Analysis of Cytokines in Response to Treatment of Diabetic Macular Edema With 0.3mg Lucentis

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PROTOCOL

STUDY TITLE:	*Analysis of Cytokines in Response to Treatment of Diabetic Macular Edema with 0.3mg Lucentis*
STUDY DRUG	Recombinant humanized anti-VEGF monoclonal antibody fragment (rhuFab V2 [ranibizumab]) 0.3mg
SPONSOR	*Vitreo-Retinal Associates and Michigan State University* *2505 East Paris Ave. SE Suite 100* *Grand Rapids, MI 49546*
IND:	*Insert IND Number*
INVESTIGATOR	*Louis C. Glazer, MD, PI* *Susanne Mohr, PhD, Co-PI*
SUB-INVESTIGATORS	*Kathleen DeHorn, MD, Jeffrey Zheutlin, MD, Frank Garber, MD*
DATE FINAL:	*November 7, 2016*
AMENDMENT:	

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1. BACKGROUND

1.1 PATHOPHYSIOLOGY

The current standard of care treatment for diabetic macular edema is anti VEGF therapy. Both 0.3mg Lucentis and Eylea have been shown to be superior to Avastin for treatment of diabetic macular edema in eyes with more advanced edema and poorer vision. 1.(DRCR Protocol T)

While there is a paucity of data of aqueous VEGF levels after Lucentis injection for DME, one study tested aqueous cytokines in response to bevacizumab injection in diabetic macular edema. Costagliola, et. al. demonstrated that aqueous VEGF levels remain lower but are still elevated compared to control patients in response to Avastin injections for DME(2). Based on Lucentis' tighter binding to VEGF (lower kd) and greater penetration of the retina, we postulate that Lucentis injections will be much more effective at lowering aqueous VEGF levels explaining Lucentis' greater efficacy compared to Avastin in DRCR Protocol T. In the present protocol, we plan to use Lucentis 0.3 mg to demonstrate the VEGF levels will be lower compared to published results of aqueous VEGF levels after Avastin use. Furthermore we have preliminary data from a clinical trial at the Van Andel Institute demonstrating VEGF levels can predict response to anti-VEGF therapy. Clinical response will be measured by ETDRS visual acuity and spectral domain OCT central thickness. Finally, there is evidence that anti-VEGF therapy can normalize the pathologic intraocular environment beyond neutralizing VEGF. It is also well documented that anti VEGF therapy effects not only vascular endothelial growth factor but may also lower other inflammatory cytokines such as IL5, IL10 and interferon alpha. (3. Suzuki, et. al)

The present protocol will measure a number of cytokines in addition to VEGF in response to 0.3mg Lucentis therapy for diabetic macular edema. We have previously demonstrated a large number of cytokines can be measured in a small quantity of ocular fluid using aqueous or vitreous samples with a microbead assay. (4. J.White, L.Glazer, et.al). We propose that use of 0.3mg Lucentis, concurrently with aqueous taps for diabetic macular edema, will

document lower aqueous VEGF levels compared to other anti VEGF inhibitors (vs that in Costagliola, et. al [2]), will decrease the level of inflammatory cytokines, and will predict clinical response to Lucentis therapy demonstrated by visual acuity and OCT. Finally, showing efficacy with this dose of Lucentis should provide a good rationale for use of 0.3mg Lucentis in further DRCR studies.

1.2 TREATMENT OF SEVERE DIABETIC MACULAR EDEMA (DISEASE SPECIFIC)

The current best treatment options for these patients include Lucentis 0.3 or 0.5mg or Eylea. Present study will use the 0.3mg dose of Lucentis and perform an anterior chamber tap measuring aqueous cytokines before the first injection of Lucentis and just before the fourth intravitreal injection of 0.3mg Lucentis.

1.3 RANIBIZUMAB FOR SEVERE DIABETIC MACULAR EDEMA (DISEASE SPECIFIC)

Both Lucentis 0.3mg and 0.5mg dose and Eylea have been shown to be highly effective for severe diabetic macular edema with poor vision. The DRCR Protocol T did not test the 0.5mg dose of Lucentis against Eylea. We believe the 0.3mg dose will be especially effective in patients with recalcitrant diabetic macular edema (with probable markedly elevated VEGF levels.)

A positive result from the current study will give the DRCR and NEI further rationale to test the 0.3mg Lucentis dose in future protocols and also to compare the 0.3mg dose to Eylea.

1.4 NONCLINICAL EXPERIENCE WITH RANIBIZUMAB

1.4.1 Nonclinical Pharmacokinetics

The pharmacokinetics of ranibizumab have been investigated in rabbits and cynomolgus monkeys following intravitreal and intravenous administration. In both species, following intravitreal administration, ranibizumab was cleared from the vitreous humor with a half-life of 2–3 days. Following single intravitreal administration to cynomolgus monkeys, retinal concentrations of ranibizumab were approximately one-third of vitreous concentrations and declined in parallel with vitreous concentrations. In humans, the intravitreal

half-life of ranibizumab is estimated to be 7-8 days. Repeated intravitreal injections of ranibizumab can lead to detectable antibodies in serum in rabbits and cynomolgus monkeys.

1.4.2 Nonclinical Toxicology

A series of nonclinical studies of ranibizumab administered by intravitreal injection to cynomolgus monkeys have been performed (details regarding study design and results can be found in the Investigator Brochure).

1.4.3 Nonclinical Data Supporting the Anti-Edema Activity of Ranibizumab

In Studies 01-401E-1757 and 01-401G-1757, the effect of ranibizumab on vascular leakage was explored using a modified Miles assay in the guinea pig. Ranibizumab demonstrated a concentration-dependent effect of blunting the vascular permeability induced by VEGF. These results are consistent with the decrease in retinal vascular permeability as observed on optical coherence tomography (OCT) and fluorescein angiography in AMD and diabetic macular edema studies and further support the rationale for the use of ranibizumab in CRVO and BRVO, in which vascular permeability plays a significant role in the pathology

1.5 CLINICAL EXPERIENCE WITH RANIBIZUMAB

Ranibizumab has been or is being studied in more than 5000 subjects with diabetic macular edema and neovascular AMD in a number of Phase I, I/II, II, III, and IIIb clinical trials. Ranibizumab is contraindicated in patients with ocular or periocular infections and in those with known hypersensitivity to ranibizumab or any of the excipients in ranibizumab. Intravitreal injections, including those with ranibizumab, have been associated with endophthalmitis and retinal detatchment. Proper aseptic injection technique should always be used when administering ranibizumab. Increases in IOP have been noted within 60 minutes of intravitreal injection with ranibizumab. Therefore, IOP as well as perfusion of the optic nerve head should be monitored and managed appropriately. Serious adverse events related to the injection procedure have occurred in <0.1% of intravitreal injections include endophthalmitis, rhegmatogenous retinal detachment, and iatrogenic traumatic cataract. Other serous ocular adverse events observed among ranibizumab-treated

subjects and occurring in <2% of subjects included intraocular inflammation and increased IOP. The most common adverse reactions (reported ≥ 6% higher in ranibizumab-treated subjects than control subjects) were conjunctival hemorrhage, eye pain, vitreous floaters, increased IOP, and intraocular inflammation.

