

THE POST-VITRECTOMY LENSTATIN™ STUDY:

A RANDOMIZED DOUBLE BLIND HUMAN CLINICAL TRIAL TESTING THE EFFICACY OF LENSTATIN, AN ORAL ANTIOXIDANT NUTRITIONAL SUPPLEMENT, IN INHIBITING NUCLEAR CATARACT PROGRESSION AFTER PARS PLANA VITRECTOMY

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ABSTRACT

The Post-Vitrectomy Lenstatin™ Study was a randomized, prospective, double-blind, placebo controlled human clinical trial to test the efficacy of Lenstatin, a proprietary antioxidant nutritional supplement, in inhibiting nuclear cataract progression after Pars Plana Vitrectomy surgery.

Twenty patients ages 33-74 scheduled to undergo Pars Plana Vitrectomy (PPV) underwent pre-enrollment random allocation in equal blocks to treatment with Lenstatin versus placebo for six months following surgery. Allocation was concealed from investigators, participants, study personnel, and data collectors. The study was carried out at a single private office location in 2014-15. Participants took Lenstatin two capsules per day versus placebo for six months following PPV. Pentacam Nuclear Staging (PNS) lens densitometry measurements were taken at baseline and at six months postoperatively. No adverse reactions were encountered. Eighteen of twenty participants completed the study.

Nuclear cataract density measurements increased in nearly all patients (19/20) at six months in both Lenstatin and placebo groups. PNS Zone 1 mean density increase was 1.25% in placebo and 1.16% in Lenstatin, Average All Zones mean density increase was 1.54% in placebo and 1.19% in Lenstatin.

Analysis of covariance (ANCOVA) was used to compare baseline and six month PNS Zone 1 and Average All Zones densities between Lenstatin and placebo treatment groups, using baseline density as the covariate. The difference between final Zone 1 adjusted mean density placebo (11.8) and Lenstatin (11.5) was not significant ($F=.4$, $p=.5$). The difference between final Average All Zones adjusted mean density placebo (12.5) and Lenstatin (11.8) was not significant ($F=1.30$, $p=.27$). The latter effect size was in the range of clinical relevance, with Lenstatin showing less increase in density. In participants with initial baseline densities of less than 11%, the difference between Lenstatin and placebo groups for both Zone 1 density ($F=1.22$, $p=0.30$, $ES=0.63$) and Average All Zones density ($F=1.79$, $p=0.21$, $ES=0.84$) showed a clinically relevant effect size with Lenstatin showing less increase in density.

We employed a novel, quantitative, and reproducible method to study the effects of an interventional agent on nuclear cataract progression. Future studies with similar methods utilizing larger sample sizes may provide

significant additional useful information with respect to the potential beneficial effects of nutritional antioxidants in inhibiting cataract progression.

The clinical trial is registered at [ClinicaTrials.gov](https://clinicaltrials.gov), Identifier Number NCT02131194. The study was funded in by Lenstatin™ LLC, a limited liability company. The Principal Investigator of the study, Scott W. Tunis MD FACS, is the majority owner of Lenstatin™ LLC.

BACKGROUND AND RATIONALE

There are conflicting reports on the effectiveness of nutritional antioxidants in preventing or slowing the growth of age related cataracts, despite extensive laboratory evidence suggesting that oxidative damage to lens epithelial cells is a common underlying etiologic factor in cataractogenesis. One reported review of nine clinical trials involving over 117,000 patients suggested that beta-carotene, Vitamin E, and Vitamin C had no effect in preventing or slowing the progression of age-related cataract.¹ Conversely, a randomized trial of over 14,000 US male physicians indicated that long-term daily multivitamin use modestly and significantly decreased the risk of cataract.²

There are significant limitations in interpreting the results of long term studies on the efficacy of interventional agents in inhibiting senile cataract progression. A number of uncontrolled variables are present in participant populations over long periods of observation which may contribute to or mitigate cataract progression. Differences among participants in total cumulative exposure to ultraviolet light, dietary anti-oxidant content, family history, trauma, and pharmacologic history may all affect the observed rates of cataract progression, yet accurate data collection and statistical accounting of these variables is difficult or impossible.

