AMERICAN ACADEMY OF OPHTHALMOLOGY®

Subthreshold Nanosecond Laser Intervention in Age-Related Macular Degeneration

The LEAD Randomized Controlled Clinical Trial

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Purpose: There is an urgent need for a more effective intervention to slow or prevent progression of age-related macular degeneration (AMD) from its early stages to vision-threatening late complications. Subthreshold nanosecond laser (SNL) treatment has shown promise in preclinical studies and a pilot study in intermediate AMD (iAMD) as a potential treatment. We aimed to evaluate the safety of SNL treatment in iAMD and its efficacy for slowing progression to late AMD.

Design: The Laser Intervention in Early Stages of Age-Related Macular Degeneration (LEAD) study is a 36-month, multicenter, randomized, sham-controlled trial.

Participants: Two hundred ninety-two participants with bilateral large drusen and without OCT signs of atrophy.

Methods: Participants were assigned randomly to receive Retinal Rejuvenation Therapy (2RT®; Ellex Pty Ltd, Adelaide, Australia) SNL or sham treatment to the study eye at 6-monthly intervals.

Main Outcome Measures: The primary efficacy outcome was the time to development of late AMD defined by multimodal imaging (MMI). Safety was assessed by adverse events.

Results: Overall, progression to late AMD was not slowed significantly with SNL treatment compared with sham treatment (adjusted hazard ratio [HR], 0.61; 95% confidence interval [CI], 0.33−1.14; $P = 0.122$). However, a post hoc analysis showed evidence of effect modification based on the coexistence of reticular pseudodrusen (RPD; adjusted interaction $P = 0.002$), where progression was slowed for the 222 participants (76.0%) without coexistent RPD at baseline (adjusted HR, 0.23; 95% CI, 0.09−0.59; $P = 0.002$), whereas an increased progression rate (adjusted HR, 2.56; 95% CI, 0.80−8.18; $P = 0.112$) was observed for the 70 participants (24.0%) with RPD with SNL treatment. Differences between the groups in serious adverse events were not significant.

Conclusions: In participants with iAMD without MMI-detected signs of late AMD, no significant difference in the overall progression rate to late AMD between those receiving SNL and sham treatment were observed. However, SNL treatment may have a role in slowing progression for those without coexistent RPD and may be inappropriate in those with RPD, warranting caution when considering treatment in clinical phenotypes with RPD. Our findings provide compelling evidence for further trials of the 2RT® laser, but they should not be extrapolated to other short-pulse lasers. *Ophthalmology 2019;126:829-838 © 2018 by the American Academy of Ophthalmology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).*

See Commentary on page 839.

Supplemental material available at www.aaojournal.org.

In the last decade, advances in the treatment of the neovascular form of late age-related macular degeneration (AMD) with intraocular injections of anti−vascular endothelial growth factor have reduced vision loss from this complication dramatically.[1] However, delivering this treatment has imposed an enormous financial burden on health systems worldwide because of the need for ongoing repeated treatment at frequent intervals for an ever-increasing number of patients. Furthermore, long-term visual benefits are not always maintained despite treatment, with vision loss continuing to occur through atrophy or scar formation.[2]

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Currently, no treatment exists for the other late AMD complication of atrophy, where progressive degeneration and death of the outer retinal cells—the photoreceptors and retinal pigment epithelium (RPE)—occurs. Apart from dietary supplements, such as the Age-Related Eye Disease Study formulations, for subsets of individuals with AMD[3,4] and general lifestyle modification,[5] there is no specific intervention that prevents or slows progression from earlier, asymptomatic stages of AMD to the visually devastating complications of late AMD. As such, there is an urgent need for an effective intervention to slow or prevent the progression of the disease in its early stages.

The early, asymptomatic stages of AMD are diagnosed clinically by the presence of drusen—focal collections of extracellular lipid-rich waste material that accumulate between the RPE basal lamina and the inner collagenous layer of Bruch's membrane (BM). The size and extent of drusen in the macula have been shown to increase the risk of disease progression,[6] where patients with large drusen (>125-μm diameter) are considered to have intermediate AMD (iAMD). A pathologic hallmark of AMD and key component of its pathogenesis is a thickened, structurally and molecularly altered BM, with increased deposition of extracellular matrix (ECM) deposits and lipids, all resulting in a less permeable membrane. These changes result in a barrier to the transport of nutrients to the retina and removal of waste across BM to the choroid, which in turn contributes to RPE and photoreceptor degeneration.[7] OCT imaging techniques recently have revealed debris located above the RPE, or subretinal drusenoid deposits, that are more prevalent than previously appreciated.[8] Clinically, these deposits are called reticular pseudodrusen (RPD) and are considered important in disease pathogenesis because they are present frequently in late-stage disease.[9,10] Although their cause is not well understood, they are thought to reflect RPE dysfunction[11] and possibly dysregulation of retinal lipid or retinoid metabolism.[12]

As a result of the overwhelming picture of abnormal accumulation of debris in AMD, it has been hypothesized that an intervention that triggers a process to reduce debris accumulation within the outer retina could slow AMD progression effectively. In 1971, it was observed serendipitously that drusen regression occurred after thermal (continuous-wave) laser photocoagulation to the retina.[13] After this, a series of thermal laser studies in AMD were conducted.[14] A Cochrane review of these studies concluded that although drusen regression did occur, there was no reduction in progression to late AMD, nor any increase, as initially observed.[15] Energy from the thermal burn is absorbed by the melanin in RPE cells, elevating local temperature and leading to coagulative necrosis at the level of RPE, BM, and choroid. However, the mechanism by which it induces drusen regression remains unknown. Recently, short-pulse lasers that can deliver subthreshold (nonvisible laser spot) energy levels have been developed potentially to harness the positive effects of laser treatment on the RPE by seeking to induce debris removal while minimizing collateral damage to neighboring tissues.[16–19]

