanded to include other types of image evaluations where additional clinical information is provided to the readers. If sequential unblinding is used, we recommend that the protocol specify the hypothesis that is to be evaluated at each step. Also, we recommend that the protocol specify which image evaluation will be the primary one for determining efficacy.[11]

[11] The labeling should reflect the image methods (blinded, sequentially unblinded, or unblinded, as appropriate) that provided substantial evidence that the Agency used to reach an approval decision and to develop appropriate labeling recommendations for use.

d. Unblinded image evaluations

In an *unblinded image evaluation*, readers are aware of the results of patient evaluation with the truth standard, of the final diagnosis, or of patient outcome. Unblinded readers also typically are aware of patient-specific information (e.g., history, physical exam, laboratory results, results of other imaging studies), of treatment identity where images obtained by different treatments (including no treatment) are being evaluated, of inclusion and exclusion criteria for patient enrollment, other details of the protocol, and of anatomic orientation to the images.

Unblinded image evaluations can be used to show consistency with the results of fully blinded image evaluations or image evaluations blinded to outcome. We recommend that these blinded and unblinded image evaluations use the same endpoints so that the results can be compared. However, we recommend that unblinded image evaluations not be used as the principal image evaluation for demonstration of efficacy. The unblinded readers may have access to additional information that may alter the readers' diagnostic assessments and may confound or bias the image evaluation by these readers.

8. Independent Image Evaluations

Two events are independent if knowing the outcome of one event says nothing about the outcome of the other. Therefore, as used in this guidance, *independent readers* are readers that are completely unaware of findings of other readers (including findings of other blinded readers and onsite investigators) and are readers who are not otherwise influenced by the findings of other readers. To ensure that blinded reader's evaluations remain independent, we recommend that each blinded reader's evaluation be locked in the dataset shortly after it is obtained and before additional types of image evaluations are performed (see section IV.B.7.c).

a. Consensus image evaluations

As used in this guidance, c*onsensus image evaluations* (*consensus reads*) are image evaluations during which readers convene to evaluate images together. Consensus image evaluations can be performed after the individual readings are completed and locked. However, readers are not considered independent during consensus reads and therefore we recommend that such reads not serve as the primary image evaluation used to demonstrate the efficacy of medical imaging agents. Although a consensus read is performed by several readers, it is actually a single image-evaluation and is unlikely to fulfill our interest in image evaluations by multiple blinded readers. As with the individual blinded evaluations, we

recommend that the consensus reads be locked once obtained and before additional types of blinded readings are performed.

b. Repeated image evaluations by the same reader

In studies where readers evaluate the same image multiple times (e.g., as in sequential unblinding, or in readings designed to assess *intra*reader variability), we recommend that the readings be performed independently of one another to the fullest extent practical. The goal is to minimize *recall bias*. We further recommend that readers be unaware, to the fullest extent practical, of their own previous image findings and not be otherwise influenced by those previous findings.

We recommend that different pages in the CRF be used for the two image evaluations and that each image evaluation be performed with sufficient time between readings to decrease recall and without reference to prior results.

9. Offsite and Onsite Image Evaluations

As used in this guidance, *offsite image evaluations* are image evaluations performed at sites that have not otherwise been involved in the conduct of the study and by readers who have not had contact with patients, investigators, or other individuals involved in the study. We recommend that Phase 3 trials include offsite image evaluations that are performed at a limited number of sites (or preferably at a centralized site). In such offsite evaluations, it is usually easier to control factors that can compromise the integrity of the blinded image evaluations and to ensure that the blinded readers perform their image evaluations independently of other image evaluations.

As used in this guidance, o*nsite image evaluations* are image evaluations performed by investigators involved in the conduct of the protocol or in the care of the patient. The term also can refer to blinded image evaluations performed at sites involved with the conduct of the study. Onsite investigators may have additional information about the patients that was not predefined in the clinical trial protocol. Such additional information may alter the investigators' diagnostic assessments and may confound or bias the image evaluation by the investigators. Therefore, we recommend that onsite image evaluations usually not be used as the principal image evaluation for demonstration of efficacy, but be regarded as supportive of the blinded image evaluations.

However, we suggest onsite investigators who are blinded to *truth* (e.g., blinded to any test result that makes up the truth standard, to the final diagnosis, and to patient final outcome as in an image evaluation blinded to outcome see (section IV.B.7.b)) can be used for principal image evaluation. In such instances, we recommend that all clinical information available to the investigator at the time of the image evaluation be clearly specified and fully documented. We also recommend that a critical assessment of how such information might have influenced the readings be performed. In addition, we

recommend that an independent blinded evaluation that is supportive of the finding of efficacy be performed.