There are also significant limitations in interpreting the results of long term observational studies in which the main outcomes used for analysis are subjective, such as visual acuities or observed rates of cataract surgery, which may vary greatly among participants, observers, data collectors, and surgery providers.

Cataract formation following PPV is a well-recognized post-operative complication of the procedure, with the reported incidence of clinically significant cataract development as high as 80% within 2 years after surgery³⁻⁵. Evaluating the efficacy of an interventional agent to inhibit nuclear cataract progression after PPV allows a much shorter interval of observation than in senescent nuclear cataract, minimizing participants' potential exposure to uncontrolled variables.

Pentacam Scheimpflug images have been shown to provide an immediate, quantitative, examiner-independent measurement of lens density which correlates with the LOCS III cataract grade.⁶ Assessing the effect of an interventional agent on nuclear cataract progression using serial Pentacam Nuclear Staging lens density measurements allows an objective, reproducible, and examiner-independent analysis.

PURPOSE OF THE STUDY

To determine the efficacy of Lenstatin™, an over-the-counter anti-cataract nutritional supplement, in preventing or inhibiting the formation and progression of cataract in eyes after pars plana vitrectomy.

INVESTIGATIONAL AGENT

Lenstatin™ is a proprietary formulated nutritional supplement containing eleven compounds with reported cataract inhibitory properties: Riboflavin, L-Glutathione, C-Phycocyanin (as Spirulina Algae Extract), Lipoic Acid, Pyruvate (as Calcium Pyruvate), Alpha Lipoic Acid, Quercetin, Turmeric, Silybin (as Milk Thistle Extract), Lutein, Zeaxanthin, and Astaxanthin.

In accordance with the [Dietary Supplement Health and Education Act of 1994](#), the sale and use of Lenstatin™ is not subject to FDA regulations which govern the distribution and sale of pharmaceuticals. As a nutritional supplement, Lenstatin™ is available for purchase over-the-counter without a prescription.

Nutricap Labs (Farmingdale, NY), the manufacturer of Lenstatin™, is a GMP and NSF certified dietary supplement manufacturing facility. These certifications indicate that the facility is in compliance with a 2007 FDA policy statement which outlines good manufacturing practices for manufacturers of dietary supplements.

DESIGN

The study design was a single center, randomized, prospective, double-blind, placebo controlled human clinical trial with a total of twenty study participants and study duration of six months.

ELIGIBILITY / RECRUITMENT

Eligible participants were adult patients age 18 or over undergoing PPV. Pregnant women were excluded on the basis that Lenstatin™ is not recommended during pregnancy. Eligible study participants were not excluded or targeted based on nationality, native language, socio-economic status, or gender.

The first phase of recruitment began with introductory information being supplied to potential participants by retina surgeons who perform PPV. A personal consultation with Principal Investigator Scott W. Tunis M.D. was required by all potential participants who were considering enrollment. The first twenty consecutive patients electing to participate after meeting with the Principal Investigator were enrolled.

ALLOCATION AND ALLOCATION CONCEALMENT

Prior to enrollment of the first participant, the entire block of 20 treatment courses were randomly allocated to treatment with Lenstatin™ or placebo. Balanced randomization with a block size of twenty and a 1:1 allocation ratio were used generating two equal groups of Lenstatin™ treatment and placebo treatment arms, each group having 10 participants.

Placebo was prepared by an independent compounding pharmacy (Medical Center Pharmacy, Wilmington NC). Capsules, bottles and labels used to formulate placebo were identical to Lenstatin capsules, bottles and labels, and were obtained from the same manufacturer.

Pre-enrollment random allocation and allocation concealment were conducted by the independent accounting firm Earney and Company LLP, Wilmington, NC (EAC) in the following manner:

Ten sealed cartons each containing six bottles of Lenstatin™ intended for a six month course of treatment of Lenstatin™ for one participant, each labelled externally with a removable post-it note indicating “L” for Lenstatin were delivered to EAC. Simultaneously, ten identical sealed cartons each containing six bottles of placebo intended for a six month treatment course for one participant, each labelled externally with a removable post-it note “P” for

placebo were delivered to EAC. Other than the post-it notes indicating Lenstatin™ or placebo, all sealed cartons were identical and indistinguishable in external appearance from one another. The external post-it note labels were easily removable and left no distinguishing mark on the cartons.