The effect of a short-pulse, nanosecond laser delivering a speckled-beam profile at subthreshold energy levels has been investigated using the retinal rejuvenation therapy laser device 2RT® (Ellex Pty Ltd, Adelaide, Australia). In an in vitro study,[19] this laser induced RPE migration and an increase in matrix metalloproteinases (MMPs) involved in ECM turnover. In an animal model with a thickened BM (ApoE-null mouse), application of this laser treatment resulted in a significant reduction in BM thickness and upregulation of gene expression for a range of genes involved in ECM turnover.[20] In AMD patients, a single application of nanosecond laser resulted in a reduction in drusen load[21] without any evidence of damage to overlying photoreceptors.[20] These preliminary results warranted further investigation, and as such, a randomized controlled clinical trial of subthreshold nanosecond laser (SNL) in iAMD was undertaken to determine if this novel intervention could reduce progression to late AMD.

The Laser Intervention in Early Stages of Age-Related Macular Degeneration (LEAD) study is a world-first, 36-month, investigator-initiated, multicenter, double-masked, randomized, controlled, medical device clinical trial. The LEAD study was designed as a proof-of-concept study to investigate the efficacy and safety of SNL treatment in patients with bilateral large drusen. The primary objective was to demonstrate that SNL treatment at 6-month intervals over 36 months slows disease progression to endpoint late AMD compared with sham treatment, with late AMD defined using novel multimodal imaging (MMI) techniques, such as OCT, fundus autofluorescence, and near-infrared imaging.[22,23]

Methods

Study Design and Participants

The LEAD study was a multicenter, randomized, sham-controlled clinical trial conducted at 6 sites, 5 in Australia and 1 in Northern Ireland. The coordinating center and sponsor was the Centre for Eye Research Australia and the study is registered with the Australian New Zealand Clinical Trials Registry (identifier, ACTRN12612000704897) and clinicaltrials.gov (identifier, NCT01790802). This study was conducted according to the International Conference on Harmonization Guidelines for Good Clinical Practice and the tenets of the Declaration of Helsinki, with the protocol approved at all sites by the local institutional review boards. All study participants provided written informed consent. An independent and masked endpoint adjudication committee determined the development of all cases of late AMD, and an independent data and safety monitoring committee provided oversight and reviewed the safety profile of the study.

The full description of the LEAD study design and baseline participant characteristics were published previously,[24] and a complete list of the inclusion and exclusion criteria is presented in Supplement 1 (available at www.aaojournal.org). In brief, eligible participants were 50 years of age or older with a diagnosis of AMD and having at least 1 druse more than 125 μm in diameter, within 1500 μm from the fovea, in both eyes, as determined on color fundus photography. These phenotypic characteristics meet the definition of iAMD.[6] Individuals with late AMD, either neovascular AMD (nAMD) or drusen-associated atrophy detected on MMI, were excluded.[22,23] The full MMI definition of late AMD is available in Table 1. All participants were required to have a best-corrected visual acuity (BCVA) of 20/40 or better (≥69 letters read) in both eyes.

Randomization and Masking

The study eye was assigned to the worse eye defined by BCVA, or if identical, on other functional criteria.[24] After baseline measurements, eligible participants gave informed consent after consultation with the site's treating clinician, then were stratified by smoking status (never smoked vs. present or past smoker) and allocated randomly to receive SNL or sham treatment in a 1:1 ratio according to a computer-generated list with variable block sizes (4 to 6; generated by a study project manager). Allocations were placed in sealed, consecutive envelopes, with a separate set of sequentially numbered envelopes for each participating center, resulting in stratification by center. The participants and study staff, other than the treating clinician, remained masked to treatment allocation throughout the study. No inadvertent unmasking was recorded.

Procedures

Subthreshold nanosecond laser treatment was performed using the 2RT® cases, which is a 532-nm Q-switched neodymium-doped yttrium−aluminum−garnet laser with 3-nanosecond pulse duration and a speckled-beam profile. Subthreshold laser spots of 400 μm in diameter were delivered at 12 locations on the retina: 6 in an arc just below the superior vascular arcade and 6 in an arc just above the inferior arcade. Test spots were used to determine the threshold for each individual.[24] For sham treatment, the exact laser procedure was performed, except that short bursts of light from the retinal illumination system on the laser device were used instead of the laser beam. Treatments were performed on the day of randomization, and each participant was reviewed at follow-up visits that occurred every subsequent 6 months (±2 weeks) from randomization up to 36 months. If a participant remained eligible, re-treatment occurred within a period of 4 weeks after the follow-up visits up to the 30-month visit, with no treatment performed at the 36-month visit. The description of study procedures and schedule of assessments are published previously, with the schedule of assessments available in Supplement 1.[24] Briefly, demographic data and ocular and systemic medical history were collected from each participant at baseline. BCVA measurement, MMI, and clinical examination were performed at baseline, at each 6-month follow-up visit, and at unscheduled visits if a participant reported new ocular symptoms (to determine the development of late AMD).

