

Tab 5 Efficacy Review

5.1 Overview of Phase 3 clinical program

In the original NDA submission, the sponsor was seeking both lymph node imaging and liver/spleen imaging indications. During our previous review, the deficiencies were identified from both indications. Since then, the sponsor had decided not to seek a liver/spleen indication. Therefore the lymph node indication is the only one under the consideration with this NDA resubmission (Please see the proposed indication under Section 4.1 of this briefing document).

To support the efficacy of Combidex in differentiating metastatic vs. non-metastatic lymph nodes, the sponsor conducted a prospectively designed Phase 3 clinical study in the US (Protocol 38804-10). The protocol was submitted initially in August 1996 and a revised statistical analysis plan, including blinded evaluation manual, was finalized and resubmitted in July and September of 1998.

In June 1999, the sponsor also proposed to conduct a predefined pooled analysis of blinded evaluation data collected from three efficacy trials conducted in Europe by Guerbet. Those studies, using the identical products, were originally designed to meet the registration and marketing strategies of Guerbet in Europe.

While the studies were conducted by different sponsors, they used the same drug product, imaging sequences, and route of administration. The patient population also appeared to be similar (Table 5.1). In the US, patients with different types of primary tumors were enrolled into a single protocol while each tumor type was enrolled under a different protocol in the European trials.

In addition to those pivotal trials, Dr. Weissleder and his colleagues from Massachusetts General Hospital and Harvard Medical School, and University Medical Center, Nijmegen, the Netherlands conducted a pooled analysis based on a subset of patients from an ongoing US study (38804-19) and an ongoing European study (ALS3-31A). Data from 40 patients from each study (80 total) with clinically resectable prostate cancer scheduled for radical prostatectomy with a limited lymph node dissection were analyzed. The results were reported in the New England Journal of Medicine (Appendix 7.3). Full study reports for the original studies were not available because those studies are still ongoing. The sponsor requested the Agency use this publication to address deficiencies of a need for additional efficacy data and usefulness of Combidex in a clinical setting

Table 5.1 – List of pivotal Phase 3 trials from the original NDA submission and re-evaluated in this review cycle

Analysis	Type of Analysis	Protocol #	Original Study Design
#1	Prospectively defined analysis from a single US study	38804-10	Open-label, multicenter study in patients with highly suspected or confirmed cancer with possible metastasis to lymph nodes in at least one of the following body areas: head & neck, lung, breast, abdomen, and pelvic
#2	Prospectively defined pooled analysis from three European studies	ALS-3-2-A	Open label, multicenter study in patients with squamous cell carcinoma of upper aerodigestive tract (Final Report: August 1999)
		ALS-3-7-A	Open label, multicenter study in patients with gynecologic or urologic cancer (Final Report: September 1999)
		ALS-3-10-A	Open label, multicenter study in patients with breast cancer (Final Report: September 1999)

Source data: Modified from ISE, Table 1, page 20, Original NDA submission, Volume 1.180

5.2 Study Design

Two analyses were conducted based on data collected from the US and European trials, respectively.

5.2.1 A prospectively defined analysis from a single US Phase 3 trial

The Phase 3 US study (protocol 38804-10) was an open-label, multicenter study conducted between November 1996 and October 1998 by 26 Investigators at 22 study sites in the US.

The study was designed to evaluate the efficacy of Combidex for the diagnosis of lymph node metastases in patients with head & neck, lung, breast, abdomen, and pelvis carcinoma. A single 2.6 mg Fe/kg dose Combidex was used to obtain the post-dose contrast-enhanced MR images. The sensitivity and specificity of a radiological interpretation based on post-dose MR images were then compared to that of pre-dose (non-contrast) MR images at a nodal level, by using histopathology as the "truth standard". A detailed description of the study design, including statistical plan, are summarized as follows:

Inclusion and Exclusion Criteria:

Each study site was allowed to enroll up to 12 patients, who met the following inclusion criteria:

- 18 years of age or older
- Had highly suspected or confirmed cancer with possible metastasis to lymph nodes in at least one of the following body areas: head & neck, lung, breast, abdomen, and pelvis
- Had at least to one lymph node, lymph node group, or coalescence of lymph nodes visualized on pre-dose MR images by site investigators;
- Scheduled for surgery or needle biopsy within three weeks following Combidex administration

The main exclusion criteria included patients with lymphoma or small cell lung carcinoma. The patients, who were currently undergoing radiation treatment or chemotherapy or who had undergone these treatments within six months prior to enrollment, were also excluded from the study.

Comment: *The inclusion/exclusion criteria are important in determining the generalizability of the study results. By restricting study population to those with visible lymph nodes on pre-dose MR images, it would suggest that a meaningful interpretation can only be assured when the node showed on post-dose image is also present at pre-dose images as well. In other words, a meaningful interpretation of Combidex-enhanced MR requires presence of two MRI scans (non-contrast and contrast-enhanced images).*

Excluding patients with lymphoma, small cell lung carcinoma, prior chemotherapy or radiation treatment may also have implications in determining which population the product is not indicated for. The sponsor has stated that excluding those two types of tumors was primarily due to the difficulty in obtaining pathology confirmation.

Dose and Administration

Combidex was administered intravenously to each patient at a dose of 2.6 mg Fe/kg and at a rate of 4 mL/min. Prior to the administration, the drug was diluted with 50 mL of normal saline.

MR Imaging Procedures

Each patient had two MR scans of the area of suspected lymph node involvement, i.e., pre-dose scan (within 14 days) and post-dose scan (within 24-36 hours post Combidex administration). Table 5.2.1.1 shows body areas scanned by the location of the primary tumors.

Pre-dose and post-dose images, each including three MR sequences - T1 Weighted Spin Echo, T2 Weighted Spin Echo, and Heavily Weighted T2*

Gradient Echo, were obtained from the same machine with the same parameters and coil(s). A detailed description of imaging procedures can be found in Appendix 7.4.