EAC arranged the unopened, sealed cartons in alternating fashion L-P-L-P etc. A random sequence of numbers was generated from 1-20 inclusive, using <http://www.random.org/sequences/>, and a printed record of the random sequence was retained. The numbers generated in the random sequence were used to re-label the cartons in the following manner:

If the random sequence generated were 3, 18, 6..., the “L” post-it note from the first carton was removed and the carton was re-labeled to (#3) in permanent ink, the “P” post-it note from was removed from the second carton and it was re-labeled to (#18) in permanent ink, and the “L” post-it note from the third carton was removed and it was re-labeled to (#6) in permanent ink. The process continued until all of the cartons had been re-labeled, all “L” and “P” post it notes were removed and all 20 cartons were assigned a treatment number.

Treatment was assigned to each participant as they were enrolled in the study in the consecutive numeric order as labeled in permanent ink on each carton, i.e. the first patient enrolled was assigned carton (#1), the second patient carton (#2) etc., until all twenty cartons each containing a six month course of therapy were assigned. The random sequence generated and the corresponding legend which recorded the original treatment type of each carton was not revealed to anyone outside EAC until the study was concluded.

PRE STUDY

Upon enrollment each participant was given a test dose of one capsule of their numbered study treatment course at the Principal Investigator’s office and was observed for one hour for any adverse or allergic reaction. No adverse reactions were observed. The participants were asked to notify the Principal Investigator of any adverse reactions occurring at any time during the study.

TREATMENT

Participants took two capsules of treatment modality per day for six months beginning one day after PPV surgery. A one month course of treatment (one bottle of sixty capsules) was dispensed on the first day of enrollment to each participant, and one bottle at each subsequent monthly visit for the ensuing month’s treatment. All treatment cartons were maintained in a locked storage cabinet and dispensed from the Principal Investigator’s office to each participant according to their pre-determined treatment number at each monthly visit.

DATA COLLECTION

The Pentacam Scheimpflug imaging system is a non-contact, non-invasive anterior segment imaging device which takes less than two minutes to acquire a detailed photographic (tomographic) image of the anterior segment of the human eye. Additional manufacturer information on the Pentacam can be accessed at <http://pentacam.com/sites/hr.php>.

Pentacam scans were performed on participants at baseline pre- PPV and at monthly intervals for six months. Two trained Pentacam operators utilized a single Pentacam at one location to perform scans and collect data. PNS nuclear density measurements were used from the Scheimpflug images nearest to axis 90 degrees, which have been shown to provide the most reliable and reproducible results.

STUDY DATA

| PATIENT NUMBER | BASELINE ZONE 1 DENSITY | FINAL ZONE 1 DENSITY | BASELINE AVERAGE DENSITY | FINAL AVERAGE DENSITY | LENSTATIN (LS) OR PLACEBO (P) |
|----------------|-------------------------|----------------------|--------------------------|-----------------------|-------------------------------|
| 1 | 9.6 | 10.9 | 10.3 | 11.7 | P |
| 2 | 11.1 | 11.9 | 11.6 | 12.9 | P |
| 3 | 8.4 | 8.8 | 8.1 | 8.7 | LS |
| 4 | 9.9 | 10.0 | 10.5 | 10.7 | LS |
| 5 | 11.4 | 13.0 | 12.0 | 13.4 | LS |
| 6 | 8.5 | 8.9 | 8.1 | 8.5 | P |
| 7 | 8.7 | 8.9 | 8.8 | 8.8 | LS |
| 8 | 10.1 | 10.4 | 10.4 | 10.5 | LS |
| 9 | 11.3 | 12.5 | 11.8 | 12.8 | LS |
| 10 | 10.1 | 12.3 | 10.6 | 13.3 | P |
| 11 | 10.5 | 11.4 | 11.1 | 12.3 | P |
| 12 | 10.6 | 12.4 | 11.2 | 13.0 | LS |
| 13 | 12.0 | 15.0 | 12.6 | 16.1 | LS |
| 14 | 13.6 | 14.3 | 13.9 | 14.8 | LS |
| 15 | 9.9 | 12.6 | 10.2 | 13.4 | P |
| 16 | 8.5 | 8.5 | 8.0 | 8.2 | P |
| 17 | 11.8 | 12.9 | 12.2 | 13.5 | P |
| 18 | 9.5 | 12.9 | 9.8 | 13.8 | P |
| 19 | 12.4 | 14.6 | 13.6 | 16.0 | LS |
| 20 | 10.5 | 10.2 | 10.9 | 10.6 | P |