The Image Reading Centre based at Centre for Eye Research Australia assessed all MMI for enrollment and re-treatment eligibility as well as signs indicative of late AMD. Patients flagged as potentially having late AMD develop were adjudicated by the endpoint adjudication committee. Those who were determined as having late AMD in the study eye were ineligible for re-treatment, but remained in the study for observation. However, participants in whom late AMD developed only in the nonstudy eye continued to receive their allocated treatment in the study eye. The Image Reading Centre also graded for the presence of RPE pigmentary abnormalities on color fundus photography and RPD on MMI (detailed definition in Supplement 1).

Outcomes

The primary efficacy outcome was the time to development of late AMD in the study eye within a 36-month follow-up period. Late AMD was defined as either nAMD or drusen-associated atrophy as determined on MMI[22,23] (described in Table 1). Secondary exploratory outcomes (not reported here because they mainly inform the mechanism of laser action, not progression of disease) included time to late AMD development in the nonstudy eye and change in drusen volume and visual function (based on BCVA, low luminance visual acuity, and microperimetric sensitivity) over time in both the study and nonstudy eye. Safety outcomes included (serious) adverse events.

Statistical Analyses

The sample size calculation of the LEAD study was based on an estimated hazard ratio of 0.5 for participants receiving the SNL treatment compared with those receiving sham treatment with 80% power ($\alpha = 0.05$, 2-tailed) using Cox regression. No previous studies have used an MMI-based eligibility criterion nor definitions of late AMD to help inform the sample size required. In this proof-of-concept study, we estimated that 26% of participants in the sham treatment group would progress to late AMD in the study eye after 3 years. Allowing for 10% attrition and 2% incomplete block allocation, we estimated that 141 participants would be required in each treatment arm to observe a total of 66 endpoint of late AMD in 36 months.

Efficacy analyses were based on the intention-to-treat set that included all randomized participants analyzed according to their randomly allocated treatment. A Cox proportional hazards model was used to estimate the treatment effect for progression to late AMD in the study eye, accounting for the stratification factors of smoking status and study site (via covariate adjustment) as well as the potential confounders of baseline age (as a continuous measure), gender, intake of Lutein-Vision or Macu-Vision (Blackmores Limited, Warriewood, Australia) at baseline (yes vs. no for each), and presence of RPD and pigmentary abnormalities (definitely present vs. absent or questionable) in a fully adjusted model as specified a priori[24] (referred to as *adjusted*). The same model accounting only for the stratification factors was also used to estimate the treatment effect without adjustments for the other potential confounders (referred to as *unadjusted*). Time at risk commenced from the date of randomization, which coincided with the date of first treatment. For participants in whom late AMD did not develop, those who were lost to follow-up or died during the study were censored at the time of their last assessment or censored at 1 day if they did not attend a postrandomization visit. Participants who completed the study were censored at the 36-month visit. The proportional hazard assumption was evaluated via statistical tests of Schoenfeld residuals from the Cox model and through visual inspection of $\log(-\log[S(t)])$ plots. The 2 phenotypic forms of late AMD, namely nAMD and drusen-associated atrophy, were also analyzed separately with the same analysis as used for the primary outcome measure of late AMD.

The 2RT® laser, with its speckled-beam profile, causes selective RPE loss.[17,20] Therefore, it is biologically plausible that its impact and therapeutic effect could differ depending on the degree of RPE dysfunction, as indicated by the clinical phenotype of RPE pigmentary abnormalities,[25] RPD, or both.[11] Therefore, a post hoc analysis was performed to investigate whether treatment effect on the primary outcome differed according to the presence of coexistent RPD or pigmentary abnormalities at baseline via fit of an interaction term between treatment and RPD or pigmentary abnormalities in the Cox model. Contrasts of the interaction effect between treatment and study site were assessed to explore heterogeneity of treatment effects across sites.

We performed secondary analyses for the per-protocol set that excluded participants with a clinically significant protocol deviation (defined as missing completely or receiving treatment outside of the 1-month treatment window at more than 1 visit) or that did not receive at least 5 of the 6 treatments in this study for other reasons (e.g., lost to follow-up). Safety was evaluated in all randomized participants who received at least 1 treatment and was

Table 1. Definition of Late Age-Related Macular Degeneration

Neovascular Age-Related Macular Degeneration
Lesions showing hyperfluorescence in the early phases on FA, with late leakage that obscures its boundaries. Indocyanine green angiography was performed when clinically applicable. If FA was not performed, subretinal hemorrhage with corresponding OCT features consistent with neovascular AMD was accepted.
Note: Subretinal fluid detected on OCT imaging that did not show corresponding leakage on FA or polyps on ICGA was not considered as neovascular AMD, but instead as nonexudative detachment of the neurosensory retina. Participants with such features continued receiving treatments as per protocol and underwent re-imaging if further signs indicative of neovascular AMD developed.
Drusen-Associated Atrophy (Any of the Following)
CFP: any area >175 μm in diameter of partial or complete RPE hypopigmentation with visible underlying large choroidal vessels that was either roughly round or oval and showed sharp margins (geographic atrophy).
Fundus autofluorescence: any sharp-edged, roughly round or oval hypoautofluorescent area that corresponded with an area of hypopigmentation on CFP.
OCT: (1) drusen-associated loss of the RPE, ellipsoid zone, external limiting membrane, and outer nuclear layer accompanied by increased signal transmission beneath Bruch's membrane; or (2) drusen-associated subsidence of the OPL and inner nuclear layer toward a region of RPE disruption and presence of a hyporeflective wedge-shaped band in the OPL (nascent geographic atrophy).

