Supplemental Online Content

Freedman SF, Beck AD, Nizam A, et al; the Infant Aphakia Treatment Study Group. Glaucoma-related adverse events at 10 years in the Infant Aphakia Treatment Study: a secondary analysis of a randomized clinical trial. *JAMA Ophthalmol*. Published online December 17, 2020. doi:10.1001/jamaophthalmol.2020.5664

This supplemental material has been provided by the authors to give readers additional information about their work.

eAppendix. Optic Nerve Head Photography and Retinal Nerve Fiber Layer Imaging Protocol, Statistical Methods and Supplementary Results

Retinal Nerve Fiber Layer and Optic Nerve Head Photography Imaging Protocol

Retinal Nerve Fiber Layer (RNFL) imaging by Spectral Domain Optical Coherence Tomography (SD-OCT)
For the IATS 10-year visit, RNFL measurements of both eyes were taken using the Heidelberg Spectralis OCT (Heidelberg Engineering GmbH, Heidelberg Germany) by a trained OCT imager, with a baseline signal strength of at least 20dB. The highest quality RNFL image was chosen for each study and each fellow eye, by one experienced reader (Allen Beck). We did not manually correct any segmentation of the RNFL, but did choose the scan with the most accurate segmentation and the highest quality from among several scans usually acquired and submitted from the site for grading for any given eye. Most artefact, when present, was related to either movement or segmentation. We did not include any imaging unless the quality and segmentation were adequate to allow quadrant-level analysis by the software. Most of the included images were well segmented. As stated, minimum accepted signal strength was 20 dB or higher. Sample RNFL images for both eyes of an IATS subject with a diagnosis, by IATS criteria described in eTable 1, of glaucoma in the treated eye, are shown in eFigures 4A and 4B. The case report form (CRF) used to record image readings is shown (eFigure 5). The global average reading in microns along with the superior, inferior, superior temporal, superior nasal, inferior temporal, and inferior nasal sector readings were recorded on the CRF. Average and sector readings were compared among study eyes with clinical diagnoses of glaucoma, glaucoma suspect, or neither. Additionally, these same parameters (average and sector readings) were compared between the study and the fellow eyes for all subjects, with the latter serving as the control group. These same readings were compared among study eyes with glaucoma suspect or glaucoma status, and those without a glaucoma-related adverse event. An attempt was made to correlate average RNFL readings with ONH grading, but was unfortunately too limited in numbers to be valid or useful (see manuscript Results).

Optic Nerve Head (ONH) Photographs
Images centered on the ONH were performed on both eyes using a digital fundus camera, and after attempted pupil dilation (with cycloplegic/sympathometic chosen by each site's investigator). The resolution of an ONH photograph was considered acceptable if it clearly showed the ONH margins and vasculature.

ONH photographs were reviewed and graded independently by three ophthalmologists with expertise in pediatric glaucoma (Sharon Freedman, Allen Beck, and David Plager). Reviewers were blinded as to previous clinical determinations of normal, glaucoma suspect, or glaucoma for the treated eye. The treated vs. fellow eye was not labeled in any fashion for the graders. Photographs were graded as unreadable, normal, glaucoma suspect, or glaucoma primarily by comparing the appearance of the study eye to the fellow eye. Sample ONH images for both eyes of an IATS subject are shown in Figures 4A and 4B. The images are of the same study participant for whom RNFL imaging is shown in eFigures 4A and 4B. The treated eye was diagnosed with glaucoma based both on the IATS criteria (eTable 1) and ONH imaging, while the fellow eye was graded as 'Normal' (neither glaucomatous nor glaucoma suspect). The CRF used to record ONH image readings is also shown (eFigure 6). Cup disc asymmetry of 0.2 or greater was the primary determinant of glaucomatous optic neuropathy, along with other classic features such as rim thinning. ONH photographs were not part of the 1-year or 5-year studies or the follow-up glaucoma-related endpoints, [1,2] due to the anticipated difficulty obtaining high quality photographs in very young children, so it was not possible to compare to prior photographs for a change in cupping or disc appearance consistent with glaucomatous optic neuropathy.