RESULTS / INTERPRETATION

Analysis of covariance (ANCOVA) was used to compare Pentacam PNS Zone 1 and Average All Zones mean density measurements from baseline to six months in Lenstatin versus placebo groups using baseline density measurements as the covariate. Measurements of Zone 1 nuclear cataract density baseline versus final and measurements of Average nuclear cataract density baseline versus final are presented descriptively in scatter plots and analyzed with Pearson correlation. The data were considered as Intention to treat with two subjects dropping out early (placebo at five months and Lenstatin at three months). Their final measurements were included in the analysis.

In Figures 1 and 2 the black line of identity illustrates that nearly all patients increased nuclear density from baseline to six months.

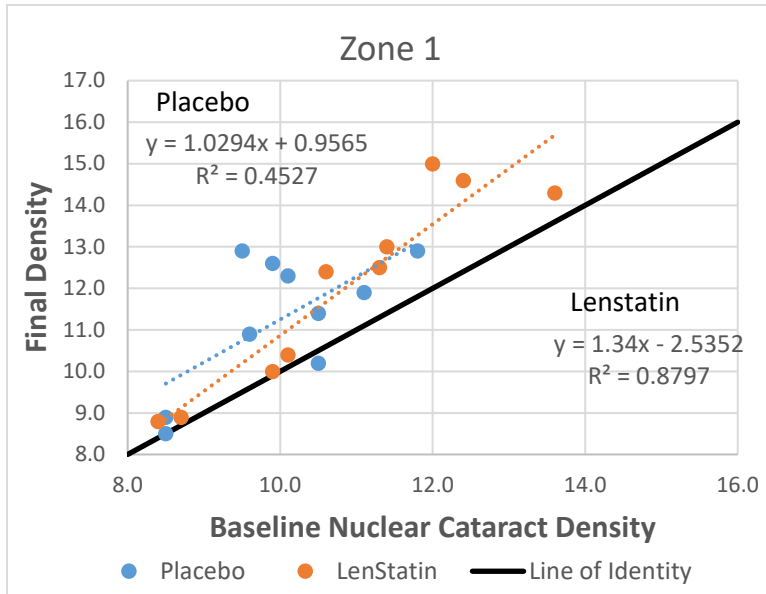


Figure 1. Comparison of Lenstatin and placebo in Zone 1.

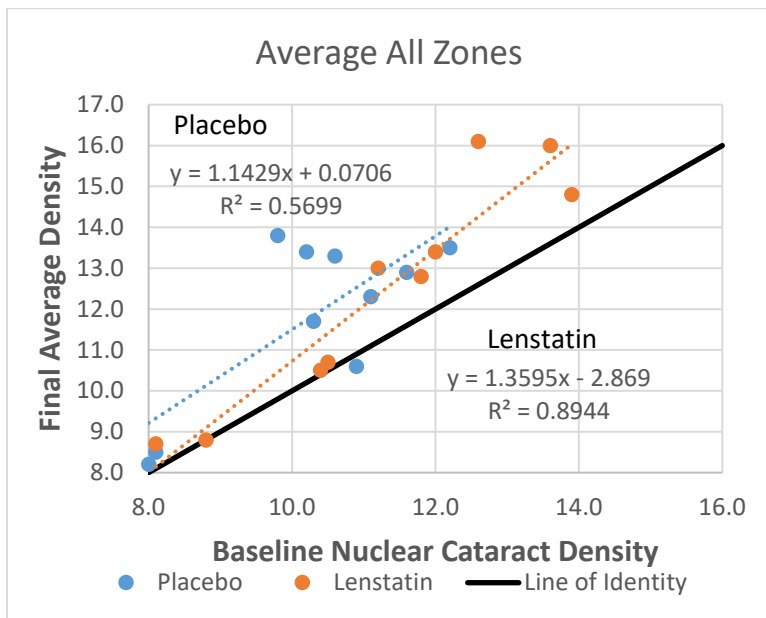


Figure 2. Comparison of Lenstatin and placebo averaged over all zones.