Although there was a low rate (<4%) of arterial thromboembolic events (ATEs) observed in the ranibizumab clinical trials there is a potential risk of ATEs following intravitreal use of inhibitors of VEGF. The rate of ATEs in three studies (FVF2598g, FVF2587g, and FVF3192g) in the first year was 1.9% of subjects in the combined group of subjects treated with 0.3 mg or 0.5 mg ranibizumab compared with 1.1% of subjects in the control arms of the studies. In the second year of Study FVF2598g and FVF2587g, the rate of ATEs was 2.6% of subjects in the combined group of those treated with 0.3 mg or 0.5 mg ranibizumab compared with 2.9% of subjects in the control arm. The most common non-ocular adverse reactions observed in ≥ 15% of ranibizumab-treated subjects that occurred more frequently than in control subjects included, nasopharyngitis, headache, and upper respiratory tract infection.

The Sailor study (FVF3689g) evaluated the safety of intravitreal ranibizumab in a large population of subjects with CNV secondary to AMD. Subjects in Cohort 1 (N=2378) were randomized (1:1) to receive ranibizumab at a dose level of 0.3 mg or 0.5 mg; subjects were masked to these dose levels. Treatment was administered monthly for three initial doses (Day 0, Month 1, and Month 2), with scheduled follow-up visits on Months 3,6,9, and 12. Retreatment after the first three injections was performed as needed, on the basis of predefined criteria with injections no more frequently than every 30 days.

Cohort 2 (N=1992) consisted of subjects enrolled after the majority of Cohort 1 subjects had been enrolled, with enrollment continuing until ranibizumab was approved or denied by the FDA for US marketing, and if approved, until commercially available or 30 September 2006, whichever was earlier. Subjects in Cohort 2 received open-label ranibizumab at the 0.5 mg dose level, with an initial injection on Day 0 followed by retreatment at the physician's discretion, no more frequently than every 30 days. Subjects were monitored for safety for a total of 12 months; safety information, including both serious and nonserious

adverse events, was collected at every clinic visit, with two formal safety visits scheduled at Months 6 and 12.

The study consisted of a 30-day screening period and a 1-year treatment period. Treatment duration was approximately 197 days for both dose groups in Cohort 1 and 144 days for subjects in Cohort 2. The mean follow-up time differed between Cohort 1 and Cohort 2, 337 days versus 254 days, respectively.

Ranibizumab was well tolerated, and the incidence of ocular SAEs and AEs was low and unrelated to dose. The rates of individual key ocular SAEs in Cohort 1 were < 1% and were similar across dose groups. Endophthalmitis or presumed endophthalmitis developed in 0.2% subjects in the 0.3-mg group and 0.4% subjects in the 0.5-mg group. The incidence of ocular inflammation, including iritis, uveitis, vitritis, and irdocylitis was 1.9% in the 0.3-mg group and 1.5% in the 0.5-mg group. Overall cataract rates were 5.4% (0.3 mg) and 6.0% (0.5 mg) and were similar when broken down by nuclear, subcapsular, and cortical subtypes. The rates of individual key ocular SAEs in Cohort 2 were <1%.

The rates of key non-ocular SAEs and AEs, including Antiplatelet Trialists' Collaboration (APTC) ATEs, MI, and vascular death were similar for cohorts 1 and 2 and 0.3- and 0.5-mg dose groups. The incidence of MI and non-ocular hemorrhage was similar across Cohort 1 dose groups. APTC ATEs, including vascular and unknown deaths, nonfatal MI, and nonfatal cardiovascular accidents, were similar across dose groups. During the 12-month study period, 0.7% of subjects in the 0.3-mg group and 1.2% of subjects in the 0.5-mg group suffered a stroke. The number of vascular deaths and deaths due to unknown cause did not differ across dose groups. Rates of key non-ocular SAEs in Cohort 2 were generally lower than those in Cohort 1.

Refer to the Ranibizumab Investigator Brochure or Lucentis® Package Insert for additional details regarding clinical safety experience with ranibizumab.

2. OBJECTIVES

2.1 Primary Objective

1. Measure aqueous VEGF levels in response to 0.3mg Lucentis dose for diabetic macular edema
2. Measure other cytokines response to 0.3 mg Lucentis dose for diabetic macular edema
3. Correlate clinical response (vision, spectral domain OCT retinal thickness level) to VEGF levels

2.2 Secondary Objectives

3. STUDY DESIGN

3.1 DESCRIPTION OF THE STUDY

Lucentis injections are the current standard of care for diabetic macular edema. The only deviation from the standard of care in the current protocol is an aqueous tap before the first Lucentis injection and the fourth Lucentis injection for diabetic macular edema. Such an approach has been shown to be extremely low risk for the patient. In a study from Pfahler et. al, (5), with 578 vitreous taps there were minimal complications. Vitreous taps are considered riskier than aqueous taps.

3.2 RATIONALE FOR STUDY DESIGN

Measuring aqueous anti VEGF levels may predict response to anti VEGF therapy. While there is not much data in DME, Compachario et al, (6), found that aqueous VEGF levels are higher in DME compared to retinal vein occlusions. A higher molar dose of Lucentis should theoretically be more effective in patients with recalcitrant macular edema and higher VEGF levels.