10. Assessment of Interreader and Intrareader Variability

We recommend that at least two blinded readers (and preferably three or more) evaluate images for each study that is intended to demonstrate efficacy. (The truth standard, however, may be read by a single blinded reader.) The use of multiple readers allows for an evaluation of the reproducibility of the readings (i.e., interreader variability) and provides a better basis for subsequent generalization of any findings. Ideally, we recommend that each reader view all of the images intended to demonstrate efficacy, both for the investigational imaging agent and the truth standard, so that interreader agreement can be measured. In large studies, where it may be impractical to have every image read by each reader, a properly chosen subset of images can be selected for such duplicate image evaluations. We recommend that consistency among readers be measured quantitatively (e.g., with the kappa statistic).

We recommend that *intra*reader variability be assessed during the development of medical imaging agents. This can be accomplished by having individual blinded readers perform repeated image evaluations on some or all images (see section IV.B.8.b).

11. Protocol and Nonprotocol Images

Images obtained in a clinical trial of a medical imaging agent can generally be considered either protocol or nonprotocol images.

a. Protocol images

As used in this guidance, *protocol images* are images obtained under protocol-specified conditions and at protocol-specified time points with the goal of demonstrating or supporting efficacy. We recommend that efficacy evaluations be based on the evaluations of such protocol images. We also recommend that all protocol images (e.g., not just those images determined to be evaluable) be evaluated by the blinded readers, including images of test patients, control patients, and normal subjects. In addition, we recommend that evaluation of the protocol images be completed before other images, such as nonprotocol images, are reviewed by the readers (see section IV.B.11.b).

In some cases where large numbers of images are obtained or where image tapes are obtained (e.g., cardiac echocardiography), sponsors have used image selection procedures. This is discouraged because the selection of images can introduce the bias of the selector.

We recommend that sponsors specify prospectively in protocols of efficacy studies how missing images (and images that are technically inadequate, uninterpretable or show results that are indeterminate or intermediate) will be

handled in the data analysis. Sponsors are encouraged to incorporate analyses in the statistical analysis plan that incorporate the principle of *intention-to-treat*, but that are adapted to a diagnostic setting (e.g., *intention-to-diagnose* considers all subjects enrolled in a diagnostic study regardless of whether they were imaged with the test drug and regardless of the image quality).[12] Images (including truth standard images) may be missing from analysis for many reasons, including patient withdrawal from the study, technical problems with imaging, protocol violations, and image selection procedures. We suggest that appropriate methods be prospectively developed to deal with missing values in the primary response variable analysis.[13]

b. Nonprotocol images

As used in this guidance, *nonprotocol image* refers to an image that is not a protocol image, as defined above (see section IV.B.11.a). These are sometimes obtained for exploratory purposes and are excluded from the locked phase 3 datasets.

12. Separate or Combined Image Evaluations

Performance of a separate image evaluation does not preclude performance of a combined image evaluation, and vice versa. If multiple image evaluations are performed, however, we recommend that the protocol specify which image evaluation will serve as the primary evaluation and which image evaluations are secondary.

a. Separate image evaluations

As used in this guidance, a *separate* image evaluation has a reader evaluate test images obtained from a patient independently of other test images obtained from that patient, to the fullest degree practical.[14] A reader evaluates each test image for a patient on its own merits without reference to, or recall of, any other test images obtained from that patient, to the fullest degree practical.

A separate image evaluation often can be performed by combining test images obtained under different conditions (or at different times) into an intermixed set. Images in this intermixed set can then be evaluated individually in random order so that multiple images are not viewed simultaneously, and so that images are not

[12] The *intention-to-treat principle* is defined as the principle that asserts that the effect of a treatment policy can be best assessed by evaluating on the basis of the intention to treat a subject (i.e., the planned treatment regimen) rather than the actual treatment given. As a consequence, we recommend that subjects allocated to a treatment group be followed up, assessed, and analyzed as members of that group irrespective of their compliance with the planned course of treatment (see *E9 Statistical Principles for Clinical Trials*, p. 28).

[13] See *E9 Statistical Principles for Clinical Trials*, p. 31.

[14] In the special case where only two test images are being evaluated, a *separate* image evaluation may also be referred to as an *unpaired* image evaluation.

evaluated sequentially within patients. Alternatively, test images obtained under one condition (or at a particular time) can be evaluated individually in a random order, followed by an evaluation in random order of the individual test images obtained under different conditions (or at different times).