AMD = age-related macular degeneration; CFP = color fundus photography; FA = fluorescein angiography; ICGA = indocyanine green angiography; MMI = multimodal imaging; OPL = outer plexiform layer; RPE = retinal pigment epithelium.

analyzed according to the received treatment. The number and proportion of patients with adverse events (ocular and general) in each treatment group and study eye were determined. *P* values are reported as 2-sided without adjustment for multiple testing. All analyses were conducted using STATA/SE software version 15.1 for Windows (StataCorp, College Station, TX).

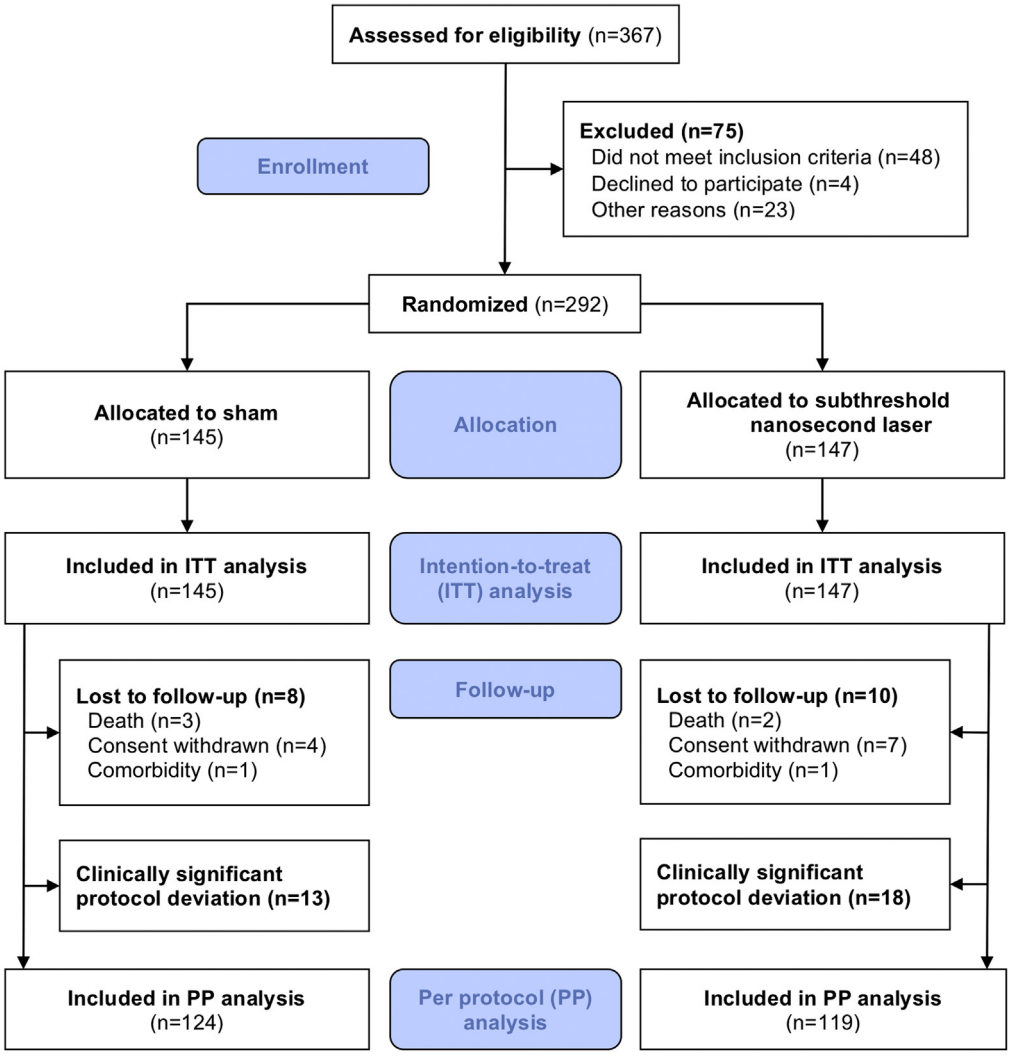

Figure 1. Flowchart showing the study profile. ITT = intention to treat; PP = per protocol.

Table 2. Demographics and Baseline Ocular Characteristics

	Subthreshold Nanosecond Laser Treatment (n = 147)	Sham Treatment (n = 145)
Demographics		
Age (yrs), mean (SD)	70.3 (7.0)	69.8 (8.1)
Gender (female), no. (%)	103 (70.1)	112 (77.2)
Ethnicity (white), no. (%)	134 (91.2)	128 (88.3)
Smoking history, no. (%)		
Never	77 (52.4)	77 (53.1)
Past or current	70 (47.6)	68 (46.9)
Macu-Vision* intake (yes), no. (%)	50 (34.0)	45 (31.0)
Lutein-Vision† intake (yes), no. (%)	9 (6.1)	24 (16.6)
Study eye ocular characteristics		
BCVA (number of letters), median (25th–75th percentile)	83 (80–87)	84 (79–88)
Pigmentary abnormalities, no. (%)		
Definitely present	46 (31.3)	51 (35.2)
Absent or questionable	101 (68.7)	94 (64.8)
Reticular pseudodrusen, no. (%)		
Definitely present	35 (23.8)	35 (24.1)
Absent or questionable	112 (76.2)	110 (75.9)

BCVA = best-corrected visual acuity; SD = standard deviation.
*Active ingredients of Macu-Vision include vitamin C, vitamin E, zinc oxide, and cupric oxide.
†Active ingredients of Lutein-Vision include lutein, selenomethionine, zeaxanthin, and omega-3 triglycerides fish oil.