Table 5.2.1.1 Body Area Scanned by the Location of Cancer (Study 38804-10)

Location of Primary Tumor	Area Scanned
Head and Neck	Base of the skull to the supraclavicular region
Lung	Thoracic inlet to the top of the diaphragm
Breast	Axillary or internal mammary lymph nodes
Abdominal	Diaphragm to the iliac crest to bottom of the kidneys
Pelvic	Iliac crest through the pubic symphysis

Source data: Study report of 38804-10, page 45, Original NDA submission, Volume 1.57

Evaluation of MR Images

There were two (unblinded and blinded) evaluations of MR images. MR images were first evaluated in an unblinded fashion by the Investigator at the study site. The purpose of this review was to identify and mark the lymph nodes on MR images that would be biopsied and ultimately provide the "truth standard" against which the blinded results were compared. Since the results of that evaluation were not included in the primary efficacy analysis, the focus of this discussion is the blinded evaluation of MR images.

Blinded evaluation was carried out at a centralized reading facility. Pre-dose and post-dose MR images were assessed by two teams of independent radiologist/oncologist, who were blinded to patient medical history and all clinical information as well as the pathology results except primary tumor location (if known). Each team first evaluated the MR images without knowing which nodes were identified by the site investigators (unmarked images). After this process was completed, the same teams were asked to conduct a blinded overread to evaluate those nodes that were identified by the site investigators but were missed during their initial evaluation of unmarked images. Those two reading procedures are discussed below:

Evaluation of unmarked images: The MR images used in the blinded evaluation were identical to what was evaluated by the site investigators. Blinded readers (two teams of radiologist/oncologist) were first presented with the images without nodes identified (unmarked images) and there were two reading sessions which were held at least two weeks apart. At the first

session, pre-dose images of a particular patient was selected randomly and presented to the readers. After the pre-dose image assessment was made, recorded and collected, the post-dose images of the same subject were placed side-by-side for a paired evaluation. At the second session, a blinded evaluation of post-dose images only was made. Each teams made their own independent evaluation of lymph nodes that they had identified on the images. The radiological interpretation was made by the radiologists only. The role of oncologist was to make a relevant clinical decision based on the information provided by the radiologist.

Blinded overread: Since the blinded readers may miss some nodes that were originally identified by the site investigator, the blinded readers were allowed to perform a second reading (blinded overread) of the same MR images. While the readers were still blinded to the pathology results and other clinical information, the set of MR images that were originally used by the site investigators were provided and the purpose of this overread was to ensure that all nodes initially identified by the site investigators were evaluated by the blinded readers. The blinded readers were only allowed to evaluate the nodes that were missed during their initial evaluation of the unmarked images.

Table 5.2.1.2 summarizes the type of image evaluation and their purpose

Table 5.2.1.2 Type of Imaging Evaluation and Their Purposes

Type of Evaluation	Reviewer	Purpose	MR Images Used in Evaluation		
			Pre-dose read	Post-dose read	Paired read
Unblinded review of unmarked MR images	Site investigators	Identify the node so that pathology results can be obtained during surgery or via biopsy	X		X
Blinded review of unmarked MR images	Two teams of radiologist/oncologist	Provide data for primary efficacy analysis	X	X	X
Blinded Overread	The same teams of Radiologist /oncologist	Evaluate the node missed during the initial blinded review (data were not used in primary analysis)	X	X	X

Source data: Study report of 38804-10, page 46, Original NDA submission, Volume 1.57

Combidex Imaging Guideline Development

The sponsor developed the Combidex Imaging Guideline, a standardized instrument used by the blinded readers to make a radiological interpretation on whether or not a lymph node is metastatic (Table 5.2.1.3).

Table 5.2.1.3. Combidex Imaging Guidelines

Post Dose	Description	Diagnosis
	No blackening of node or node is hyperintense to surrounding tissue; heterogenous or homogenous architecture	Metastatic
	Node has central high signal with darkening along the peripheral rim; heterogenous architecture	Metastatic
	Partial darkening whereby more than 50% of the node has area of high signal intensity; heterogenous architecture	Metastatic
	Less than 50% of node has high signal intensity; heterogenous architecture	Possibly Metastatic
	Node having an overall dark signal other than a central or hilar area of fat seen on T1 sequence; heterogenous architecture	Nonmetastatic
	Node having an overall dark signal with speckles of subtle granularities; homogenous architecture	Nonmetastatic
	Node having an overall dark signal intensity; homogenous architecture	Nonmetastatic

Comment: Based on the statements in the original NDA submission (Section 2.3.2.4, page 37, Volume 1.180), it appears that the Combidex Imaging Guidelines were developed from the same database (i.e., US Phase 3 trial – Protocol 38804-10) which was later used to confirm the efficacy of Combidex-enhanced MR imaging. On January 26, 2005, the sponsor revised this statement and said that only small number of nodes in Study 38804-10 was used to verify the accuracy of Combidex Imaging Guidelines. The Guidelines were actually developed by using Phase 2 clinical trial data. The Agency is currently seeking the documents to support the actual timeline in the development of the Combidex Imaging Guidelines, and seeking clarification on how many nodes from US Phase 3 study (protocol 38804-10) were actually reviewed in the development of the Guidelines. Those nodes can then be excluded from the primary analyses.