As described in the first example below, we recommend that an appropriately designed separate image evaluation be performed when a goal of a study is to make comparative inferences about product performance (e.g., to compare the diagnostic performance of one medical imaging agent with another). As described in the second example, an appropriately designed separate image evaluation also can be used to demonstrate that a contrast agent contributes additional information to images obtained with the device alone.

Example 1: Comparative inferences of product performance

In a comparative study designed to show that the diagnostic performance of a new medical imaging agent is superior to that of an approved agent and that the new agent can replace the approved agent (see section IV.D.1), we recommend that an appropriate separate image evaluation of test images be performed as the principal image analysis. The *test images* in this case are the images obtained with the new and the approved medical imaging agents. The two agents are not intended to be used together in actual clinical practice, and we therefore recommend that the goal of such an *unpaired* image evaluation be to show that the information obtained with the new agent is clinically and statistically superior to the information obtained with the approved agent. For any given patient, we recommend that images obtained with the new agent be evaluated independently of the evaluation of the images obtained with the approved agent, to the fullest degree practical.

If desired, a side-by-side (*paired)* comparison of images obtained with the new agent and the approved agent can be performed as a secondary image analysis. However, such a side-by-side comparison may yield estimates of diagnostic performance that are biased. The blinded reader may tend to *overread* the presence of masses on the image obtained with the new agent in such a paired comparison. Similarly, the blinded reader may tend to *underread* the image obtained with the new agent in a paired evaluation where a mass is not seen clearly on the image obtained with the approved agent.

In general, these procedures for image evaluation also are applicable to studies designed to show noninferiority. We recommend that sponsors seek Agency comment on proposed study designs and analytical plans before enrolling patients in such studies (see also section IV.D.1 for additional discussion).

Example 2: Contribution of additional information by a contrast agent

In a study intended to demonstrate that a contrast agent contributes additional information to images obtained with the device alone, it is often highly desirable to perform an appropriate separate image evaluation of test images as the principal image analysis (see the next section for an alternative approach). The *test images*, in this case, include both the images obtained before administration of contrast (the *unenhanced* images) and those obtained after administration of contrast (the *enhanced* images). We recommend that the goal of such an unpaired image evaluation be to show that the information obtained from the enhanced image is clinically and statistically superior to the information obtained from the unenhanced image.

b. Combined image evaluations

As used in this guidance, a *combined* image evaluation has a reader simultaneously evaluate two or more test images that were obtained under different conditions or at different times with respect to agent administration.[15] A combined image evaluation may resemble the conditions under which the product will be used clinically. For example, in some clinical situations both unenhanced and enhanced imaging studies are typically performed in patients.[16] If so, such images often are evaluated concurrently in a comparative fashion.[17] However, as noted above, such combined image evaluations may increase the likelihood that bias will be introduced into the image evaluations (e.g., by systematic overreading or underreading particular findings on images).

A combined image evaluation can be performed by creating a set of combined images for each patient. These sets can then be presented to the blinded readers in random sequence.

When this type of reading is performed, however, we recommend that an additional independent *separate* image evaluation be completed on at least one of the members of the combination. We recommend that the member chosen be the member that usually is obtained under the current standard of practice (e.g., the unenhanced image). In this way, differences in the evaluations of the combined reading with those of the separate reading can be assessed. When the goal is to show that the medical imaging agent adds information to images, we suggest that these differences demonstrate that the information from the combined images is

[15] In the special case where only two test images are being evaluated, a *combined* image evaluation can also be referred to as a *paired* image evaluation.

[16] Also, combined images may refer to results from the test drug and modality plus images from a different modality.

[17] Under sections 505 and 502 of the Act, if images are evaluated only in a combined fashion, the approved labeling of the medical imaging agent likely will have to specify that combined evaluations should be performed in clinical practice. If such labeling restrictions are not desired, we recommend that additional separate image evaluations be performed.

clinically and statistically superior to information obtained from the separate image alone. The results of the combined and separate image evaluations can be analyzed statistically using paired comparisons.

For example, when a two-dimensional ultrasound study of blood vessels is performed with a microbubble contrast agent, a combined image evaluation could be performed by evaluating for each patient the unenhanced and enhanced images side-by-side (or in close temporal proximity). A separate independent evaluation of the unenhanced image of the blood vessel (i.e., images obtained with the device alone) for each patient could also be performed. Assessing the differences for each patient between the results of the combined reading with those of the separate readings could allow the effects of the microbubble on the images to be determined.