3.3 OUTCOME MEASURES

3.3.1 Primary Outcome Measures

1. VEGF levels before and after Lucentis injections

2. In addition to measuring VEGF, Multiplex bead-based immunoassay will be used for simultaneous measurement of 39 cytokines and chemokines in the aqueous samples, before and after Lucentis injections. (See White and Glazer. 4)

3.3.2 Secondary Outcome Measures

1. ETDRS vision before and after Lucentis injection

2. Spectral domain OCT retinal thickness before and after Lucentis injection

3.3 SAFETY PLAN

A current IRB exists with Dr. Mohr's lab at MSU to analyze vitreous specimens. See attached. The study is funded by Genentech, and an IRB will be obtained for the current protocol and material transfer agreement will be consummated with Dr. Mohr to send the aqueous samples directly to her lab.

Safety concerns, the only deviation from current standard of care in the current protocol is an aqueous tap before the first Lucentis injection and before the fourth Lucentis injection. We have previously conducted a clinical trial similar to this with the Van Andel Institute for macular degeneration without any complications. Furthermore, Pfahler (5) performed more than 578 vitreous taps without significant morbidity, which are considered riskier than aqueous taps. Compachairo et al (7) performed aqueous taps on 59 patients receiving Lucentis without morbidity. This is six times the number of patients proposed in this clinical trial.

3.5 compliance with Laws and Regulations

This study will be conducted in accordance with current U.S. Food and Drug Administration (FDA) Good Clinical Practices (GCPs), and local ethical and legal requirements.

4. MATERIALS AND METHODS

4.1 SUBJECTS

4.1.1 Subject Selection

Ten patients will be selected with diabetic macular edema using entry criteria from DRCR protocol T.

4.1.2 Inclusion Criteria

Age ≥ 18 years

- Diagnosis of diabetes mellitus (type 1 or type 2)
- Able and willing to provide informed consent
- Best corrected E-ETDRS visual acuity score ≤78 (ie 20/32 or worse) and ≥24 (ie 20/320 or better)
- On clinical exam, definite retinal thickening due to diabetic macular edema involving the center of the macula
- DME present on OCT (central subfield thickness on OCT ≥250 microns)
- Media clarity, pupillary dilation, and individual cooperation sufficient for adequate fundus photographs

- DME with DR score DRSS 47-53

4.1.3 Exclusion Criteria

Significant renal disease, defined as a history of chronic renal failure requiring dialysis or kidney transplant

- A condition that, in the opinion of the investigator, would preclude participation in the study (e.g. unstable medical status including blood pressure, cardiovascular disease, and glycemic control)
- Individuals in poor glycemic control who, within the last four months, initiated intensive insulin treatment (a pump or multiple daily injections) or plan to do so in the next four months should not be enrolled
- Participation in an investigational trial within 30 days of randomization that involved treatment with any drug that has not received regulatory approval for the indication being studied at time of study entry
- Known allergy to any component of the study drug
- Blood pressure >180/100 (systolic above 180 OR diastolic above 110)
- Myocardial infarction, other acute cardiac event requiring hospitalization, stroke, transient ischemic attack, or treatment for acute congestive heart failure within 4 months prior to randomization
- Systemic anti-VEGF or pro-VEGF treatment within 4 months prior to randomization or anticipated use during the study
- For women of childbearing potential: pregnant or lactating or intending to become pregnant within the next 24 months
- Individual is expecting to move out of the area of the clinical center to an area not covered by another clinical center during the first 12 months of the study
- Macular edema is considered to be due to a cause other than diabetic macular edema
- An ocular condition is present such that, in the opinion of the investigator, visual acuity loss would not improve from resolution of

macular edema (eg foveal atrophy, pigment abnormalities, dense subfoveal hard exudates, nonretinal condition)

- An ocular condition is present (other than diabetes) that, in the opinion of the investigator, might affect macular edema or alter visual acuity during the course of the study (e.g. vein occlusion, uveitis or other ocular inflammatory disease, neovascular glaucoma, etc.)
- Substantial cataract that, in the opinion of the investigator, is likely to be decreasing visual acuity by three lines or more
- History of an anti-VEGF treatment for DME in the past 12 months or history of any other treatment for DME at any time in the past four months (such as focal grid macular photocoagulation, intravitreal or peribulbar corticosteroids)
- History of pan-retinal photocoagulation within four months prior to randomization or anticipated need for pan-retinal photocoagulation in the six months following randomization
- History of anti-VEGF treatment for a disease other than DME in the past 12 months
- History of major ocular surgery (including vitrectomy, cataract extraction, scleral buckle, any intraocular surgery, etc.) within four months or anticipated within the next six months following randomization
- History of YAG capsulotomy performed within 2 months prior to randomization
- Aphakia
- Exam evidence of external ocular infection, including conjunctivitis, chalazion, or significant blepharitis

4.3 STUDY TREATMENT

4.3.1 Formulation

Ranibizumab is formulated as a sterile solution aseptically filled in a sterile, 3-mL stoppered glass vial. Each single-use vial is designed to deliver 0.05 mL of 10 mg/mL

ranibizumab aqueous solution with 10 mM histidine *HCl,* 10%, α-trehalose dihydrate, and 0.01% polysorbate *20, pH 5.5.* The results in the delivery of a 0.5 mg dose of ranbizumab. Each vial contains no preservative and is suitable for **single use only.**

Further details and molecule characterization will be included in the Investigator Brochure.

4.3.2 Dosage, Administration, and Storage

Dosage

0.3mg Lucentis dose used

b. Administration

Intravitreal injection

c. Storage

Upon receipt, study drug kits should be refrigerated at 2°C - 8°C (36°F - 46°F). DO NOT FREEZE. Do not use beyond the expiration date. Ranibizumab vials should remain refrigerated. Protect vials from direct light. Store in original carton until time of use.

RANIBIZUMAB VIALS ARE FOR SINGLE USE ONLY. Vials used for one subject may not be used for any other subject.

4.4 CONCOMITANT AND EXCLUDED THERAPIES

Standard preinjection betadine given along with lid speculum.

4.5 STUDY ASSESSMENTS

4.5.1 Assessments during the Treatment Period

Patients will be seen once per month, see attached study flow sheet. At visit 0 and at 3 month visit patient will also have an aqueous tap prior to Lucentis injection.

4.5.2 Early Termination Assessments

Subjects who withdraw from the study prior to completion should return for an early termination evaluation **10** days (±**X** days) following the last injection/study visit for monitoring of all adverse events (serious and

nonserious). The schedule of assessments for early termination is the same as that for the final visit.