Results

Of the 367 participants screened for eligibility at 6 clinical sites (between July 26, 2012, and April 30, 2015), 292 participants were randomized to receive SNL (n = 147) or sham (n =145) treatment and were included in the intention-to-treat set (Fig 1). All randomized participants received at least 1 treatment, and thus all were included in the safety analysis. Among all randomized participants, 274 (93.8%) attended the 36-month follow-up visit.

Ten participants in the SNL treatment group and 8 participants in the sham treatment group were lost to follow-up. A clinically significant protocol deviation was recorded for 18 participants in the SNL treatment group and 13 participants in the sham treatment group. Therefore, the per-protocol set included 119 participants in the SNL treatment group and 124 participants in the sham treatment group (Fig 1).

Baseline characteristics of the participants were comparable between treatment groups, except for a larger proportion of participants reporting Lutein-Vision intake in the sham treatment group (Table 2). The average age of the participants at baseline was 70 years, and they were predominantly women (73.6%) and of white descent (89.7%), with 47.3% being past or current smokers. In the study eye, the median BCVA was 83 letters read, 33.2% of the participants demonstrated definite RPE pigmentary abnormalities, and 24.0% showed definite RPD. Further details on participant demographics and ocular characteristics at baseline are outlined in our previous publication.[24]

After 36 months of follow-up, 45 randomized participants (15.4%) developed late AMD in the study eye, occurring in 20 participants (13.6%) in the SNL treatment group and 25 participants (17.2%) in the sham treatment group (unadjusted hazard ratio [HR], 0.78; 95% confidence interval [CI], 0.43−1.41; $P = 0.412$; adjusted HR, 0.61; 95% CI, 0.33−1.14; $P = 0.122$; Fig 2).

There were 7 (4.8%) and 5 (3.4%) randomized participants in whom nAMD developed as their first, late AMD endpoint in the SNL and sham treatment groups, respectively (unadjusted HR, 1.37; 95% CI, 0.43−4·31; $P = 0.594$; adjusted HR, 1.21; 95% CI, 0.37−3.93; $P = 0.753$). Thirteen patients (8.8%) demonstrated drusen-associated atrophy as their first, late AMD endpoint in the SNL treatment group compared with 20 patients (13.8%) in the sham treatment group (unadjusted HR, 0.63; 95% CI, 0.31−1.27; $P = 0.194$; adjusted HR, 0.53; 95% CI, 0.25−1.13; $P = 0.101$). Findings from the analyses of the per-protocol set were consistent with those based on the intention-to-treat set (Fig 3; see full description in Supplement 1).

There was strong evidence of significant treatment effect modification according to the presence of coexisting RPD at baseline (adjusted interaction $P = 0.002$). Among the 222 participants (76.0%) without RPD in the study eye at baseline, the rate of progression to late AMD was reduced in the SNL treatment group compared with the sham treatment group (adjusted HR, 0.23; 95% CI, 0.09−0.59; $P = 0.002$; Fig 4). Conversely, among the 70 participants (24.0%) with RPD in the study eye at baseline, there was an increased rate of progression to late AMD for the SNL treatment group compared with the sham treatment group (adjusted HR, 2.56; 95% CI, 0.80−8.18; $P = 0.112$; Fig 4). There was no evidence of a significant treatment effect modification based on the presence of pigmentary abnormalities at baseline (adjusted interaction $P = 0.251$) or by study site (contrast $P = 0.777$). These findings regarding effect modification were consistent in the analysis of the per-protocol analysis set (see supplemental analyses in Supplement 1; available at www.aaojournal.org). There was also no evidence of a violation of the proportional hazards assumption for any of the models presented.

There were no device-related serious adverse events in this study. However, the treating ophthalmologist noted a deep retinal hemorrhage at the same location where the laser was delivered in the SNL treatment group in 10 participants (6.8%) on 11 occasions, and no such cases were present in the sham treatment group. These hemorrhages resolved in all patients without any untoward

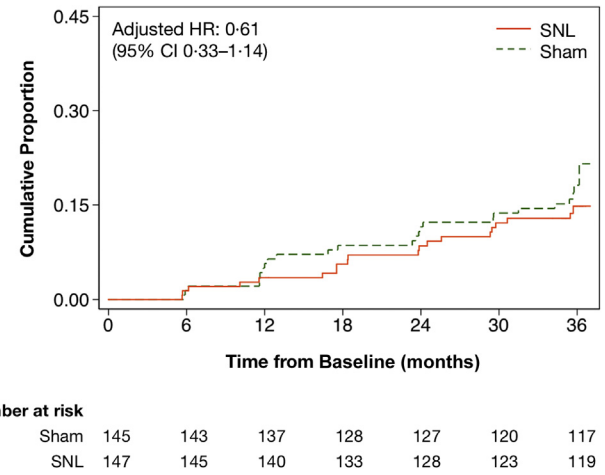

Figure 2. Kaplan-Meier failure plot showing progression to late age-related macular degeneration for the subthreshold nanosecond laser (SNL) and sham treatment groups separately. Estimated hazard ratio (HR) is from a model adjusted for baseline covariates in the intention-to-treat set (n = 292). CI = confidence interval.