Nodal Mapping – Matching the Nodes from MR images to that of histopathology

The nodal mapping was critical to ensure that the blinded evaluations of the MR images and histopathology results were made for the same lymph nodes. In this study, the nodal mapping was achieved by the procedures implemented as follows:

- Investigator at the study site conducted a unblinded read of pre-dose images, and provided the surgeons with the actual MR images or diagrams on which the nodes were visualized and labeled
- During surgery, the surgeons identified and labeled the study nodes (tissue specimen) by referencing MR images and/or diagrams. Often the Investigator was present during the surgery to assist in labeling nodes
- All nodes removed at surgery were given to a pathologist for histologic examination
- After receiving the pathology reports, the site investigators correlated the pathology results to each node identified by him/her during the unblinded review of MR images
- After the blinded evaluation of the unmarked images was completed, a third party Adjudicator, compared the nodes identified by the blinded readers with the nodes identified by the site investigator to establish the link.

The sponsor stated that these procedures permitted nodes sampled at surgery/biopsy with histology to be matched to those identified by the blinded readers.

Comment: *It is possible that many nodes visualized by the site investigators could not be used in the primary analysis simply because either those nodes were not correlated to pathology specimens or the blinded readers did not perceive some of those nodes during their blinded evaluation of MR images. As the results, validity and generalizability of the study results could be affected.*

Statistical Plan and Analysis

(1) Primary Endpoint:

The primary efficacy endpoint was the presence or absence of a metastatic lesion in a lymph node. MR's ability to detect the metastatic lesion in a lymph node is measured by the sensitivity and specificity, using histopathology (a histopathological diagnosis) as the truth standard. The

calculation of sensitivity and specificity of a radiological interpretation is illustrated in Table 5.2.1.4.

Table 5.2.1.4. Method for the calculation of sensitivity and specificity of a radiological interpretation, by using a histopathological diagnosis as the "truth" standard

Radiological interpretation	A histopathological diagnosis	
	Metastatic	Non-Metastatic
Metastatic	TP	FP
Non-metastatic	FN	TN

TP=true positive, FP=false positive, TN=true negative and FN=false negative

Here sensitivity (true positive) is defined as the ability of a radiological (MR) imaging test to identify correctly those lymph nodes that are determined by pathology as metastatic, i.e., sensitivity = TP/(TP+FN)

Specificity (true negative) is defined as the ability of a radiological (MR) imaging test to identify those lymph nodes that are determined by pathology as non-metastatic, i.e., specificity = TN/(TN+FP)

Comments: In practice, clinicians are more interested in the likelihood of a patient having a metastatic lesion in lymph nodes if a MR imaging test is positive, or the likelihood of a patient having no metastatic lesion in lymph nodes if a MR imaging test is negative, which are referred as the predictive value of a positive test or predictive value of a negative test, respectively. It is important to realize that the ability of a test to predict the presence or absence of a metastatic lesion is dependent on not only the sensitivity and specificity, but also the prevalence of metastatic lymph nodes in the population tested. Since the prevalence of metastatic lymph nodes can differ greatly from patient to patient, FDA only accepts sensitivity and specificity as the valid primary endpoints in evaluating the performance of a contrast agent. Please refer to FDA's Guidance for Industry - Developing Medical Imaging Drug and Biological Products: Part 3: Design, Analysis, and Interpretation of Clinical Studies for a detailed discussion (Appendix 7.5).

A histopathology diagnosis: A histopathological diagnosis is considered the truth standard for confirming whether or not a lymph node is metastatic. In this study, histopathology diagnosis for each lymph node was made at each study site and there was no centralized read. The pathology diagnosis included the following four categories: normal, inflammatory, metastatic, and other. Inflammatory nodes were treated as "normal" in the primary analysis and those nodes classified as "other" were excluded from the primary analysis.

A radiological interpretation: A radiological interpretation of the presence or absence of a metastatic lesion in the lymph nodes was made by the two blinded radiologists who evaluated MR images. Three sets of MR images were presented to each of the two radiologists for the assessment, i.e., (1) pre-dose images alone, (2) post-dose images alone, and (3) pre-dose and post-dose images side by side (paired read). Under each of those three scenarios, the blinded readers were first asked to make an instrument-based interpretation, and then a skill-based interpretation.

- Instrument-based radiological interpretation: For pre-dose images, the blinded readers were asked to measure the size of the lymph nodes. Any nodes > 10 mm (short axis) were considered metastatic. For post-dose alone or paired read, the readers were instructed to use only the Combidex Imaging Guidelines to determine whether or not a node was metastatic. In the analysis of sensitivity and specificity, a diagnosis of "metastatic" and "possibly metastatic" from the Combidex Imaging Guideline were combined as metastatic (Table 5.1.2.3).
- Skill-based radiological interpretation: The readers were also asked to provide overall assessment of a node (metastatic vs. non-metastatic) based on all information obtained from the MR images (as known as Blinded Reader Diagnosis or Skill-based diagnosis). After the radiological interpretation was made, the blinded readers were then asked to rate the importance of the nodal characteristics in making the diagnosis. Table 5.2.1.5 listed the factors that were considered in making an instrument-based or a skill-based radiological interpretation on whether or not a node was metastatic.

Table 5.2.1.5 Factors that were used in making an instrument-based or skill-based radiological interpretation

Radiological Interpretation	Type of MR Images		
	Pre-dose	Post-dose	Paired
Instrument-based (More objective)			
Short Axis (mm)	X		
Imaging Guideline		X	X
Skill-based (More subjective)			
Size	X	X	X
Shape	X	X	X
Consistency with the guideline		X	X
Signal intensity		X	X
Change in signal intensity			X
Location with respect to primary tumor	X	X	X
Architecture	X	X	X
Fatty replaced	X	X	X

Data Source: Appendix 7.1 and 7.2 of ISE, original NDA submission, Volume 1.182.

(2) Primary Analysis:

The primary analysis was performed at a nodal level, and the blinded evaluation of unmarked images was used in the primary analysis in this study.

As stated earlier, the study was prospectively designed to demonstrate the improved sensitivity and specificity of post-dose MR over that of pre-dose MR in differentiating metastatic from non-metastatic lymph nodes. The sensitivity and specificity calculated from the pre-dose images were compared with that calculated from the Combidex-enhanced images, and the differences were analyzed using McNemar's test of proportions. A significance level of < 0.05 was used to determine whether the difference were statistically significant.