4.5 SUBJECT DISCONTINUATION

Subjects have a right to withdraw from the study at any time.

The subject may be withdrawn from the study for any reasons: if it is in the best interest of the subject, intercurrent illness, adverse events, or worsening condition. Dr. Glazer may request the withdrawal of a subject because of protocol violations, administrative reasons, or any other valid and ethical reasons.

If a subject discontinues from the study, he or she will not be allowed to re-enter the study.

Reasons for subject discontinuation may include, but are not limited to, the following:

- Sensory rhegmatogenous retinal detachment or Stage 3 or 4 macular hole
- Investigator determination that it is not in the best interest of the subject to continue participation
- Pregnancy
- Endophthalmitis
- Need for anti-VEGF therapy other than ranibizumab in the study eye, unless as a part of the prospective investigational study design
- SAE
- Any other safety concerns

In the event of an adverse event in the study eye that is considered by the investigator to be severe in intensity, serious consideration should be given to discontinuing the subject from the study.

4.7 STUDY DISCONTINUATION

This study may be terminated by **Vitreo-Retinal Associates** or Genentech at any time. Reasons for terminating the study may include the following:

- The incidence or severity of adverse events in this or other studies indicates a potential health hazard to subjects
- Subject enrollment is unsatisfactory
- Data recording is inaccurate or incomplete

4.8 STATISTICAL METHODS

4.8.1 Analysis of the Conduct of the Study

There is no formal sample size calculation in a pharmacokinetic/pharmacodynamics study of this size. A sample size of **10** patients is chosen, making sure that it is feasible financially to conduct the study and logistically to complete the study within **6 months**.

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4.8.2 Safety Analyses

Any adverse events, laboratory assessments, physical examinations, vital signs, ocular examinations and measurements from all **10** subjects will be utilized to summarize safety data for this pilot study.

4.8.3 Efficacy Analyses

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a. Primary Endpoint

Aqueous cytokines and VEGF will be measured with an aqueous tap before the first and after the fourth Lucentis injection.

b. Secondary Endpoints

ETDRS vision will be measured at each visit along with spectral domain OCT.

After the fourth Lucentis injection, the patient will be exited from the study. The patients will continue to receive anti VEGF injections for DME based on clinical need. Dr. Glazer, Zheutlin, Garber, and DeHorn will continue to follow all patients

4.8.4 Missing Data

Analyses of efficacy and safety will be based on available cases, without imputation for missing values.

4.8.5 Interim Analyses

No formal schedule of interim analyses is planned. Reports of adverse events from this study may be reviewed and summarized periodically while the study is ongoing to ensure the safety of subjects.

4.9 DATA QUALITY ASSURANCE

Accurate, consistent, and reliable data will be ensured through the use of standard practices and procedures.

5. ASSESSMENT OF SAFETY

Specification of Safety Variables

Safety assessments will consist of monitoring and reporting adverse events (AEs) and serious adverse events (SAEs) per protocol. This includes all events of death, and any study specific issue of concern.

5.1 ADVERSE EVENTS

An AE is any unfavorable and unintended sign, symptom, or disease temporally associated with the use of an investigational medicinal product (IMP) or other protocol-imposed intervention, regardless of attribution.

This includes the following:

- AEs not previously observed in the subject that emerge during the protocol-specified AE reporting period, including signs or symptoms associated with DME that were not present prior to the AE reporting period.
- Complications that occur as a result of protocol-mandated interventions (aqueous taps) will be immediately reported to the IRB.
- If applicable, AEs that occur [rior to assignment of study treatment associated with medication washout, no treatment run-in, or other protocol-mandated intervention
- Preexising medical conditions (other than the condition being studied) judged by the investigator to have worsened in severity or frequency or changed in character during the protocol-specified AE reporting period.

5.2 SERIOUS ADVERSE EVENTS

An AE should be classified as an SAE if the following criteria are met:

- It results in death (i.e., the AE actually causes or leads to death).
- It is life threatening (i.e., the AE, in the view of the investigator, places the subject at immediate risk of death. It does not include an AE that, had it occurred in a more severe form, might have caused death.).
- It requires or prolongs inpatient hospitalization.
- It results in persistent or significant disability/incapacity (i.e., the AE results in substantial disruption of the subject's ability to conduct normal life functions).
- It results in a congenital anomaly/birth defect in a neonate/infant born to a mother exposed to the IMP.
- It is considered a significant medical event by the investigator based on medical judgment (e.g., may jeopardize the subject or may require medical/surgical intervention to prevent one of the outcomes listed above).

5.3 METHODS AND TIMING FOR ASSESSING AND RECORDING SAFETY VARIABLES

The investigator is responsible for ensuring that all AEs and SAEs that are observed or reported during the study are collected and reported to the FDA, appropriate IRB(s), and Genentech, Inc. in accordance with CFR 312.32 (IND Safety Reports).

Adverse Event Reporting Period

The study period during which all AEs and SAEs must be reported begins after informed consent is obtained and initiation of study treatment and ends 30 days following the last administration of study treatment or study discontinuation/termination, whichever is earlier. After this period, investigators should only report SAEs that are attributed to prior study treatment.

Assessment of Adverse Events

All AEs and SAEs whether volunteered by the subject, discovered by study personnel during questioning, or detected through physical examination, laboratory test, or other means will be reported appropriately. Each reported AE or SAE will be described by its duration (i.e., start and end dates), regulatory seriousness criteria if applicable,

suspected relationship to the 0.3mg Lucentis (see following guidance), and actions taken.

To ensure consistency of AE and SAE causality assessments, investigators should apply the following general guidelines:

Yes

There is a plausible temporal relationship between the onset of the AE and administration of the Lucentis or aqueous tap, and the AE cannot be readily explained by the subject's clinical state, intercurrent illness, or concomitant therapies; and/or the AE follows a known pattern of response to the {study drug}; and/or the AE abates or resolves upon discontinuation of 0.3mg Lucentis or dose reduction and, if applicable, reappears upon re-challenge.

No

Evidence exists that the AE has an etiology other than the 0.3mg Lucentis (e.g., preexisting medical condition, underlying disease, intercurrent illness, or concomitant medication); and/or the AE has no plausible temporal relationship to 0.3mg Lucentis administration (e.g., cancer diagnosed 2 days after first dose of study drug).