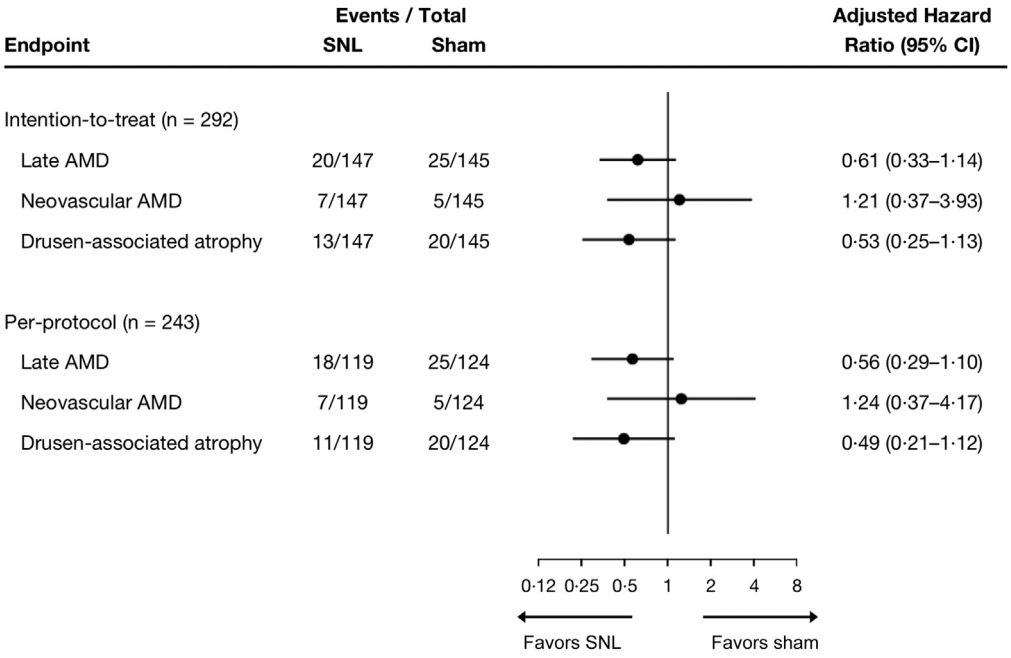

Figure 3. Forest plot of treatment effect on late age-related macular degeneration (AMD) and its 2 clinical phenotypes (neovascular AMD and drusen-associated atrophy). The forest plot displays the estimated effect of subthreshold nanosecond laser (SNL) compared with sham treatment on the rate of progression to late AMD from models adjusted for baseline covariates, as well as for the rate of progression to neovascular AMD and drusen-associated atrophy as their first, late AMD endpoint separately. CI = confidence interval.

sequelae, such as the development of neovascularization at the laser location. In addition, 6 participants (4.1%) and 1 participant (0.7%) reported persistent after-images (visible for more than 1 day) after SNL and sham treatment, respectively. Only 1 participant in the SNL treatment group reported its persistence to the degree that no further treatments were performed. Otherwise, there was no difference between the 2 groups in the proportion of participants with systemic or ocular (in the study eye) serious adverse events or adverse events, unrelated to the progression to late AMD (Table 3). Five participants died, 2 (1.4%) in the SNL treatment group and 3 (2.1%) in the sham treatment group.

Discussion

One in 8 people older than 50 years in Western countries have drusen[26] and are at risk of vision-threatening late AMD developing. We report herein the results of a first sham-controlled randomized clinical trial of a novel nanosecond laser intervention, delivered at subthreshold energy levels, in participants with bilateral large drusen without any baseline MMI signs of late AMD. Overall, the time to late-stage AMD development was not significantly different between those who received SNL or sham treatment over the 3-year trial duration, although the direction of the treatment effect was for a reduction in progression rate for those in the SNL group. From a clinical safety viewpoint, there was no overall significant increase in either form of late-stage AMD, particularly given the initial concerns from thermal laser studies in the 1990s about an increased rate of nAMD.[27–29] Overall, SNL treatment as performed in this study seemed to be safe and not associated with an increase in systemic or non-AMD–related adverse events. However, our post hoc analyses suggested that the effect of the SNL treatment on progression to late AMD is modified by the clinical phenotype of iAMD, specifically by the baseline presence of RPD.

We observed a 4-fold reduction in the rate of progression to late AMD with the SNL treatment compared with sham treatment for eyes without RPD at baseline, but a more than 2-fold increase in progression rate for eyes with RPD at baseline, considering the adjusted time-to-event analyses. The same effect modification by the presence of RPD was not observed when considering the status of RPE pigmentary abnormalities at baseline. These results, which require confirmation from further studies, suggest that SNL treatment has the potential to reduce the rate of progression to late AMD in eyes without RPD, but this intervention currently should be considered with caution in eyes with RPD because of the potential for this treatment to increase the rate of progression for such eyes.