As noted above, we recommend that combined and separate image evaluations be performed independently of one another to decrease recall bias (see section IV.B.8.b). We recommend that different pages in the CRF be used for the combined and separate evaluations and that the combined and separate image evaluations be performed at different times without reference to prior results.

We recommend that when differences between the combined and separate images are to be assessed, the combined CRF and separate CRF contain items or questions that are identical so that differences can be calculated and biases can be reduced by avoiding questions asking for comparative judgment.

C. Truth Standards (Gold Standards)

A truth standard provides an independent way of evaluating the same variable being assessed by the investigational medical imaging agent. A truth standard is known or believed to give the true state of a patient or true value of a measurement. Truth standards are used to demonstrate that the results obtained with the medical imaging agent are valid and reliable and to define summary test statistics (e.g., sensitivity, specificity, positive and negative predictive value). We recommend that the following general principles be incorporated prospectively into the design, conduct, and analysis of the phase 3 efficacy trials for medical imaging agents:

1. We recommend that the test results obtained with the medical imaging agent be evaluated without knowledge of the results obtained with the truth standard and without knowledge of outcome (see section IV.B.7).

2. We recommend that the true state of the subjects (e.g., diseased or nondiseased) be determined with a truth standard without knowledge of the test results obtained with the medical imaging agent.

3. We recommend that truth standards not include as a component any test results obtained with the test medical imaging agent (i.e., to avoid *incorporation bias)*. This is because the features of the test image obtained with the test agent (e.g., the *enhanced*

image) are likely to be correlated to the features of the image obtained with the device alone (e.g., the *unenhanced image*). For example, in the case of a CT contrast agent intended to visualize abdominal masses, unenhanced abdominal CT images should not be included in the truth standard. However, components of the truth standard might include results from other imaging modalities (e.g., MRI, ultrasonography).

4. We recommend that evaluation with the truth standard be planned for all enrolled subjects, and the decision to evaluate a subject with the truth standard not be affected by the test results with the medical imaging agent under study. For example, if patients with positive results with the test agent are evaluated preferentially with the truth standard (as compared to patients with negative test results), the results of the study may be affected by *partial verification bias*. Similarly, if patients with positive results with the test agent are evaluated preferentially with the truth standard and those with negative test results are evaluated preferentially with a less rigorous standard, the results of the study may be affected by *differential verification bias.*[18]

We encourage sponsors to seek FDA comment when it is anticipated that a meaningful proportion of enrolled subjects might not be evaluated with the truth standard or might be evaluated with a less rigorous standard. In such situations, it may be appropriate to evaluate clinical outcomes for the enrolled subjects (see section IV.D.4).

From a practical perspective, diagnostic standards are derived from procedures that are considered more definitive in approximating the truth than the test agent. For example, histopathology or long-term clinical outcomes may be acceptable diagnostic standards for determining whether a mass is malignant. Diagnostic standards may not be error free, but for purposes of the clinical trial, they generally are regarded as definitive. However, misclassification of disease by the truth standard can lead to positive or negative biases in diagnostic performance measures (*misclassification bias*). Thus, we recommend that the choice of the truth standard be discussed with the Agency during design of the clinical trials to ensure that it is appropriate.

After the truth standard has been selected, we recommend that the hypothesis for the summary test statistic in reference to the truth standard be determined and prospectively incorporated into the study protocol. We recommend that the hypothesis and expected summary statistics reflect the intended clinical setting for use of the imaging agent (e.g., screening test, sequential evaluation, alternative to or replacement of another imaging study (see section V)).

D. Comparison Groups

Before selecting comparison groups, discussions with the Agency are recommended. General principles relating to the choice of control groups in clinical trials are set forth in the ICH guideline *E10 Choice of Control Group and Related Issues in Clinical Trials* (ICH *E10*), and these principles are applicable to diagnostic trials.

[18] Partial verification bias and differential verification bias are forms of *diagnostic work-up bias*.

1. Comparison to an Agent or Modality Approved for a Similar Indication

If the test agent is being developed as an advance over an approved drug, biological product, or other diagnostic modality, we recommend that a direct, concurrent comparison to the approved comparator(s) be performed. We recommend that the comparison include an evaluation of both the safety and the efficacy data for the comparator(s) and the test agent. Because of disease variability, typically such comparisons are performed in the same patient. We recommend that the image evaluation for the test product or modality be done without knowledge of the imaging results obtained from the approved products or modalities (see section IV.B.7).