Expected adverse events are those adverse events that are listed or characterized in the Package Insert or current Investigator Brochure.

Unexpected adverse events are those not listed in the Package Insert (P.I.) or current Investigator Brochure (I.B.) or not identified. This includes adverse events for which the specificity or severity is not consistent with the description in the P.I. or I.B. For example, under this definition, hepatic necrosis would be unexpected if the P.I. or I.B. only referred to elevated hepatic enzymes or hepatitis.

5.4 EVALUATIONS

Reviews of body systems will be performed.

Ophthalmologic evaluations will include slit lamp examination, dilated binocular indirect high-magnification ophthalmoscopy, measurements of BCVA and intraocular pressure, and finger-count testing

5.5 VITAL SIGNS

Pulse and blood pressure will be measured at protocol-specified study visits (see Section 4.5).

5.6 PROCEDURES FOR ELICITING, RECORDING, AND REPORTING ADVERSE EVENTS

5.6.1 Eliciting Adverse Events

A consistent methodology for eliciting AEs at all subject evaluation time points should be adopted. Examples of non-directive questions include:

- "How have you felt since your last clinical visit?"
- "Have you had any new or changed health problems since you were last here?"

5.6.2 Specific Instructions for Recording Adverse Events

Investigators should use correct medical terminology/concepts when reporting AEs or SAEs. Avoid colloquialisms and abbreviations.

a. Diagnosis vs. Signs and Symptoms

If known at the time of reporting, a diagnosis should be reported rather than individual signs and symptoms (e.g., record only liver failure or hepatitis rather than jaundice, asterixis, and elevated transaminases). However, if a constellation of signs and/or symptoms cannot be medically characterized as a single diagnosis or syndrome at the time of reporting, it is ok to report the information that is currently available. If a diagnosis is subsequently established, it should be reported as follow-up information.

b. Deaths

All deaths that occur during the protocol-specified AE reporting period (see Section 5.1), regardless of attribution, will be reported to the appropriate parties. When recording a death, the event or condition that caused or contributed to the fatal outcome should be reported as the single medical concept. If the cause of death is unknown and cannot be ascertained at the time of reporting, report "Unexplained Death".

c. Preexisting Medical Conditions

A preexisting medical condition is one that is present at the start of the study. Such conditions should be reported as medical and surgical history. A preexisting medical condition should be re-assessed throughout the trial and reported as an AE or SAE only if the frequency, severity, or character of the condition worsens during the study. When reporting such events, it is important to convey the concept that the preexisting condition has changed by including applicable descriptors (e.g., "more frequent headaches").

d. Hospitalizations for Medical or Surgical Procedures

Any AE that results in hospitalization or prolonged hospitalization should be documented and reported as an SAE. If a subject is hospitalized to undergo a medical or surgical procedure as a result of an AE, the event responsible for the procedure, not the procedure itself, should be reported as the SAE. For example, if a subject is hospitalized to undergo coronary bypass surgery, record the heart condition that necessitated the bypass as the SAE.

Hospitalizations for the following reasons do not require reporting:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for preexisting conditions
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study or
- Hospitalization or prolonged hospitalization for scheduled therapy of the target disease of the study.

e. Pregnancy

If a female subject becomes pregnant while receiving investigational therapy or within 90 days after the last dose of study drug, a report should be completed and expeditiously submitted to the Genentech, Inc. Follow-up to obtain the outcome of the pregnancy should also occur. Abortion, whether accidental, therapeutic, or spontaneous, should always be classified as serious, and expeditiously reported as an SAE. Similarly, any congenital anomaly/birth defect in a child born to a female subject exposed to the 0.3mg Lucentis should be reported as an SAE.

f. Post-Study Adverse Events

The investigator should expeditiously report any SAE occurring after a subject has completed or discontinued study participation if attributed to prior 0.3mg Lucentis exposure. If the investigator should become aware of the development of cancer or a congenital anomaly in a subsequently conceived offspring of a female subject who participated in the study, this should be reported as an SAE.

g. Reconciliation

The Sponsor agrees to conduct reconciliation for the product. Genentech and the Sponsor will agree to the reconciliation periodicity and format, but agree at minimum to exchange quarterly line listings of cases received by the other party.

If discrepancies are identified, the Sponsor and Genentech will cooperate in resolving the discrepancies. The responsible individuals for each party shall handle the matter on a case-by-case basis until satisfactory resolution. The Sponsor shall receive reconciliation guidance documents within the 'Activation Package'.

h. AEs of Special Interest (AESIs)

AEs of Special Interest are defined as a potential safety problem, identified as a result of safety monitoring of the Product.

The Lucentis Events of Special Interest are:

- Retinal pigment epithelial tear
- Increased intraocular pressure to > 30mm Hg not responsive to maximal topical IOP-lowering drugs measured on 2 separate days
- Traumatic cataract
- Endophthalmitis
- Intraocular inflammation of greater than 2+ cells (including vitritis and uveitis)
- Retinal detachment
- ATEs, including stroke

i. Adverse Event Reporting

Investigators must report all SAEs to Genentech within the timelines described below. The completed Medwatch/case report should be faxed immediately upon completion to Genentech Drug Safety at:

650-238-6067

Serious adverse events (SAEs), pregnancy reports and AEs of special interest (AESIs), where the patient has been exposed to the Product, will be sent on a MedWatch or CIOMS I form to the Roche contact specified in Addendum 2 of this Protocol. Transmission of these reports (initial and follow-up) will be either electronically or by fax and within the timelines specified below:]

- **SADRs**
  Serious AE reports that are related to the Product shall be transmitted to Roche within fifteen (15) calendar days of the awareness date.

- **Other SAEs**
  Serious AE reports that are unrelated to the Product shall be transmitted to Roche within thirty (30) calendar days of the awareness date.

- **Pregnancy reports**
  While such reports are not serious AEs or ADRs per se, as defined herein, any reports of pregnancy, where the fetus may have been exposed to the Product, shall be transmitted to Roche within thirty (30) calendar days of the awareness date. Pregnancies will be followed up until the outcome of the pregnancy is known, whenever possible, based upon due diligence taken to obtain the follow-up information.

- **AESIs**
  AESIs requiring expedited reporting shall be forwarded to Roche within fifteen (15) calendar days of the awareness date. Others shall be sent within thirty (30) calendar days.