Statistical Methods and Selected Supplemental Results

Statistical Methods
The Kaplan-Meier method was used to estimate the cumulative probability of glaucoma and glaucoma + glaucoma suspect over time for all 114 study eyes. Note: in the results, and throughout manuscript, the term 'risk', as it relates to Kaplan-Meier method estimates refers to cumulative probability or cumulative incidence. The bivariate association between the occurrence of glaucoma and glaucoma + glaucoma suspect and treatment group (contact

lens [CL] vs. intraocular lens [IOL]) was assessed by comparing the group-specific Kaplan-Meier curves using the log-rank test, and by estimating the hazard ratio for treatment group using proportional hazards regression. The association between glaucoma and glaucoma + glaucoma suspect and treatment group (CL vs. IOL) as well as baseline covariates age at surgery (age ≤48 days vs. >48 days), presence of persistent fetal vasculature, corneal diameter (≤10 mm vs. >10 mm) and intraocular pressure (≥12 mm Hg vs. <12 mm Hg) were assessed using a multivariable proportional hazards regression model.

One-way fixed effects analysis of variance was used to compare the mean 10-yr IOP, axial and refractive error for eyes with glaucoma, glaucoma suspect or neither diagnosis ('normal'). The group means were compared using the Tukey-Kramer method for pairwise comparisons. This approach was also used to compare mean RNFL quality and thickness measurements across the groups.

Paired t-tests were used to compare the RNFL measurements for treated and fellow eyes. This Analysis was stratified by subject's treated eye glaucoma status (glaucoma, glaucoma suspect and neither). Bonferroni-corrected 99.9% confidence intervals for the paired differences were produced due to the multiple comparisons being performed.

The Kruskal-Wallis test was used to compare the median logMAR visual acuity between the glaucoma, glaucoma suspect and normal groups.

The median logMAR visual acuity was compared for eyes that had surgery at ≤48 days vs. >48 days using the exact Wilcoxon two-sample test.

The association between 10-yr glaucoma status (glaucoma, glaucoma status and normal) and placement of a secondary IOL between 5- and 10-years was assessed using Fisher's Exact test.

The Kappa statistic was calculated to quantify the degree of agreement between the glaucoma diagnoses for the three readers of the optic nerve head images.

One-way fixed-effects analysis of variance was used to compare mean RNFL parameter values for eyes with optic nerve head gradings of 'glaucoma', 'glaucoma suspect' and 'neither'.

Statistical analyses were performed using SAS software version 9.4 (SAS Institute) and R version 3.6.1 (The R Foundation for Statistical Computing). A significance level of 5% (confidence level of 95%) was used in inference-making, except where otherwise noted.

Glaucoma-related adverse events and secondary IOL

Note that secondary IOL implantation prior to the 5-year exam was approved in IATS participants' aphakic eyes in the CL group only for poor adherence to CL wear. Twenty-two eyes had secondary IOL implantation between 5 and 10 years; glaucoma status was not available for two of these eyes. By the 10-year exam, 3 of 20 eyes (15%) were diagnosed as glaucoma, 6 (30%) as glaucoma suspect, and 11 (55%) as neither diagnosis (no significant association between secondary IOL placement and glaucoma status, p=0.3). Two eyes had secondary IOL before 5 years: at the 10-year exam, 1 was a glaucoma suspect, and the other still normal.

Glaucoma-related adverse events and refraction

Comparison of cycloplegic refraction among the study eyes diagnosed as Glaucoma, Glaucoma suspect, or Neither, was impaired by the fact that there was a huge range of refractive errors within each group, especially in the eyes originally assigned to remain aphakic (CL group), because by the 10-year exam, some but not all of these had received secondary IOLs, and most of these were after the age of 5 years, so that original 'refraction' was no longer as relevant because the secondary IOL was targeted to a specific, PI-chosen target refraction. Given those limitations to the data, however, the mean cycloplegic refraction was -1.2 D (±9.8, range -26.5 to 12.3) in eyes with glaucoma (n=23), 0.11 D (±8.1, range -12.5 to 15.6) in eyes with glaucoma suspect (n=20), and -0.43 D (±9.6, range -24.0 to 24.3) in eyes with neither (n=60). The means were not significantly different (eFigure 3).