Comments: The statistical plan, finalized on July 27, 1998 (Appendix 13.11 of the final study report of Protocol 38804-10) did not specify whether pre-dose diagnosis should be size-based or skill-based. Nor did it state whether results from the paired read or post-dose MR image only read would be used in the primary analysis.

(3) Analytical population in the primary analysis

The statistical plan (July 27, 1998) stated that:

"The intent-to-treat" population for efficacy will be defined as any image set that is of diagnostic quality. The evaluable population will be defined as images from patients who received at least 80% of the dose, image of diagnostic quality, and did not have any protocol violations that may have affected imaging".

Pre-dose images were considered to be of diagnostic quality if at least one MR sequence (T1, T2 or T2*) was of diagnostic quality. For post-dose images, either T2 or T2* had to be of diagnostic quality. Therefore, the sample size for efficacy analysis may be different among different readers.

Since the primary analysis was performed at a nodal level, the unit of analysis is the lymph node not the patient. To ensure the validity of study design, the sponsor emphasized in the original study protocol that:

- All lymph nodes identified on pre- and post-dose MR images by the site investigators should be correlated to histology results
- Each lymph node (regardless of size) removed should be evaluated histologically
- The extent of surgery performed will not be based on the results of post-dose MR images

Due to the inherent difficulties in the nodal mapping, however, not all lymph nodes that were surgically removed or biopsied could be included in the primary analysis. As a result, the analytical population consisted of only those lymph nodes that met the following two conditions:

- The nodes must be visualized by the site investigator, be removed surgically, and had a valid pathology result
- The same nodes must also be identified by the blinded readers from the unmarked MR images, and be mapped by the third-party Adjudicator to that visualized by the site investigator

Comments: A nodal disposition chart is provided in the Section 5.3.2 of this briefing document.

5.2.2 A prospectively defined pooled analysis from three European Phase 3 trials

As stated earlier, the sponsor proposed to conduct a pooled analysis of three European Phase 3 studies. Those three studies (ALS-3-2A, -7-A, and -10-A) were individually designed to study patients with upper aerodigestive tract, urologic/gynecologic, and breast cancers. The primary endpoint described in the European clinical trial reports was an evaluation of statistical differences in AUC (area under the curve) between pre- and post-dose images on a patient level for two of the studies and on the nodal group level for the third study.

Prior to the completion of those clinical trials, the sponsor committed to conduct a pooled analysis of nodal level data, similar to that of the US study. While the drug, dose, MR imaging procedures and the number of blinded readers were similar between US and European studies, Table 5.2.2.1 lists the major differences between the US and European trials.

Table 5.2.2.1 Comparison of the US and European trial designs

	US Study	European Studies		
	38804-10	2-A	7-A	10-A
Study protocol were reviewed by FDA prior to initiation of the study	Yes	No	No	No
Patient Population				
Risk for lymph node metastases	X			
Any TNM		X	X	
T1-T4 and any NM				X
Location of Primary Tumor				
Head & Neck	X	X		
Lung	X			
Breast	X			
Abdomen	X			X
Pelvis	X		X	
Allowing the use of Combidex results in deciding patient surgical/management strategies	No	Yes	Yes	Yes
Dose and Administration				
2.6 mg Fe/kg	X	X	X	X
Dilution	50 mL	100 mL	100 mL	100 mL

	US Study	European Studies		
	38804-10	2-A	7-A	10-A
Biopsy or surgery (post-dosing) 3 weeks 10 days	X	X	X	X
Nodal Mapping – Effort to correlate all nodes visualized by site investigator on MRI images	Yes	No (>10mm only)	No	No
Node mapping - correlation the results from blinded review of unmarked images to that of site investigators By third party radiologist By sponsor	X	X	X	X
Blinded Imaging Evaluation Use of Combidex Imaging Guidelines for the evaluation of post-dose images	Yes	No	No	No
Availability of a nodal level data				
From Unmarked Image				
Pre-dose alone	X	X	X	X
Paired	X	X	X	X
Post-dose alone	X			
From Blinded Overread				
Pre-dose alone	X			
Paired	X	X	X	X
Post-dose alone	X	X	X	X
Analytical Dataset				
Patients without diagnostic quality of MR images	Excluded	Excluded	Excluded	Excluded
Patients received < 80% intended dose	Included	Excluded	Excluded	Excluded

Initially histopathology information was only collected for those nodes having a small axis greater than 10 mm in MR images. Consequently there was a significantly higher percentage of large (>10 mm) nodes evaluated in these studies than that in the US studies (76% vs. 36%)

The Combidex Imaging Guidelines developed by the sponsor were not presented to the blinded readers during the blinded evaluation of MR images. The readers used a different guideline (called reading guideline, see Appendix 7.6) to record the signal intensity related information, including

signal changes (pre vs. post), homogeneous vs. heterogeneous, and percentage of surface with signal decrease. That information was later reviewed by the sponsor, comparing to the descriptions on the Combidex Imaging Guidelines. A Combidex Imaging Guidelines-based radiological interpretation was then made retrospectively by the sponsor, by comparing the information from blinded readers and the descriptions of Combidex Imaging Guidelines.

Comments: *It appeared that for each image, the blinded readers first answered the questions a, b, c and d of the reading guideline (Appendix 7.6). Each image was then assigned a Guerbet Guideline number (Appendix 7.7) by the sponsor based on the Guerbet Guideline description and blinded readers' answers to the question a, b, and c. The description on the Guerbet Guideline serves as the basis for correlating blinded reader's original assessment to a Combidex Imaging Guidelines-based interpretation (referred as AMI Guideline in Appendix 7.7).*

It is also noted that the procedures for nodal mapping were not specifically outlined in the European study reports. The following is the standard statement:

"On site, lymph nodes were correlated by radiologists and pathologists after discussion of the sites and sizes of the lymph nodes. For centralized reading, the correlation was performed retrospectively by the sponsor according to the specific procedure described in Annex 13.1.1. Based on the computer file (site and size), the lymph nodes described by the centralized reader were compared to the lymph nodes correlated on site (page 35 of final study report of ALS-3-2-A)".