- **Non-serious AEs**
  Non-serious AEs shall be transmitted to Roche on a periodic (e.g., monthly) line-listing containing the following elements (Protocol number, Patient ID, Patient birth date, Adverse Event/MedDRA term, Seriousness of event, Onset date of event, Death date, Product received, Date of first dose, Cause(s) of event, Adverse Event description).

Relevant follow-up information should be submitted to Genentech Drug Safety as soon as it becomes available.

Special situation reports
In addition to all AEs, pregnancy reports and AESIs, the following Special Situations Reports should be collected and transmitted to Roche even in the absence of an Adverse Event within thirty (30) calendar days:

- Data related to the Product usage during pregnancy or breastfeeding
- Data related to overdose, abuse, off-label use, misuse, inadvertent/erroneous administration, medication error or occupational exposure, with or without association with an AE/SAE unless otherwise specified in the protocol

- Data related to a suspected transmission of an infectious agent via a medicinal product (STIAMP)
- Lack of therapeutic efficacy

In addition, reasonable attempts should made to obtain and submit the age or age group of the patient, in order to be able to identify potential safety signals specific to a particular population

j. Aggregate Reports

Vitreo-Retinal will forward periodically listings of non-serious AEs originating from the Study to Roche.

Note: Investigators should also report events to their IRB as required.

5.6.3 MedWatch 3500A Reporting Guidelines

In addition to completing appropriate patient demographic and suspect medication information, the report should include the following information within the Event Description (section 5) of the MedWatch 3500A form:

- Protocol description (and number, if assigned)
- Description of event, severity, treatment, and outcome if known
- Supportive laboratory results and diagnostics
- Investigator's assessment of the relationship of the adverse event to each investigational product and suspect medication

5.6.4 Follow-up Information

Additional information may be added to a previously submitted report by any of the following methods:

- Adding to the original MedWatch 3500A report and submitting it as follow-up
- Adding supplemental summary information and submitting it as follow-up with the original MedWatch 3500A form
- Summarizing new information and faxing it with a cover letter including patient identifiers (i.e. D.O.B. initial, patient number), protocol description and number, if assigned, brief adverse event description, and notation that additional or follow-up information is being submitted (The patient identifiers are important so that the new information is added to the correct initial report)

Occasionally Genentech may contact the reporter for additional information, clarification, or current status of the patient for whom and adverse event was reported.

For questions regarding SAE reporting, you may contact the Genentech Drug Safety

representative noted above or the MSL assigned to the study. Relevant follow-up information should be submitted to Genentech Drug Safety as soon as it becomes available and/or upon request.

MedWatch 3500A (Mandatory Reporting) form is available at http://www.fda.gov/medwatch/getforms.html

5.6.5 Additional Reporting Requirements for IND Holders

For Investigator-Sponsored IND Studies, some additional reporting requirements for the FDA apply in accordance with the guidance set forth in 21 CFR § 600.80.

Events meeting the following criteria need to be submitted to the Food and Drug Administration (FDA) as expedited IND Safety Reports according to the following guidance and timelines:

7 Calendar Day Telephone or Fax Report:

The Investigator is required to notify the FDA of any fatal or life-threatening adverse event that is unexpected and assessed by the investigator to be possibly related to the use of 0.3mg Lucentis. An unexpected adverse event is one that is not already described in the 0.3mg Lucentis Investigator Brochure. Such reports are to be telephoned or faxed to the FDA and Genentech within 7 calendar days of first learning of the event.

15 Calendar Day Written Report

The Investigator is also required to notify the FDA and all participating investigators, in a written IND Safety Report, of any serious, unexpected AE that is considered reasonably or possibly related to the use of 0.3mg Lucentis. An unexpected adverse event is one that is not already described in the 0.3mg Lucentis investigator brochure.

Written IND Safety reports should include an Analysis of Similar Events in accordance with regulation 21 CFR § 312.32. All safety reports previously filed by the investigator with the IND concerning similar events should be analyzed and the significance of the new report in light of the previous, similar reports commented on.

Written IND safety reports with Analysis of Similar Events are to be submitted to the FDA, Genentech, and all participating investigators within 15 calendar days of first learning of the event. The FDA prefers these reports on a Medwatch 3500 form, but alternative formats are acceptable (e.g., summary letter).

FDA fax number for IND Safety Reports:

Fax: 1 (800) FDA 0178

All written IND Safety Reports submitted to the FDA by the Investigator must also be sent to Genentech Drug Safety:

Fax: (650) 238-6067

or

Email: usds_aereporting-d@gene.com

And to the Site IRB:

[IRB Contact info / fax here]

For questions related to safety reporting, please contact Genentech Drug Safety:

Tel: (888) 835-2555
Fax: (650) 238-6067

IND Annual Reports

Copies to Genentech:

All IND annual reports submitted to the FDA by the Sponsor-Investigator should be copied to Genentech. Copies of such reports should be faxed to Genentech Drug Safety:

Fax: (650) 238-6067

Study Close-Out

Any study report submitted to the FDA by the Sponsor-Investigator should be copied to Genentech. This includes all IND annual reports and the Clinical Study Report (final study report). Additionally, any literature articles that are a result of the study should be sent to Genentech. Copies of such reports should be sent to the following e-mail address:

Lucentisgsr_clinops-d@gene.com

Genentech
A Member of the Roche Group

5.6.6 SAFETY REPORTING FAX COVER SHEET

Genentech Supported Research

AE / SAE FAX No: (650) 238-6067

Genentech Study Number	
Principal Investigator	
Site Name	
Reporter name	
Reporter Telephone #	
Reporter Fax #	

Initial Report Date	[DD] / [MON] / [YY]
Follow-up Report Date	[DD] / [MON] / [YY]

Subject Initials (Enter a dash if patient has no middle name)	[ ] - [ ] - [ ]

SAE or Safety Reporting questions, contact Genentech Safety: (888) 835-2555

PLEASE PLACE MEDWATCH REPORT or SAFETY REPORT BEHIND THIS COVER SHEET

6.0 INVESTIGATOR REQUIREMENTS

6.1 STUDY INITIATION

Before the start of this study, the following documents must be on file with **Vitreo-Retinal Associates** or its appointed representative:

- FDA correspondence letter assigning an IND number or an IND waiver letter
- Original U.S. FDA Form 1571 (if applicable)
- Original U.S. FDA Form 1572 (for all studies conducted under U.S. Investigational New Drug [IND] regulations), signed by the Principal Investigator (if applicable)
- The names of any sub-investigators must appear on this form. Investigators must also complete all regulatory documentation as required by local and national regulations.
- Current curricula vitae of the Principal Investigator
- Medical License
- Written documentation of IRB approval of the protocol (identified by Vitreo-Retinal Associates, protocol number or title and date of approval)
- IRB Approved protocol
- Fully executed contract
- Documentation of registration into clinical research website (e.g., www.clinicaltrials.gov) (as applicable)
- Investigator Brochure Signature Receipt

6.2 STUDY COMPLETION

The following data and materials are required by **Vitreo-Retinal Associates** before a study can be considered complete or terminated:

- Laboratory findings, clinical data, and all special test results from screening through the end of the study follow-up period (if applicable)

- Case Report Forms properly completed by appropriate study personnel and signed and dated by the investigator (if applicable)
- Copies of protocol amendments and IRB approval/notification (if applicable)
- A summary of the study prepared by the Principal Investigator (will accept IRB summary close letter) (if applicable)
- All regulatory documents (e.g., curricula vitae for each Principal Investigator, U.S. FDA Form 1571 and 1572)

6.3 INFORMED CONSENT

Informed consent documents will be provided to each subject.

The informed consent document must be signed and dated by the subject or the subject's legally authorized representative before his or her participation in the study. The case history for each subject shall document that informed consent was obtained prior to participation in the study. A copy of the informed consent document must be provided to the subject or the subject's legally authorized representative. If applicable, it will be provided in a certified translation of the local language.

Signed consent forms must remain in each subject's study file and must be available for verification at any time.

The following basic elements must be included:

- A statement that the study involves research, an explanation of the purposes of the research and the expected duration of the patient's participation, a description of the procedures to be followed, and identification of any procedures or drug used for purposes which are experimental
- A description of any reasonably foreseeable risks or discomforts to the patients
- A description of any benefits to the patient or to others which may reasonably be expected from the research. A description that there may be no benefit from this research.

- A disclosure of appropriate alternative procedures or courses of treatment, if any, that might be advantageous to the patient
- A statement describing the extent, if any, to which confidentiality records identifying the patient will be maintained and that notes the possibility that the FDA and the Vitreo-Retinal Associates and the drug manufacturer may inspect the records
- For research involving more than minimal risk, an explanation as to whether any compensation and any medical treatments are available should injury occur and, if so, what they consist of or where further information may be obtained
- An explanation of whom to contact for answers to pertinent questions about the research and research patient's rights, and whom to contact in the event of a research-related injury to the patient
- A statement that participation is voluntary, that refusal to participate will involve no penalty or loss of benefits to which the patient is otherwise entitled, and that the patient may discontinue participation at any time without penalty or loss of benefits to which the patient is otherwise entitled

6.4 INSTITUTIONAL REVIEW BOARD OR ETHICS COMMITTEE APPROVAL

This protocol, the informed consent document, and relevant supporting information must be submitted to the IRB/EC for review and must be approved before the study is initiated. The study will be conducted in accordance with U.S. FDA, applicable national and local health authorities, and IRB/EC requirements.

The Principal Investigator is responsible for keeping the IRB/EC apprised of the progress of the study and of any changes made to the protocol as deemed appropriate, but in any case the IRB/EC must be updated at least once a year. The Principal Investigator must also keep the IRB/EC informed of any significant adverse events.

Investigators are required to promptly notify their respective IRB/EC of all adverse drug reactions that are both serious and unexpected. This generally

refers to serious adverse events that are not already identified in the Investigator Brochure and that are considered possibly or probably related to the study drug by the investigator. Some IRBs or ECs may have other specific adverse event requirements that investigators are expected to adhere to. Investigators must immediately forward to their IRB/EC any written safety report or update provided by Vitreo-Retinal Associates (e.g., IND safety report, Investigator Brochure, safety amendments and updates, etc.).

6.5 CASE REPORT FORMS

All CRFs should be filled out completely by appropriate personnel. The CRF should be reviewed, signed, and dated by the investigator.

All CRFs should be completed in a neat, legible manner to ensure accurate interpretation of data. Black ink is required to ensure clarity of reproduced CRF copies. When making changes or corrections, cross out the original entry with a single line, and initial and date the change. DO NOT ERASE, OVERWRITE, OR USE CORRECTION FLUID ON THE ORIGINAL.

6.6 STUDY DRUG ACCOUNTABILITY

The Investigator is responsible for the control and distribution of study drug.

All partially used or empty containers should be disposed of at the study site according to institutional standard operating procedure.

6.7 DISCLOSURE OF DATA

Subject medical information obtained by this study is confidential, and disclosure to third parties other than those noted below is prohibited.

Upon the subject's permission, medical information may be given to his or her personal physician or other appropriate medical personnel responsible for his or her welfare.

Data generated by this study must be available for inspection upon request by representatives of the U.S. FDA, national and local health authorities, the drug manufacturer and the IRB/EC for each study site, if appropriate.

6.8 RETENTION OF RECORDS

U.S. FDA regulations (21 CFR §312.62[c]) require that records and documents pertaining to the conduct of this study and the distribution of investigational drug, including CRFs, consent forms, laboratory test results, and medication inventory records, must be retained by the Principal Investigator for 2 years after the investigation is discontinued and the U.S. FDA and the applicable national and local health authorities are notified.

6.9 STUDY CLOSE-OUT

Any study report submitted to the FDA by the Sponsor-Investigator should be copied to Genentech. This includes all IND annual reports and the Clinical Study Report (final study report). Additionally, any literature articles that are a result of the study should be sent to Genentech. Copies of such reports should be faxed to the assigned Clinical Operations contact for the study:

Lucentis IST Program Fax: 1-866-728-4622

Lucentis Email: lucentisgsr_coa-d@gene.com

REFERENCES

List references alphabetically, and format them using the Vancouver style described below.