The difference between final Zone 1 adjusted mean density placebo (11.8) and Lenstatin (11.5) was not significant ($F=.4$, $p=.5$). The pooled standard deviation was 1.06 and the effect size was .29 SD. The difference between final Average All Zones adjusted mean density placebo (12.5) and Lenstatin (11.8) was not significant ($F=1.30$, $p=.27$). The pooled standard deviation was 1.19 and the effect size was .53 SD. The latter effect size was in the range of clinical relevance.

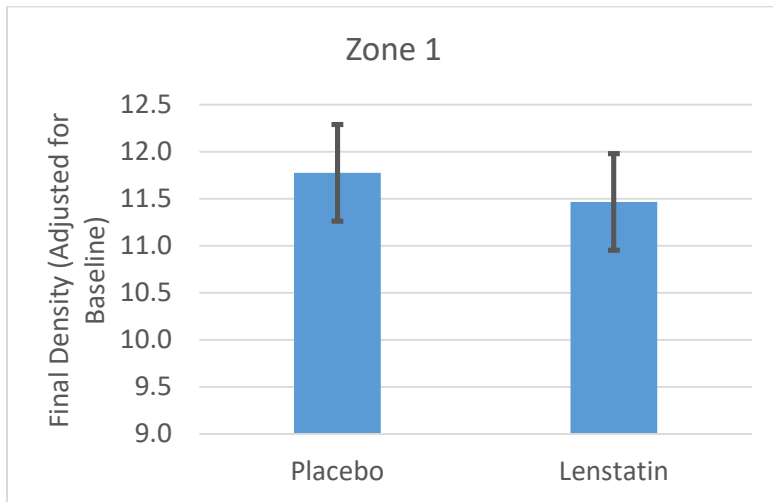


Figure 3. Comparison mean nuclear density between Lenstatin and placebo in Zone 1.

Error bars represent 84% Confidence Interval ($\pm 1.53 \times SE$)

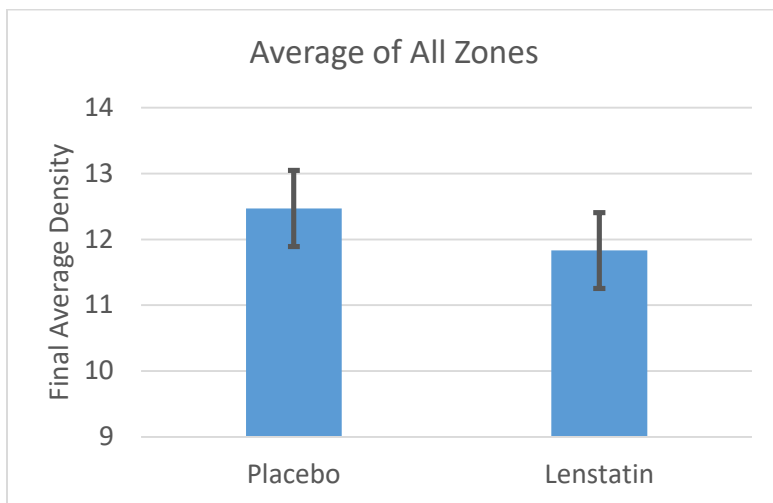


Figure 4. Comparison mean nuclear density between Lenstatin and placebo for Average of All Zones.