We recommend that information from both the test and comparator images (i.e., using the new and old methods) be compared not only to one another but also to an independent truth standard. This will facilitate an assessment of possible differences between the medical imaging agent and the comparator and will enable comparative assessments of diagnostic performance. Such assessments could be obtained, for example, by comparing estimates of sensitivity, specificity, positive and negative predictive values, likelihood ratios, related measures, or receiver operating characteristic (ROC) curves for the different diagnostic agents. Note that two medical imaging agents could have similar values for sensitivity and specificity in the same set of patients, yet have poor agreement rates with each other. Similarly, two medical imaging agents could have good agreement rates, yet both have poor sensitivity and specificity values. In ROC analysis, overall areas under the curves obtained with different agents may be comparable, but areas under partial spans of the curves may be dissimilar. Likewise, one diagnostic agent may have superior diagnostic performance characteristics over another at one point on the ROC curve, but may have inferior diagnostic performance characteristics at a different point (see section V.B).

When a medical imaging drug or biological product is being developed for an indication for which other drugs, biological products, or diagnostic modalities have already been approved, a direct, concurrent comparison to the approved drug, biological product, or diagnostic modality is encouraged. However, prior approval of a medical imaging agent for use in a particular indication does not necessarily mean that the results of a test with that agent alone can be used as a truth standard. For example, if a medical imaging agent has been approved on the basis of sufficient concordance of findings with truth as determined by histopathology, we recommend that assessment of the proposed medical imaging agent also include determination of truth by histopathology. In this case, the direct and concurrent comparison of the proposed medical imaging agent to the approved agent with histopathology serving as the truth standard best measures the performance difference between the two agents.

In studies that compare the effects of a test agent with another drug, biological product, or imaging modality, we recommend that any images obtained using a nontest agent that are taken before enrollment be used only as enrollment criteria. We recommend that these images not be part of the database used to determine test agent performance. Such

baseline enrollment images have inherent selection bias because they are unblinded and based on referral and management preferences. We recommend that test agent administration be within a time frame when the disease process is expected not to have changed significantly. This provides for a fair, balanced comparison between the test and the comparator agent.

a. Noninferiority studies

Trials can be designed to show that a new test agent is not inferior to a reference product. In general, the requirements for such studies are more stringent that the requirements for studies designed to show superiority. Imaging studies, in particular, can lack assay sensitivity for several reasons, including inappropriate study population, lack of objective imaging endpoints, and inaccuracy in the truth standard. Moreover, assay sensitivity is difficult to validate because imaging studies often lack historical evidence of sensitivity to drug effects, and it is not always clear that the conduct of the imaging procedures and the subsequent image evaluations did not undermine the trial's ability to distinguish effective treatments from less effective ones. ICH *E10* provides further guidance on these matters.

We recommend that noninferiority studies be based on a concurrent comparison of the test agent and a reference product and that such studies use objectively defined endpoints validated by an acceptable truth standard. Such designs allow comparative assessment of the diagnostic (or functional) performance of the new and reference tests. For example, if the study endpoint is the presence or absence of disease, the sensitivities and specificities of the test product and the reference product can each be compared. The statistical hypotheses may be superiority, noninferiority, or both. If the test agent is to be used primarily to rule out disease, high negative predictive value and thus high sensitivity might be more important than specificity. The objective then would be to show that the new agent, when compared to the reference test, is superior with regard to sensitivity but not inferior with regard to specificity.

When the study design includes a truth standard but no comparison to a reference product, the performance levels of the new test agent can only be compared to some fixed threshold (e.g., prespecified levels of sensitivity and specificity). The statistical objective should then be to show superiority to the threshold values. Such values should be based on substantial clinical evidence supporting the assertion that exceeding the thresholds clearly demonstrates product efficacy.

To obtain a noninferiority claim against a reference product, a sponsor should show that its test agent has been shown to have similar performance characteristics as the reference product and can be used as an alternative modality in a precisely defined clinical setting. In other situations, the noninferiority comparison might only serve as a demonstration of efficacy of the test product. Generally, non-inferiority trials are designed to show that new and comparator test performance differ at most by a clinically acceptable margin that has been

agreed to by the Agency. We recommend that noninferiority trials be carefully planned and that discussions with the Agency begin early in the development program.

b. Agreement studies

Similarity between a new test agent and a reference product can also be shown by demonstrating that both agents consistently give identical results. In this case, the use of a truth standard is not possible, and the objective is to show agreement between test and comparator outcomes even though the validity (accuracy) of the outcomes cannot be verified. High agreement between a new test product and a reference product can support a claim that the new test is an acceptable alternative to the reference product.