General Rules:

1. *DRCR Protocol T*
2. *Costagliola, Aqueous Humor Levels of VEGF and adiponectin in patients with Type II Diabetes before and after intravitreal Avastin injection, Experimental Eye Research 2013 110 pg 50-54*
3. *Suzuki, Effects of Intravitreal injection of bevacizumab on inflammatory cytokines, Retina 2014 pg 165-171*

4. White, J. and Glazer, L., Identification of VEGF independent cytokines in PDR vitreous, IOVS 2013 pg 6472-6480

5. Phaler, Retina 2009, A prospective study of in office diagnostic vitreous sampling in patients with vitreoretinal pathology, pg 1032-1035

6. Compachairo, Ranibizumab for macular edema due to retinal vein occlusions, Molecular therapeutics 2008, 16, 791-799

7. Compachairo et al, Monitoring ocular drug therapy by analysis of aqueous samples, Ophthalmology 2009, 2158-2164.

APPENDIX A

Study Flowchart

	Day 1	Week 4	Week 8	Week 12
Informed Consent	X			
Inclusion/Exclusion	X			
Visual Acuity	X	X	X	X
Refraction	X			X
IOP	X	X	X	X
Dilate	X	X	X	X
OCT	X	X	X	X
FA/FP	X			
Ophthalmic Exam	X	X	X	X
Aqueous sample	X			X
Lucentis injection	X	X	X	X
Study Exit				X
Pregnancy Test if applicable	X	X	X	X

Include a study flowchart for ease of review and understanding of the study.

Note: General note to entire table here.

[a] Footnote.

[b] Footnote.

[c] Footnote.

APPENDIX B
Pre-Injection Procedures for All Subjects

The following procedures will be implemented to minimize the risk of potential adverse events associated with serial intravitreal injections (e.g., endophthalmitis). Aseptic technique will be observed by clinic staff involved in the injection tray assembly, anesthetic preparation, and study drug preparation and administration. In addition to the procedures outlined below, added safety measures in adherence to specific institutional policies associated with intravitreal injections will be observed.

The following procedures (except where noted), will be conducted by the investigator.

- The technician assembles the supplies and prepares a sterile field. Supplies include 10% povidone iodine swabs, sterile surgical gloves, 4×4 sterile pads, pack of sterile cotton-tipped applicators, eyelid speculum, sterile ophthalmic drape, 0.5% proparacaine hydrochloride, 5% povidone iodine ophthalmic solution, 1% lidocaine for injection, ophthalmic antimicrobial solution (e.g. trimethoprim-polymyxin B ophthalmic solution, ofloxacin ophthalmic solution, ophthalmic gatifloxacin solution, ophthalmic moxifloxacin solution), and injection supplies.
- Instill 2 drops of 0.5% proparacaine hydrochloride into the study eye, followed by 2 drops of a broad-spectrum antimicrobial solution (e.g. trimethoprim-polymyxin B ophthalmic solution, ofloxacin ophthalmic solution, ophthalmic gatifloxacin solution, ophthalmic moxifloxacin solution).
- Disinfect the periocular skin and eyelid of the study eye in preparation for injection. Scrub the eyelid, lashes, and periorbital skin with 10% povidone iodine swabs, starting with the eyelid and lashes and continuing with the surrounding periocular skin. Make certain that the eyelid margins and lashes are swabbed, and proceed in a systematic fashion, from medial to temporal aspects.
- The investigator will glove, place sterile ophthalmic drape to isolate the field, and place the speculum underneath the eyelid of the study eye.
- Instill 2 drops of 5% povidone iodine ophthalmic solution in the study eye, making sure the drops cover the planned injection site on the conjunctiva.
- Wait 90 seconds.
- Saturate a sterile cotton-tipped applicator with 0.5% proparacaine hydrochloride drops and hold the swab against the planned intravitreal injection site for 10 seconds in preparation for the subconjunctival injection of 1% lidocaine hydrochloride ophthalmic solution for injection (without epinephrine).
- Use a sterile 4×4 pad in a single wipe to absorb excess liquid and to dry the periocular skin.

- ***Instruct subject to direct gaze away from syringe prior to ranibizumab injection.***

APPENDIX C

Analysis of Similar Events Template for IND Safety Reports

IND Safety Report

Case Summary

This section will be initiated by a research coordinator and may be modified by principal investigators if necessary. The case summary should describe the reported AE in detail, including a description of what happened and a summary of all relevant clinical information (e.g. medical status prior to the event, signs, symptoms, diagnoses, clinical course, treatment, outcome, etc.) The IND safety report should not identify the subject ID #, reporting investigator, or the site as this information may compromise the study blind.

PREVIOUS REPORTS

The information for this section comes from Principal Investigator and the search of similar events. This section should be written by the responsible principal investigator.

* *Select one of the following two statements after reviewing the search of similar events results.*

Under IND _____(insert IND#), the following IND safety reports of similar AEs have been previously submitted:

MCN	Reported Event	Submission Date

Or

Under IND _____ (insert IND#), no IND safety reports of similar AEs have been submitted previously.

In addition to previously submitted IND safety reports of similar events, this section can also summarize pervious serious reports of the same/similar event that were considered unrelated to the investigational product at the time of the reporting. These events would remain blinded, unless a decision to unblind is made by an Independent Monitoring Committee for reasons of subject protection. The decision on what similar events to summarize in this section should be made after reviewing the similar events report generated by Clinical Data Management. If a safety signal is particularly worrisome (e.g., a study stopping type of event), a more extensive evaluation may be required.

Assessment of Relationship

After evaluation the new case report and reviewing any relevant previous reports of similar events, the PI selects one of the following boilerplate conclusion statements, if applicable. The PI may also craft an alternative conclusion.

Based on review of available data, (Insert Institution Name) believes there is a reasonable possibility of a cause-and-effect relationship between administration of _____(insert study drug name) and the occurrence of _____(insert AE).

Additional information on risk factors and/or treatment of the AE may be provided if warranted.

Or

Based on review of available data, the (Insert Institution Name) does not believe that there is a reasonable possibility of a cause-and-effect relationship between administration of _____(insert study drug name) and the occurrence of _____(insert AE).

Explain if warranted. Do not speculate.

Or

Based on review of available data, the (Insert Institution Name) cannot establish or exclude the possibility of a cause-and-effect relationship between administration of _____(insert study drug name) and the occurrence of _____(insert AE).

Explain if warranted. Do not speculate.

After review of the clinical details and investigator's comments pertaining to this AE, and based on experience to date, the (Insert Institution Name) does not believe that changes to the conduct of this clinical trial are warranted. *This statement can be modified if changes to the conduct of the clinical trial are made.*