5.3 Primary Efficacy Analysis

5.3.1 Description of Patients and Lymph Nodes Used in the Primary Efficacy Analysis

Patients Level Information:

Of 166 patients enrolled into the US study, 152 received Combidex. In three European trials, a total of 181 patients received the drug. Table 5.3.1.1 summarizes the demographic characteristics of those patients, including disease specific information.

Table 5.3.1.1 Patient-based demographics and disease specific information

Characteristics	US Study	European Studies		
	38804-10 (n=152)	3-2-A (n=90)	3-7-A (n=56)	3-10-A (n=35)
Demographic				
Mean age	57	55	63	56
Female	44%	12%	16%	100%
White	80%	100%	100%	100%
Primary tumor site				
Head & Neck	18%	100%	--	--
Lung	22%	--	---	--
Breast	17%	--	--	100%
Abdomen	11%	--	--	--
Pelvis	32%	--	100%	--
Surgical Status				
Surgery	101 (66%)	81 (90%)	51 (91%)	30 (86%)
Biopsy	33 (22%)	--	--	--
Neither biopsy or surgery or unknown	18 (12%)	9 (10%)	5 (9%)	5 (14%)
Lymph Node Metastases by pathology*				
Yes	67 (50%)	56 (69%)	19 (37%)	14 (47%)
No	67 (50%)	25 (31%)	32 (63%)	16 (53%)

Data Source: Modified from Table 3 and 4 of final study report of 38804-10, page 79, original NDA submission, Volume 1.57, and Table 11 of ISE, page 46, original NDA submission, Volume 180, and Table IV, V and VII, page 34, final study report of ASL-3-2-A, Volume 1.86.

* based on patients who had surgery or biopsy

Of 152 patients who received Combixen in the US trial, 18 (12%) did not undergo biopsy or surgery (patient refusal, failed procedure, patient unstable etc). Of 134 patients who had surgery or biopsy, 67 (50%) had at least one positive (metastatic) lymph node.

Of 181 patients who received Combixen in the European trials, 19 (10%) did not undergo biopsy or surgery. Of the 162 patients who had surgery or biopsy, 89 (55%) had at least one positive (metastatic) lymph node (69%, 37% and 47% for the neck, pelvic and breast cancer, respectively).

Under an ideal situation, all dosed patients should be included in the primary analysis. Since Combixen Phase 3 studies required the pathology confirmation for all lymph nodes that were visualized in pre-dose MR images, 18 and 19 patients who did not undergo surgery or biopsy from US and

European studies, respectively, were excluded from the primary analysis.

In study ASL-3-7-A, 26/56 (46%) were prostate and 24 (43%) were urinary bladder cancer.

Nodal Level Information:

Pathology of lymph nodes that were surgically removed: In the US trial, a total number of 1,691 nodes were removed during the surgery or biopsy from 134 patients. Of those nodes, 231 (14%) were metastatic. In three European trials, the percentage of surgically removed lymph nodes that were metastatic varied from 8% to 64% (Table 5.3.1.2). One of the factors that affect the rate of the metastatic lesion was the size of the lymph node.

Table 5.3.1.2 Number and Percent of metastatic lymph nodes among the nodes that were surgically removed, stratified by the size of the nodes, in US and European Studies

Size during Pathology Exam	US Study 38804-10	European Studies		
		3-2-A	3-7-A	3-10-A
< 10 mm	Unknown	None	46/696 (7%)	99/466 (21%)
> 10 mm	Unknown	82/129 (64%)	17/87 (20%)	26/50 (52%)
Total	231/1,691 (14%)	82/129 (64%)	63/783 (8%)	125/516 (24%)

Data Source: Modified from Table 11.2.49 of final study report of 38804-10, page 82, original NDA submission, Volume 1.59, and Table XIX of final study report of Study ALS-3-2-A, page 55, Volume 1.86, and Table XIV of final study reports of study ALS--3-7-A, page 51, Volume 1.94, and Table XV of final study report of Study ALS-3-10-A, page 49, Volume 1.102

Lymph nodes that were used in the primary efficacy analysis: Under an ideal situation, all lymph nodes that were visualized by the site investigators from pre-dose MR images should have been included in the primary analysis. However, in actuality, the number of lymph nodes that were included in the primary analysis was much less for the following reasons:

- Not all nodes that were visualized by the site investigators were surgically removed, and matched to the pathology results;
- Not all nodes that were visualized by the site investigator and had a pathology confirmation were also visualized by the blinded readers at pre-dose or paired read of unmarked images;
- Not all nodes visualized by the blinded readers at pre-dose and paired read were the same nodes.

Table 5.3.1.3 shows the disposition of those lymph nodes identified by the site investigators.

Table 5.3.1.3 Disposition (outcomes) of those lymph nodes identified by the site investigators from MR Images

Outcomes	US Study	European Studies		
		2-3-A	2-3-7A	2-3-10A
Number of node visualized by site investigators	371	834	333	234
Number of node visualized by site investigators and correlated to pathology results	276 (74%)	86 (10%)	65 (20%)	144 (62%)

Data Source: Modified from Table 7 of final study report of 38804-10, page 84, original NDA submission, Volume 1.57, and final study report of Study ALS-3-2-A, page 58-9, Volume 1.86, and Table XIX of final study report of study ALS-3-7-A, page 54, Volume 1.94, and Table XVIII of final study report of Study ALS-3-10-A, page 50, Volume 1.102

Comments: In the US trial, 276/371 (74%) of the nodes visualized by the site investigators were matched to the pathology results. The percentages were much lower in the European studies (86/834 or 10%, 65/330 or 20%, or 144/234 or 62%). Only those nodes that were seen by the site investigators and matched to pathology (matched nodes) could be used in the primary analysis.