eTable 1: Definitions of Glaucoma, Glaucoma Suspect and Glaucoma-Related Adverse Event[a]

Term	Definition
Glaucoma	*Glaucoma* was diagnosed in a study eye if the intraocular pressure (IOP) was >21 mmHg with one or more of the following anatomical changes: a) corneal enlargement, b) asymmetrical progressive myopic shift coupled with enlargement of the corneal diameter and/or axial length, c) increased optic nerve cupping defined as an increase of ≥ 0.2 in the cup-to-disc ratio; or d) a surgical procedure was performed for IOP control.
Glaucoma suspect	*Glaucoma suspect* was diagnosed in a study eye if there was either: a) recording of two consecutive IOP measurements above 21 mmHg on different dates after topical corticosteroids had been discontinued without any of the anatomical changes listed above for *Glaucoma*; or b) glaucoma medication was used to control IOP without any of the anatomical changes listed above.
Glaucoma-related adverse event	Glaucoma and glaucoma suspect together (glaucoma+glaucoma suspect).[b]

[a] Modified from Table 1 in Freedman et al, "Glaucoma-related adverse events in the first 5 years after unilateral cataract removal in the Infant Aphakia Treatment Study," JAMA Ophthalmol 133(8):907-14, 2015. [2]

[b] In the statistical analyses, for an eye originally diagnosed as a glaucoma suspect that developed glaucoma, that eye was considered in the glaucoma group when analyzing the glaucoma outcome and in the glaucoma suspect group when analyzing the glaucoma-related adverse event outcome. For all other analyses the eye was considered in the glaucoma group. Throughout the text, eye is synonymous with child, since no fellow eye (without cataract) has developed glaucoma or glaucoma suspect in this study to date.

eTable 2: Relationship Between Baseline Characteristics and 10-year Glaucoma and Glaucoma+Glaucoma Suspect

		Glaucoma		Glaucoma + Glaucoma Suspect	
Baseline Characteristic	**n**	**# (%)**	**Hazard Ratio (95% CI)[a]**	**# (%)**	**Hazard Ratio (95% CI)[a]**
Treatment Group					
Contact Lens	57	13 (23%)	1.0 (0.4, 2.2)	26 (46%)	1.4 (0.8, 2.5)
Intraocular Lens	57	12 (21%)		20 (35%)	
Age Strata (days)					
28 – 48	50	18 (36%)	**3.2 (1.2, 8.2**)	26 (52%)	1.5 (0.8, 3.0)
49 – 210	64	7 (11%)		20 (31%)	
Persistent Fetal Vasculature[b]					
Yes	25	9 (36%)	2.1 (0.9, 4.8)	14 (56%)	1.6 (0.8, 3.0)
No	89	16 (18%)		32 (36%)	
Corneal Diameter (mm)					
≤ 10	45	14 (31%)	1.3 (0.6, 3.2)	25 (56%)	**2.0 (1.1, 3.8)**
> 10	69	11 (16%)		21 (30%)	
Intraocular Pressure (mm Hg)					
≥ 12	57	11 (19%)	0.9 (0.4, 2.0)	24 (42%)	1.2 (0.7, 2.2)
< 12[¶]	57	14 (25%)		22 (37%)	

[a] Estimated from a proportional hazard regression model including all baseline covariates
[b] To quality for inclusion in the Infant Aphakia Treatment Study, persistent fetal vasculature had to be mild and anterior only, without stretching of the ciliary processes after pupil dilation.
Omission of corneal diameter from the multivariable proportional hazards regression results in a hazard ratio of 2.0 for age [95% confidence interval 1.1-3.7].