In agreement studies, assay sensitivity is critical. In particular, outcomes should be objectively defined and the two agents should be compared in subjects who represent an appropriate spectrum of disease conditions. For example, showing that two diagnostic tests give the same positive diagnosis for a large percentage of the trial subjects might not be sufficient. We recommend that the sponsor also demonstrate that the test agent and the reference product respond similarly when a negative diagnosis prevails and that the probability of discordant outcomes is negligible. When outcomes are multivalued as opposed to dichotomous, agreement should be shown across the entire range of test values.

An agreement hypothesis should not imply that the agreement between test and comparator outcomes exceeds agreement among comparator outcomes. Thus, an understanding of intra-test and intra-reader variability should be taken into account. For example, consider a new pharmacological stress agent used with myocardial perfusion imaging to assess perfusion defects. One possible design would be to apply the comparator procedure to all subjects for a first evaluation and, for a second evaluation, randomize subjects to receive either the comparator procedure or the new test agent. This would allow the inter-test agreement to be directly compared with the intra-test agreement of the comparator using a noninferiority hypothesis.

Because agreement studies do not provide direct evidence of new test validity, they are difficult to design and execute effectively. Therefore, we recommend that sponsors pursue agreement studies in limited circumstances and consider alternative designs that employ an acceptable truth standard.

2. Comparison to Placebo

Whether the use of a placebo is appropriate in the evaluation of a medical imaging agent depends on the specific imaging agent, proposed indication, and imaging modality. In some cases, the use of placebos can help reduce potential bias in the conduct of the study and can facilitate unambiguous interpretation of efficacy or safety data. However, in

some diagnostic studies (such as ultrasonography), products that are considered to be placebos (e.g., water, saline, or vehicle) can have some diagnostic effects. We recommend that these be used as controls to demonstrate that the medical imaging agent has an effect above and beyond that of its vehicle.

V. STATISTICAL ANALYSIS

We recommend that statistical methods and the methods by which diagnostic performance will be assessed be incorporated prospectively into the statistical analysis plan for each study (see section IV.B.2). In addition, we recommend that each study protocol clearly state the hypotheses to be tested, present sample size assumptions and calculations, and describe the planned statistical methods and other data analysis considerations. The ICH guideline *E9 Statistical Principles for Clinical Trials* provides guidance on these matters.

A. Statistical Methods

One part of imaging evaluation is the determination of how well the test measures what it is intended to measure (validity). The overall diagnostic performance of the product can be measured by factors such as sensitivity, specificity, positive and negative predictive values, and likelihood ratios. Outcome validity can be demonstrated by a showing that use of the test enhances a clinical result.

The reliability of an imaging agent reflects the reproducibility of the result (i.e., the value of a measure repeated in the same individual, repeated evaluations of the same image by different readers, or repeated evaluations of the same image by the same reader). (See the glossary for other related definitions.)

Many studies of imaging agents are designed to provide dichotomous, ordered, or categorical outcomes. We think it important that appropriate assumptions and statistical methods be applied in their analysis. Statistical tests for proportions and rates are commonly used for dichotomous outcomes, and methods based on ranks are often applied to ordinal data. We recommend that study outcomes be stratified in a natural way, such as by center or other subgroup category, and the Mantel-Haenszel[19] procedures provide effective ways to examine both binomial and ordinal data. We recommend that exact methods of analysis, based on conditional inference, be employed when necessary. We recommend that the use of model-based methods also be encouraged. These models include logistic regression models for binomial data and proportional odds models for ordinal data. Log-linear models can be used to evaluate nominal outcome variables.

[19] For more on this topic, see Fleiss, Joseph, L., *Statistical Methods for Rates and Proportions*, 2nd ed., 1981, John Wiley and Sons, New York; and Woolson, Robert, *Statistical Methods for the Analysis of Biomedical Data*, 1987, John Wiley and Sons, New York.

In studies that compare images obtained after the administration of the test agent to images obtained before administration, dichotomous outcomes are often analyzed as matched pairs, where differences in treatment effects can be assessed using methods for correlated binomial outcomes. These studies, however, may be problematic because they often do not employ blinding and randomization. For active- and placebo-control studies, including dose-response studies, crossover designs can often be used to gain efficiency. We recommend that subjects be randomized to order of treatment. If subjects are not randomized to order of treatment, we otherwise recommend that the order in which images are evaluated be appropriately randomized. We recommend that study results from a crossover trial always be analyzed according to methods specifically designed for such trials.