Not all matched nodes, however, were identified by the blinded readers. Table 5.3.1.4 shows the number of cancer-positive and negative nodes that were identified by the blinded readers and therefore were used in the primary analysis.

In the US study, of those matched nodes, approximate 72-75% positive nodes and 44-54% of negative nodes were actually used in the primary analysis.

Data from the European Study, ALS-3-10-A, was not used in the primary analysis because the nodal level evaluation of unmarked images was not performed. In the other two European studies, 81 (61+17) pathology-confirmed cancer positive nodes and 70 (22+48) cancer negative nodes were matched to that visualized by the site investigators. Of those nodes, approximate 29-61% positive nodes and 10-32% of negative nodes were used in the primary analysis depending on the study and blinded reader.

Table 5.3.1.4 Number of matched nodes that were actually used in the primary analysis (Evaluation of Unmarked Images)

Outcomes	Metastatic Nodes by Pathology		Non-Metastatic Nodes by Pathology	
	Reader 1	Reader 2	Reader 1	Reader 2
US Study 38804-10 (total matched nodes N=276)				
Number of matched nodes	108		168	
Number of matched nodes included in primary analysis	78 (72%)	81 (75%)	89 (54%)	73 (44%)
European Study ASL-3-2A (total matched nodes N=86)				
Number of matched nodes	64		22	
Number of matched nodes included in primary analysis	40 (63%)	39 (61%)	7 (32%)	7 (32%)
European Study ASL-3-7A (total matched nodes N=65)				
Number of matched nodes	17		48	
Number of matched nodes included in primary analysis	5 (29%)	7 (41%)	7 (15%)	5 (10%)
European Study ASL-3-10A (total matched nodes N = 144)				
Number of matched nodes	53		91	
Number of matched nodes included in primary analysis	--	--	--	--

Data Source: Modified from Table 7 of final study report of 38804-10, page 84, original NDA submission, Volume 1.57, and Section 8.4.6.2 of final study report of Study ALS-3-2-A, page 74, Volume 1.86, and Section of 8.4.6.3 of final study report of study ALS-3-7-A, Volume 1.94, and Section of 8.4.6.1 of final study report of Study ALS-3-10-A, page 59, Volume 1.102

-- No blinded evaluation of unmarked images was conducted at nodal level

Comments:

The differences seen in number of nodes that were used in the primary analysis between blinded reader 1 and 2 reflects inter-reader variability in their ability to identify the lymph nodes on MR images and their judgment on whether the image sets were of diagnostic quality.

It is worth noting that both blinded readers have identified additional nodes that were not visualized by the site investigators. Those additional nodes could not be included in the analysis because of lack of pathology results.

There were 152 and 181 subjects with different cancer types who received Combidex in Phase 3 US trial and European trials, respectively. The number of lymph nodes used in the primary analysis, however, is small. We do not know what cancer type or non-cancer type were included. This may affect the generalizability of the study results. The Agency is currently seeking this information from the sponsor. The information will be presented at the meeting.

5.3.2 Results of Primary Efficacy Analysis

As stated earlier, the primary analysis was performed at the nodal level, and the blinded evaluation of unmarked images was the primary efficacy evaluation in this study. The comparisons between pre-dose read and paired read (i.e., pre-dose and post-dose images presented side by side) are presented for each of the two blinded readers. Our selection of such a comparison (instead of pre-dose vs. post-dose alone) is based on the following considerations:

- Paired read is likely to increase the number of nodes to be included in the primary analysis because of better correlation between pre-dose and post-dose lymph nodes;
- The presence of pre-dose images is unlikely to affect the performance of the Combidex imaging because at both paired read and post-dose alone sessions, blinded readers were instructed to use the same instrument, i.e., Combidex Imaging Guidelines to make radiological interpretation;
- In clinical practice, radiologists are most likely to use paired read to make their assessment
- Post-dose alone evaluation of unmarked images was not available in the European trials

Table 5.3.2.1 shows the differences in sensitivities between the pre-dose only evaluation, using size-based or skill-based interpretation, and the paired evaluation, using Combidex Imaging Guidelines-based interpretation, by two blinded readers in the US and European Studies. The similar comparisons for specificities are presented in Table 5.3.2.2.

Table 5.3.2.1 Difference in Sensitivity between the pre-dose read, using size-based or skill-based diagnosis, and paired read, using Combidex Imaging Guidelines-based diagnosis, by blinded readers in US and European Studies (Blinded Review of Unmarked Images)

MRI Images	US Studies		European Studies#	
	Reader 1 (n=78)	Reader 2 (n=81)	Reader 1 (n=45)	Reader 2 (n=44)
Pre-dose (size-based)	55%	56%	93%	98%
Pre-dose (skill-based)	94%	90%	96%	98%
Post-dose (Paired Read)	85%	83%	89%	86%
Post-dose vs. Pre-dose (Size-based)				
Difference	30%*	27%*	-4%	-12%
Post-dose vs. Pre-dose (Skill-based)				
Difference	-9%	-7%	-7%	-12%

Source Data: Modified from table 14 of ISE, page 56, Volume 1.180
 * One-sided test with significance level of P<0.05 (McNemar's)
 # Data from European breast cancer trial (ALS-2-10-A) is not included

Table 5.3.2.2 Difference in Specificity between the pre-dose read, using size-based or skill-based diagnosis, and paired read, using Combidex Imaging Guidelines-based diagnosis, by blinded readers in US and European Studies (Blinded Review of Unmarked Images)