Error bars represent 84% Confidence Interval ($\pm 1.53 \times SE$)

ANCOVA was used to analyze final Zone 1 adjusted mean densities and final Average All Zones adjusted mean densities separately for participants with baseline densities less than 11% summarized in Table 1.

| | % Baseline Density | | Mean | SE | n | F | p | Effect Size |
|---------|--------------------|-----------|--------|-------|---|------|------|-------------|
| Zone 1 | <11 | Placebo | 10.91 | 0.41 | 8 | 1.22 | 0.30 | -0.63 |
| | | Lenstatin | 10.18 | 0.52 | 5 | | | |
| Average | <11 | Placebo | 11.357 | 0.758 | 7 | 1.79 | 0.21 | -0.84 |
| | | Lenstatin | 9.675 | 1.003 | 4 | | | |

Table 1. Comparison of Placebo to Lenstatin with baseline density < 11%. The F and p values are from the ANCOVA comparison of groups after adjusting for baseline differences. Negative effect sizes reflect a smaller increase in density for Lenstatin relative to the Placebo control.

A baseline density less than 11% yielded a clinically relevant effect size for both Zone 1 density and the Average All Zones density with Lenstatin showing less growth in density. Chance could not be ruled out ($p > 0.2$) because, at least in part, the study was considerably underpowered in terms of sample size. A study that is powered to detect an effect size of at least 0.5SD at 80% power and has a baseline covariate (correlated $r = .8$ with follow-up) would require 23 subjects per group.

Additional data was collected on each study participant which is not contained in detail in this report. Participants were queried on the number of servings of uncooked leafy green vegetables consumed per week (greater or less than seven), the presence or absence of diabetes (IDDM, NIDDM, or none) and whether air, gas, or neither were infused during the vitrectomy. In the few situations in which there was data on these variables of sufficient size to allow meaningful statistical analysis, there was no significant difference between baseline and end point cataract densities in Zone 1 and Average All Zones between Lenstatin and placebo.

DISCUSSION / CONCLUSIONS

We have demonstrated a novel approach to investigating the effects of an interventional agent on cataract progression using an objective, quantitative, and reproducible measurement of nuclear cataract density. In addition, we have selected a study population in which cataract progression is expected to occur more predictably and rapidly than in age-related senile cataract, allowing a shorter window of observation to detect potentially meaningful changes by an interventional agent in retarding cataract progression.

Clinically relevant effect sizes were observed, particularly in participants with initial nuclear densities less than 11% and densities and graded 0 or 1 density by the PNS module, suggesting there may be more effect when initial cataract development is minimal.

The main limitation of the study is that it was underpowered in sample size. Future studies which are larger could be undertaken which could more effectively examine the observed findings of clinical relevance. A larger sample size would also more effectively isolate the contribution of other factors identified herein, such as dietary intake of antioxidants, the presence or absence of diabetes, and whether air or gas infusion performed at the time of vitrectomy play a role in the observed rates of cataract progression between interventional agent and placebo.

Another limitation of interpreting the study is despite the fact that age-related senile nuclear cataract and post-PPV nuclear cataract are morphologically identical, they are fundamentally different in causation, and as a consequence, an observed inhibitory effect by an interventional agent on post-PPV cataract may not be translatable to the senile form. Nonetheless, the advantage of studying nuclear cataract progression with quantitative analysis over a relatively short period of time relative to the normal time frame of nuclear cataract progression makes this a worthwhile investigation.

ADVERSE REACTIONS

There were no adverse effects reported by any participant during the duration of the study.

REGISTRATION

This clinical trial is registered at ClinicaTrials.gov, Identifier Number NCT02131194. It was conducted with the approval and under the supervision of the Institutional Review Board of New Hanover Regional Medical Center, Wilmington, NC and was compliant with National Institute of Health policies insuring the protection of human research subjects.

FUNDING

The study was funded in by Lenstatin™ LLC, a limited liability company. The Principal Investigator of the study, Scott W. Tunis MD FACS, is the majority owner of Lenstatin™ LLC. There existed a potential conflict of interest in that the majority owner of Lenstatin™ LLC is also the Principal Investigator of the study. The design of the study was intended to nullify any potential conflict of interest that the Principal Investigator might have had in the outcome of the study.

PARTICIPANT COMPENSATION

Participants were compensated \$500 for completion of the study. The intent of reimbursement was not to create a financial incentive to participate, which might otherwise unfairly bias the participant pool based on economic status, but rather, to reimburse participants for the costs of travel and time away from work.

TEXT REFERENCES

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