B. Diagnostic Performance

Diagnostic validity can be assessed in a number of ways. For example, both the unenhanced and enhanced images could be compared to the truth standard, and the sensitivity and specificity of the unenhanced image could be compared to that of the enhanced image. Two different active agents can be compared in the same manner. Diagnostic comparisons can also be made when there are more than two outcomes to the diagnostic test results. Common methods used to test for differences in diagnosis include the McNemar test and the Stuart Maxwell test.[20] In addition, we recommend that confidence intervals for sensitivity, specificity, and other measures be provided in the analyses. ROC analysis also may be useful in assessing the diagnostic performance of medical imaging agents over a range of threshold values.[21] For example, ROC analysis can be used to describe the relative diagnostic performance of two medical imaging agents if each test can be interpreted using several thresholds to define a positive (or negative) test result (see section IV.D.1). For all planned statistical analyses, we recommend that details of the analysis methods and specific hypotheses to be tested be stated prospectively in the protocol as part of the statistical analysis plan. We recommend that sponsors seek Agency comment on the design of and statistical approach to analyses before the protocols are finalized.

[20] Ibid.

[21] For an introduction to this topic, see Metz, Charles E., *Basic Principles of ROC Analysis*, Seminars in Nuclear Medicine 1978;VIII(4):283-298. For a current treatment of statistical issues in diagnostic trials, see Zhou, Xiao-Hua, et al., *Statistical Methods in Diagnostic Medicine*, 2002, John Wiley and Sons, New York.

GLOSSARY

Note: Subjects in trials of medical imaging agents are often classified into one of four groups depending on (1) whether disease is present (often determined with a truth standard or *gold standard*) and (2) the results of the diagnostic test of interest (positive or negative). The following table identifies the variables that are used to estimate the parameters defined below.

Test Result:	**Disease: Present (+)**	**Absent (-)**	
Positive (+)	**TP (a)** true positive=TP	**FP (b)** false positive=FP	**m1 = a+b** = TP+FP total with positive test
Negative (-)	**FN (c)** false negative=FN	**TN (d)** true negative=TN	**m2 = c+d** = FN+TN total with negative test
	n1 = a+c = TP+FN total with disease	**n2 = b+d** = FP+TN total without disease	**N = a+b+c+d** = TP+FP+FN+TN total in study

Accuracy: (1) In common usage, *accuracy* is the quality of being true or correct. (2) As a measure of diagnostic performance, *accuracy* is a measure of how faithfully the information obtained using a medical imaging agent reflects reality or *truth* as measured by a truth standard or *gold standard*. Accuracy is the proportion of cases, considering both positive and negative test results, for which the test results are correct (i.e., concordant with the truth standard or *gold standard*). Accuracy = (a+d)/N = (TP+TN)/(TP+FP+FN+TN).

Comparator: An established test against which a proposed test is compared to evaluate the effectiveness of the proposed test. A comparator usually means an agent or modality approved for a similar indication. (See also the definition of *reference product*.)

Likelihood ratio: A measure that can be interpreted either as (a) the relative *odds* of a diagnosis, such as being diseased or nondiseased, for a given test result, or (b) the relative *probabilities* of a given test result in subjects with and without the disease. This latter interpretation is analogous to a relative risk or risk ratio.

1. For tests with dichotomous results (e.g., positive or negative test results), the likelihood ratio of a positive test result can be expressed as LR(+), and the likelihood of a negative test result can be expressed as LR(-). See the equations below:

$$LR(+) = \frac{\frac{a}{n1}}{\frac{b}{n2}} = \frac{sensitivity}{1 - specificity} = \frac{TruePositiveRate}{FalsePositiveRate} = \frac{\frac{a}{b}}{\frac{n1}{n2}} = \frac{PostTestOdds(+)}{PreTestOdds}$$

$$LR(-) = \frac{\frac{c}{n1}}{\frac{d}{n2}} = \frac{1 - sensitivity}{specificity} = \frac{FalseNegativeRate}{TrueNegativeRate} = \frac{\frac{c}{d}}{\frac{n1}{n2}} = \frac{PostTestOdds(-)}{PreTestOdds}$$

LR(+): *Interpreted as relative odds:* LR(+) is the post-test odds of the disease (among those with a positive test result) compared to the pretest odds of the disease.

Interpreted as relative probabilities: LR(+) is the probability of a positive test result in subjects with the disease compared to the probability of a positive test result in subjects without the disease.

LR(-): *Interpreted as relative odds*: LR(-) is the post-test odds of the disease (among those with a negative test result) compared to the pretest odds of the disease.

Interpreted as relative probabilities: LR(-) is the probability of a negative test result in subjects with the disease compared to the probability of a negative test result in subjects without the disease.