MRI Images	US Studies		European Studies#	
	Reader 1 (n=89)	Reader 2 (n=73)	Reader 1 (n=13)	Reader 2 (n=13)
Pre-dose (size-based)	82%	81%	46%	46%
Pre-dose (skill-based)	40%	64%	38%	46%
Post-dose (Paired Read)	76%	77%	92%	85%
Post-dose vs. Pre-dose (Size-based)				
Difference	-6%	-4%	46%	39%
Post-dose vs. Pre-dose (Skill-based)				
Difference	36%*	13%*	54%*	39%*

Source Data: Modified from table 14 of ISE, page 56, Volume 1.180
 * One-sided test with significance level of P<0.05 (McNemar's)
 # Data from European breast cancer trial (ALS-2-10-A) is not included

The results suggest that:

- There was 27%-30% (point estimates) improvement in sensitivity with

post-dose MR images (using Combidex Imaging Guidelines), compared to that of size-based interpretation from pre-dose images. The improvement, however, was not observed in the European studies;

- There was no improvement in sensitivity was observed with post-dose MR images in both US and European studies when pre-dose images were evaluated using reader's skill (i.e., the readers were allowed to use all information (in addition to size) on the MR mages)
- There was 13-54% (point estimates) improvement in specificity with post-dose MR images (using Combidex Imaging Guidelines), compared to that of skill-based interpretation from pre-dose images in both US and European studies.

Two efficacy issues identified during the Agency's previous review were as follows:

- Issue #1: Inconsistent results noted between the US and European studies. The US study shows a significant improvement (27%-30%) in sensitivity while the European studies did not
- Issue #2: No improvement in sensitivity of post-dose MR images when compared to that of skill-based pre-dose images.

Comments:

Issue #1: Inconsistent results between the US and European studies. Using size-based criteria as a comparator at pre-dose baseline may be problematic. The sensitivity and specificity of the size-based MR interpretation is dependent on the size distribution of the lymph nodes that are included in the primary analysis. A sponsor can easily win on the sensitivity endpoint if the study is limited to nodes < 10 mm because by the definition the sensitivity at baseline (pre-dose) is 0%. In the contrary, a sponsor will never win on the sensitivity if all nodes that are studied are > 10 mm because by the same definition sensitivity at baseline (pre-dose) is 100%.

The sponsor has provided data in this resubmission showing that 36% and 76% of lymph nodes, in the US and European trials, respectively, were >10 mm. Thus pre-dose sensitivities were only 55-56% in the US study while the sensitivities in the European studies reached 98%. The data has clearly explained the reasons behind the "inconsistent" results between the US and European studies. Since the sensitivities of Combidex-enhanced MR imaging were relatively stable and consistent between the US and European studies (83%-89%), the "inconsistent" results between the US and European studies

are most likely due to instability of the baseline (size-based MR interpretation at pre-dose) results, rather than the lack of performance of Combidex-enhanced MR imaging procedure.

The potential value of Combidex-enhanced imaging over size-based interpretation of non-contrast MR imaging might be better explained by data presented in Table 5.3.2.3.

Table 5.3.2.3 Sensitivity and Specificity of Combidex-enhanced MR (paired read, using Combidex Imaging Guidelines-based interpretation) by Node Size seen on Pre-dose MR in Study 38804-10

Node Size	Reader 1		Reader 2	
	Sensitivity	Specificity	Sensitivity	Specificity
Nodes < 10 mm	69%	81%	66%	78%
Nodes > 10 mm	93%	56%	98%	71%

Source Data: Modified from NDA resubmission (dated September 29, 2004), ISE, table 13, p. 25, Vol 1.1

Table 5.3.2.3 shows the sensitivity and specificity of Combidex-enhanced MR imaging by nodal size seen on pre-dose MR images in the US study (Study 38804-10). For those nodes less than 10 mm, Combidex-enhanced MR imaging is able to detect 66%-69% of metastatic lymph nodes (please refer to the sensitivities from the two blinded readers) that would otherwise be missed by those radiologists who use size of the lymph node only in making a radiological interpretation. It is important to emphasize that Combidex-enhanced MR imaging cannot be used to replace biopsy for confirming the nodal status because the imaging procedure is still associated with approximately 20% false negative rate (1-specificity, i.e., 1-0.81 or 1-0.78) for those nodes less than 10 mm. It is conceivable, however, that Combidex enhanced imaging could be helpful in identifying the nodes less than 10 mm that are most appropriate for biopsy.

For those nodes larger than 10 mm, the use of Combidex-enhanced imaging may be limited. Since the probability of being metastatic is relatively high for any nodes that are greater than 10 mm, clinician may still want to perform the biopsy even if the Combidex-enhanced imaging shows a negative result for a node that is greater than 10 mm. The high sensitivity (low false negative rate) of the imaging procedure (93%-98%) for the nodes greater than 10 mm is very encouraging. However, there is still reasonable uncertainty because of the small sample size.

Issue #2: Lack of observed improvement in sensitivity of Combidex-enhanced MR imaging over the pre-dose reader's skill based interpretation. In clinical practice the consequences of a false positive or a false negative reading may influence the way that MRI scans are actually read by radiologists. The worst consequence of a false positive is an unnecessary biopsy whereas the consequences of a false negative may be mis-diagnosis, mis-treatment and failure to cure the patient's cancer. Knowing this radiologists may tend to over-read the scans and may be willing to accept a low specificity in exchange for a very high sensitivity. This tendency is clearly shown in Table 5.3.2.2 where the specificities for skill-based diagnosis ranged from 38% to 64% while the sensitivities reached over 90%.

Requiring improved sensitivity over radiologist's skill under this scenario may have little gain since (1) some radiologists tend to over-read to avoid false negative, and (2) the sensitivity of skill-based diagnosis can vary from reader to reader.