The SNL used in this study (the 2RT®) uses the principle of selective photothermolysis to restrict injury to the RPE (sparing the overlying retina) and to induce beneficial RPE changes in eyes with the early stages of AMD.[17,20] Indeed, ultrastructural analysis of mouse retinae has revealed specific changes in melanosomes within the RPE in areas that received SNL treatment compared with adjacent areas, confirming that SNL has a targeted action on the RPE.[16] Enzymes implicated in ECM turnover, MMP-2 and MMP-9, are known to be increased in cultured human RPE cells after nanosecond laser treatment.[19] In aged ApoE-null mice (which exhibit thickened BM), a single nanosecond treatment led to a significantly thinner BM at 3 months and also an increase in MMP-2 and MMP-3 gene expression levels,

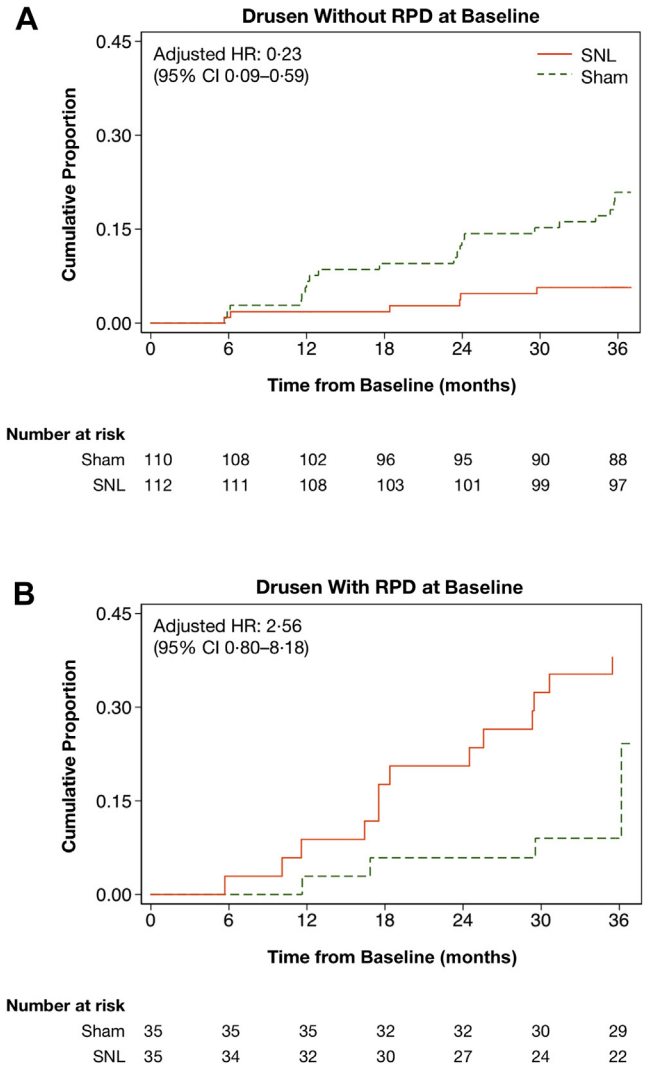

Figure 4. Kaplan-Meier failure plot showing progression to late age-related macular degeneration for the subthreshold nanosecond laser (SNL) and sham treatment groups separately: (**A**) for participants without coexisting reticular pseudodrusen (RPD) at baseline (n = 222) and (**B**) for participants with coexisting RPD at baseline (n = 70). Estimated hazard ratios (HRs) are from models adjusted for baseline covariates in the intention-to-treat set, and the interaction test was statistically significant ($P = 0.002$). CI = confidence interval.

Table 3. Number and Proportion of Patients with Adverse Events

	Subthreshold Nanosecond Laser Treatment (n = 147)	Sham Treatment (n = 145)
Definitely related ocular adverse events		
Participants reporting 1 or more adverse events	15 (10.2)	1 (0.7)
After-images (visible for more than 1 day)	6 (4.1)	1 (0.7)
Retinal hemorrhage	10 (6.8)	0 (0.0)
Possibly related ocular adverse events		
Participants reporting 1 or more adverse events	27 (18.4)	24 (16.6)
Epiretinal membrane	1 (0.7)	4 (2.8)
Symptomatic PVD or floaters	9 (6.1)	5 (3.4)
Ocular discomfort following treatment	10 (6.8)	11 (7.6)
Cataract requiring surgery	1 (0.7)	2 (1.4)
Migraine (visual aura)	4 (2.7)	5 (3.4)
Other	6 (4.1)	3 (2.1)
Other adverse events		
Unrelated ocular adverse events	92 (62.6)	80 (55.2)
Nonocular adverse events	103 (70.1)	110 (75.9)
Serious adverse events		
Participants reporting one or more serious adverse events	56 (38.1)	50 (34.5)
Cardiovascular or cerebrovascular disorders	14 (9.5)	13 (9.0)
Death (unknown cause)	0 (0.0)	1 (0.7)
Infections	8 (5.4)	2 (1.4)
Injury and procedural complications	10 (6.8)	9 (6.2)
Neoplasms (benign and malignant)	7 (4.8)	8 (5.5)
Nervous system disorder	3 (2.0)	3 (2.1)
Respiratory disorder	5 (3.4)	3 (2.1)
Surgery and medical procedures	7 (4.8)	5 (3.4)
Other (medical)	23 (15.6)	22 (15.2)

PVD = posterior vitreous detachment.
Data are number of participants (%), and ocular adverse events refer to those associated with the study eye.

as well as a number of other ECM genes, including collagen, laminin, and components of elastic fibers and several integrin subunits.[20] Overall, these preclinical studies suggest that SNL may slow the degenerative process in AMD by modulating RPE-mediated turnover of Bruch's membrane,[7] so as to reduce outflow resistance across BM, to improve RPE health, and to slow progression to late AMD, as we have reported here.