2. For tests with several levels of results, such as tests with results expressed on ordinal or continuous scales, the likelihood ratio can be used to compare the proportions of subjects with and without the disease at different levels of the test result. Alternatively, the likelihood ratio can be used to compare the post-test odds of disease at a particular level of test result compared with the pretest odds of disease. Thus, the generalized likelihood ratio can reflect diagnostic information at any level of the test result.

Negative predictive value: The probability that a subject does not have the disease when the test result is negative. Synonyms include *predictive value negative*. Negative predictive value = d/m2 = TN/(TN+FN).

By application of Bayes' Rule, the negative predictive value also can be defined as a function of pretest probability of disease (p), sensitivity, and specificity:

Negative predictive value = [(1-p) • specificity]/[(1-p) • specificity + p • (1-sensitivity)]

Odds: The probability that an event will occur compared to the probability that the event will not occur. Odds = (probability of the event)/(1 - probability of the event).

Positive predictive value: The probability that a subject has disease when the test result is positive. Synonyms include *predictive value positive*. Positive predictive value = a/m1 = TP/(TP+FP).

By application of Bayes' Rule, the positive predictive value also can be defined as a function of pretest probability of disease (p), sensitivity, and specificity:

Positive predictive value = (p • sensitivity)/[p • sensitivity + (1-p) • (1-specificity)]

Post-test odds of disease: The odds of disease in a subject after the diagnostic test results are known. Synonyms include *posterior odds of disease*. For subjects with a positive test result, the post-test odds of disease = a/b = TP/FP. For subjects with a negative test result, the post-test odds of disease = c/d = FN/TN. The following expression shows the general relationship between the post-test odds and the likelihood ratio: Post-test odds of disease = Pretest odds of disease x Likelihood ratio.

Post-test probability of disease: The probability of disease in a subject after the diagnostic test results are known. Synonyms include *posterior probability of disease*. For subjects with a positive test result, the post-test probability of disease = a/m1 = TP/(TP+FP). For subjects with a negative test result, the post-test probability of disease = c/m2 = FN/(TN+FN).

Precision: A measure of the reproducibility of a test, including reproducibility within and across doses, rates of administration, routes of administration, timings of imaging after product administration, instruments, instrument operators, patients, and image interpreters, and possibly other variables. Precision is usually expressed in terms of variability, using such measures as confidence intervals and/or standard deviations. Precise tests have relatively narrow confidence intervals (or relatively small standard deviations).

Pretest odds of disease: The odds of disease in a subject before doing a diagnostic test. Synonyms include *prior odds of disease.* Pretest odds of disease = n1/n2 = (TP+FN)/(TN+FP).

Pretest probability of disease: The probability of disease in a subject before doing a diagnostic test. Synonyms include *prevalence of disease* and *prior probability of disease.* Pretest probability of disease = n1/N = (TP+FN)/(TP+FP+FN+TN).

Probability: The likelihood of occurrence of an event, expressed as a number between 0 and 1 (inclusive).

Receiver operating characteristic (ROC) curve: A graphical representation of pairs of values for *true positive rate* (or sensitivity) and the corresponding *false positive rate* (or 1-specificity) for a diagnostic test. Each pair is established by classifying the test result as *positive* when the test outcome equals or exceeds the value set by a given threshold, and *negative* when the test outcome is less than this threshold value. For example, if a five-point ordinal scale is used to rate the likelihood of malignancy for a tumor (e.g., definitely benign, probably benign, equivocal, probably malignant, definitely malignant), setting the threshold at *equivocal* will classify tumors as malignant (i.e., a *positive* test result) when the test outcome is at this level or higher and will classify tumors as nonmalignant (i.e., a *negative* test result) when the test outcome is less than this level. To generate an ROC curve, the sensitivity and specificity of the diagnostic test are calculated and graphed for several thresholds (e.g., all values of the rating scale). In a typical ROC curve, values for *true positive rate* (or sensitivity) are plotted on the vertical axis, and the corresponding values for *false positive rate* (or 1-specificity) are plotted on the horizontal axis.

Reference product: An FDA-approved drug product having an indication similar to that of an investigational drug or biological product to which it is being compared for the purpose of evaluating the effectiveness of the investigational drug or biological product.

Sensitivity: The probability that a test result is positive when the subject has the disease. Synonyms include *true positive rate*. Sensitivity = a/n1 = TP/(TP+FN).

Specificity: The probability that a test result is negative when the subject does not have the disease. Synonyms include *true negative rate*. Specificity = d/n2 = TN/(TN+FP).

Truth standard (gold standard): An independent method of measuring the same variable being measured by the investigational drug or biological product that is known or believed to give the *true* value of a measurement.