Combixd-enhanced MR imaging might be still useful here. Because of the low specificity associated with those radiologists who over read, it is possible that many false positive nodes, along with true positive nodes will be identified. Because of the improvement in specificity, again Combixd-enhanced MR might be helpful in selecting the node that is the most appropriate for biopsy. However, there will be a time delay to acquire the Combixd-enhanced MR images (24-36 hours) versus proceeding to biopsy directly.

The potential uses of Combixd-enhanced MR imaging discussed here are only valid if there is reasonable certainty about

- The validity of development and use of Combixd Imaging Guidelines in the US and European trials
- Performance of Combixd enhanced imaging in the setting of a relatively small sample size (number of lymph nodes) in the primary analysis

Both the US and European study protocols included only those subjects who had at least one lymph node, lymph node group, or coalescence of lymph nodes visualized on pre-dose MR images by site investigators. Thus it would be anticipated that Combixd-enhanced imaging would only be used in the setting of post non-contrast MR imaging.

It is also yet to be determined whether the efficacy results observed from

the US and European studies, if they are valid, are applicable to other types of tumors that have not been studied.

5.3.3 Results of Alternative Analyses (Subgroup Analyses)

The following analyses were conducted to provide more stable estimates on the sensitivity and specificity of Combidex-enhanced MR imaging on the target population, and to explore the factors that may affect the performance (sensitivity and specificity) of combidex-enhanced MR imaging.

Table 5.3.3.1 Sensitivity and Specificity of Combidex-enhanced MR imaging (paired read, using the Combidex Imaging Guidelines-based interpretation), by blinded readers in US and European Studies

MRI Images	US Studies		European Studies	
	Reader 1 (n=263)	Reader 2 (n=283)	Reader 1 (n=261)	Reader 2 (n=273)
Sensitivity	80%	77%	83%	83%
Specificity	84%	86%	81%	90%

Source Data: Modified from table 16 and 17 of ISE, page 59 and 60,, Volume 1:180

Comments: *To stabilize the estimates of sensitivity and specificity of the Combidex-enhanced MR imaging test, we have added to the analysis those additional nodes that were identified during the blinded overread session, i.e., the nodes that were missed during the original blinded read of unmarked images. During the blinded overread, readers were still blinded to the pathology results. As stated earlier, the sensitivity and specificity of Combidex-enhanced MR imaging by cancer types will be provided at AC meeting.*

5.4 Discussion of a study published in New England Journal of Medicine (NEJM)

An analysis of a subset of patients from an ongoing US study and an ongoing European study were performed by the researchers from Massachusetts General Hospital and Harvard Medical School, and University Medical Center, Nijmegen, the Netherlands. Data from 40 patients from each study (80 total) with clinically resectable prostate cancer scheduled for radical prostatectomy with a limited lymph node dissection were analyzed. The results were reported in an article in the New England Journal of Medicine (Appendix 7.3). Full study reports were not available because the studies were still ongoing. There was an on-site investigator's evaluation, and an evaluation at a centralized site by two blinded readers.

A total of 334 nodes were evaluated from 80 subjects. Of those nodes, 93% were less than 10 mm, and 19% metastatic. The results showed that Combixd-enhanced MR imaging was associated with 91% of sensitivity and 97% of specificity. The author also stated that in this study, 14 patients were spared unnecessary surgery because of the results of the Combixd scan.

Comments: *The results from the NEJM article are impressive. Since the sponsor had little control over how the analyses were designed and performed, no pre-defined statistical plan, no essential source documents, and full study reports were able to be provided to FDA upon request. The lack of adequate documentation, as required by the federal regulation (21 CFR 314.50(f)(1)-(3)), prevents us from considering those data as the evidence collected from adequate and well-controlled clinical studies, in determining the efficacy of Combixd.*

Nevertheless it is worth noting that with the advance of MR technology, increased numbers of small lymph nodes (< 10mm) are being identified on non-contrast MR imaging, which might increase role of Combixd-enhanced MR imaging in identifying the appropriate node for biopsy. Also there were anecdotal examples that Combixd MR scan helped patients by sparing them from unnecessary surgery.

5.5 Summary

The US and European studies suggest that a radiological interpretation made by using Combixd Imaging Guidelines on Combixd-enhanced MR images was associated with 83-85% sensitivity and 76-92% specificity (point estimates) for differentiating metastatic vs. non-metastatic lymph nodes. It also appears that Combixd-enhanced MR imaging is associated with a statistically significant improvement in specificity over a radiological interpretation based on the radiologist's overall assessment on non-contrast MR images. Those findings may make Combixd-enhanced MR imaging particularly attractive to be used in conjunction with non-contrast MR imaging in selecting lymph nodes for further evaluation, if appropriate. We are not certain, however, Combixd should be indicated for all type of primary tumors given that only selective types of primary tumors were studied and the numbers of patients/lymph nodes included in the primary analysis were relatively small. We also have concerns over how the Combixd Imaging Guidelines were developed and how it was applied in the European studies. It appears that the Guidelines were developed by using the same data on which the efficacy was later being tested (US study 38804-10). In the European studies, the Guidelines were not used directly by the blinded readers. The Combixd Imaging Guidelines-based

radiological interpretation was later made by the sponsor by comparing the node's characteristic recorded on the case report form to the diagnosis criteria stated on the Combidex Imaging Guidelines. This process leads to concerns about whether the original information recorded by the blinded readers could be accurately translated into the Combidex Imaging Guidelines based interpretation.

The results from NEJM article are impressive. Based on 334 nodes from 80 subjects with prostate cancer, both sensitivity and specificity of Combidex-enhanced MR imaging reached over 95%. Unfortunately the lack of adequate documentation, as required by the federal regulation (21 CFR 314.50(f)(1)-(3)), prevents us from considering those data as the evidence collected from adequate and well-controlled clinical studies, in determining the efficacy of Combidex.