However, because the SNL treatment depends on selective loss and subsequent healing of the RPE, there may be a stage of AMD disease at which RPE integrity is so greatly compromised as to render the treatment ineffective. This could occur in a stage of the disease where RPD can be detected. Reticular pseudodrusen are deposits that are distinct from conventional drusen, consisting of a variety of lipid, photoreceptor debris, and immune cell fragments.[9,10] The subretinal drusenoid debris has similarities with respect to its location, appearance, and effect on dark adaptation to the earliest lesions that form in patients manifesting vitamin A deficiency or those carrying mutations in genes encoding critical components of the retinoid cycle such as retinol dehydrogenase (e.g., retinitis fundus albipunctatus).[30,31] This debris also has similarities to the earliest lesions that form in animal models and patients carrying mutations in the scavenger receptor merTK,[32] a receptor that is critical for removal of spent photoreceptors by the RPE. Evaluation of RPE melanin with near-infrared fundus autofluorescence imaging suggests that RPE dysfunction is more severe in RPD areas than in areas free of RPD.[33] Moreover, histopathologic analysis of human eyes shows significant morphologic defects within the RPE at sites adjacent RPD lesions compared with RPD-free regions.[11] In the context of

this study, therefore, it is biologically plausible that the SNL treatment with the 2RT® laser could hasten disease progression in eyes with RPD, which already have significant RPE dysfunction. Conversely, it is also biologically plausible that SNL treatment indeed could be beneficial in AMD eyes without this degree of RPE compromise. Therefore, the results of this study suggest that caution is warranted when considering treating iAMD cases with RPD until further studies are performed.

The LEAD study is the first randomized controlled clinical trial of an intervention for iAMD using MMI techniques, particularly OCT, to detect and define late AMD. With MMI, the atrophic complications of AMD in particular could be detected at a much earlier time point than on color fundus photography, which has been used historically,[23] thus providing greater power to detect treatment effects. However, these earlier anatomic signs are not yet accepted by regulatory authorities, and as such currently cannot be used to gain marketing approval. Typically, regulatory authorities have accepted visual acuity as the primary outcome for AMD treatment trials. However, these trials have involved treating late AMD, where vision is lost without intervention. In the earlier stages of AMD, visual acuity is unaffected and does not provide a useful efficacy outcome measure. In addition, MMI was also used to exclude participants who already showed these signs of late AMD at baseline, thus ensuring a homogeneous iAMD cohort at a time point before any atrophy had developed. The results of this trial need to be interpreted taking into consideration that only iAMD participants with bilateral large drusen without any MMI-detected signs of late AMD were included in this study. Our study does not provide information on whether SNL treatment is beneficial or harmful in participants with late AMD, especially those with early signs of drusen-associated atrophy detected using MMI.

If subsequently validated, the findings of this study will be highly clinically meaningful. Treatment with SNL could reduce the rate of progression to late AMD—the most common cause of vision loss in elderly populations—in iAMD eyes without RPD, which make up most patients (76%) in our study. However, the current evidence raises some concern for its use of SNL treatment for iAMD eyes with RPD. The importance of the RPD phenotype in this study adds to the growing body of evidence about their potential role in AMD.[12,34] As such, all clinicians involved in the management of AMD would benefit from becoming familiarized with the detection of RPD. Our findings also highlight the need for further investigations into the underlying pathologic processes of RPD. Future studies are also needed to validate the apparent beneficial effect in eyes without RPD to investigate whether treatment with larger numbers of laser shots could reduce the rate of progression further and to determine if repeated treatments (compared with a single treatment) are necessary. However, note that our results cannot be extrapolated to other subthreshold short-pulse lasers because their mechanism of action may be entirely different. The findings of this study also provide the crucial information about effect size and natural history progression rates that will inform future trial designs for SNL treatment and other interventions aiming to slow progression to late AMD and wishing to use MMI-based definitions of late AMD. If the beneficial effect of the SNL treatment in eyes without RPD can be confirmed, it will have major implications for millions of people worldwide with the earliest stages of AMD.

The strengths of this study include the large sample size achieved for this proof-of-concept study, the low attrition rate, and the low proportion of participants with a clinically significant protocol deviation. A limitation of this study is that it was not originally designed or powered to demonstrate effect modification by the coexistence or not of RPD with drusen. However, this analysis was performed as a clinical imperative to investigate any potential harm of this intervention, based on an increased understanding of the disease biology, particularly relating to RPD and the mechanism of action of the 2RT® laser that made this effect modification biologically plausible.

In conclusion, SNL treatment using the 2RT® laser did not significantly reduce the overall rate of progression to late AMD compared with sham treatment in iAMD patients with bilateral large drusen without any MMI signs of late AMD at baseline. However, post hoc analyses revealed a potential beneficial effect of SNL treatment in eyes without RPD at baseline that warrants confirmation in a future trial. It also revealed that SNL treatment may increase the rate of progression to late AMD in eyes with RPD at baseline, thus highlighting the current need for caution if considering the use of SNL treatment in such eyes. These results cannot be extrapolated to other subthreshold short-pulse lasers because their mechanism of action may be entirely different.

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Footnotes and Financial Disclosures

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Abbreviations and Acronyms:
AMD = age-related macular degeneration; **BCVA** = best-corrected visual acuity; **BM** = Bruch's membrane; **CI** = confidence interval; **ECM** = extracellular matrix; **HR** = hazard ratio; **iAMD** = intermediate age-related macular degeneration; **MMP** = matrix metalloproteinase; **nAMD** = neovascular age-related macular degeneration; **LEAD** = Laser Intervention in Early Stages of Age-Related Macular Degeneration; **MMI** = multimodal imaging; **RPD** = reticular pseudodrusen; **SNL** = subthreshold nanosecond laser; **2RT®** = retinal rejuvenation therapy.

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