eTable 3: Glaucoma Surgeries for Eyes with Glaucomatous Eyes

Subject Number	Group (IOL or CL)	Glaucoma Diagnosis Date	Age at Glaucoma Diagnosis (Years)	Glaucoma Surgery Date	Age at Glaucoma Surgery (Years)	Surgery	10-Yr IOP (mm Hg)	10-Yr Visual (logMAR)	10-Yr Overall RNFL[a] (μm)
1	IOL	2/14/2006	0.3	3/8/2006	0.4	Trabeculotomy	15.5	0.2	97
2	IOL	7/7/2006	0.3	9/20/2006	0.5	Trabeculotomy	20.5	0.1	128
3	CL	8/28/2007	0.3	9/21/2007	0.4	Trabeculotomy	17.5	0.4	95
4	CL	4/12/2012	3.3	12/20/2013	5.0	Trabeculotomy	19.0	0.2	73
				6/24/2015	6.5	Trabeculotomy, visco-canulostomy			
5	IOL	3/28/2006	0.5	4/12/2006	0.5	Glaucoma drainage device	21.3	2.6	--
6	IOL	9/8/2008	0.3	9/16/2008	0.3	Glaucoma drainage device	38.5	1.7	--
				1/12/2010	1.6	Glaucoma drainage device revision			
7[b]	CL	3/30/2005	0.3	4/8/2005	0.3	Peripheral Iridectomy, Vitrectomy	6.5	2.9	--
8	CL	3/15/2012	7.7	3/15/2012	5.4	Iridectomy	18.0	0.2	98
				5/15/2014	7.6	Trabeculectomy			
9	CL	10/31/2007	0.6	8/18/2010	3.4	Trabeculotomy	14.0	1.6	--
				12/3/2014	7.7	Glaucoma drainage device			
10	IOL	9/21/2010	3.7	9/21/2010	3.7	Endocyclophotocoagulation	21.0	1.1	--
11	CL	7/1/2014	7	12/22/2014	7.5	Glaucoma drainage device	14.0	0.3	55

[a] The missing values were either unable to be obtained or deemed unreadable by the expert grader (ADB).
[b] This eye later developed a retinal detachment, and despite surgical intervention, developed phthisis and blindness.

eTable 4: Means and 99.9%[a] Confidence Intervals for Paired Differences Between Fellow and Treated Eye[b] Retinal Nerve Fiber Layer OCT Quality and Thickness Measurements, by Glaucoma Status

	Glaucomatous Subjects (n=15)	Glaucoma Suspect Subjects (n=12)	Neither (n=37)
Image Quality (1-40)	4.2 (-1.9, 10.3)	3.7 (-6.8, 14.2)	3.3 (-1.1, 7.7)
Overall (µm)	12.7 (-7.5, 32.9)	-0.8 (-20.3, 18.8)	-1.0 (-8.9, 6.9)
Nasal (µm)	12.8 (-9.6, 35.2)	0.6 (-25.4, 26.6)	0.5 (-12.4, 13.4)
Nasal Inferior (µm)	**30.8 (0.2, 61.4)**	17.8 (-26.9, 62.6)	-5.8 (-32.2, 20.6)
Nasal Superior (µm)	24.4 (-16.8, 65.6)	1.4 (-20.3, 23.1)	2.5 (-16.7, 21.6)
Temporal (µm)	-7.1 (-26.9, 12.7)	**-15.8 (-31.6, -0.1)**	**-9.2 (-16.6, -1.8)**
Temporal Inferior (µm)	11.7 (-22.3, 45.6)	0.6 (-51.7, 52.9)	-1.2 (-15.4, 12.9)
Temporal Superior (µm)	22.4 (-23.7, 68.5)	8.1 (-23.8, 39.9)	8.8 (-8.0, 25.7)

a. Bonferroni correction applied due to multiple comparisons being performed
b. Difference = fellow eye measurement minus treated eye measurement

Clinical interpretation of the results in the table: the results suggest that indeed there was no significant difference in overall RNFL between the study eyes in each respective grouping (glaucoma, glaucoma suspect, and neither) compared to the respective fellow eyes. The small differences identified in selected sectors between study and fellow eyes (see bolded numbers above) are likely not clinically relevant, especially given the large confidence intervals, and the fact that the overall RNFL was not significantly different among the groups.

eTable 5: Glaucoma Status by IATS Criteria vs. by Optic Nerve Head

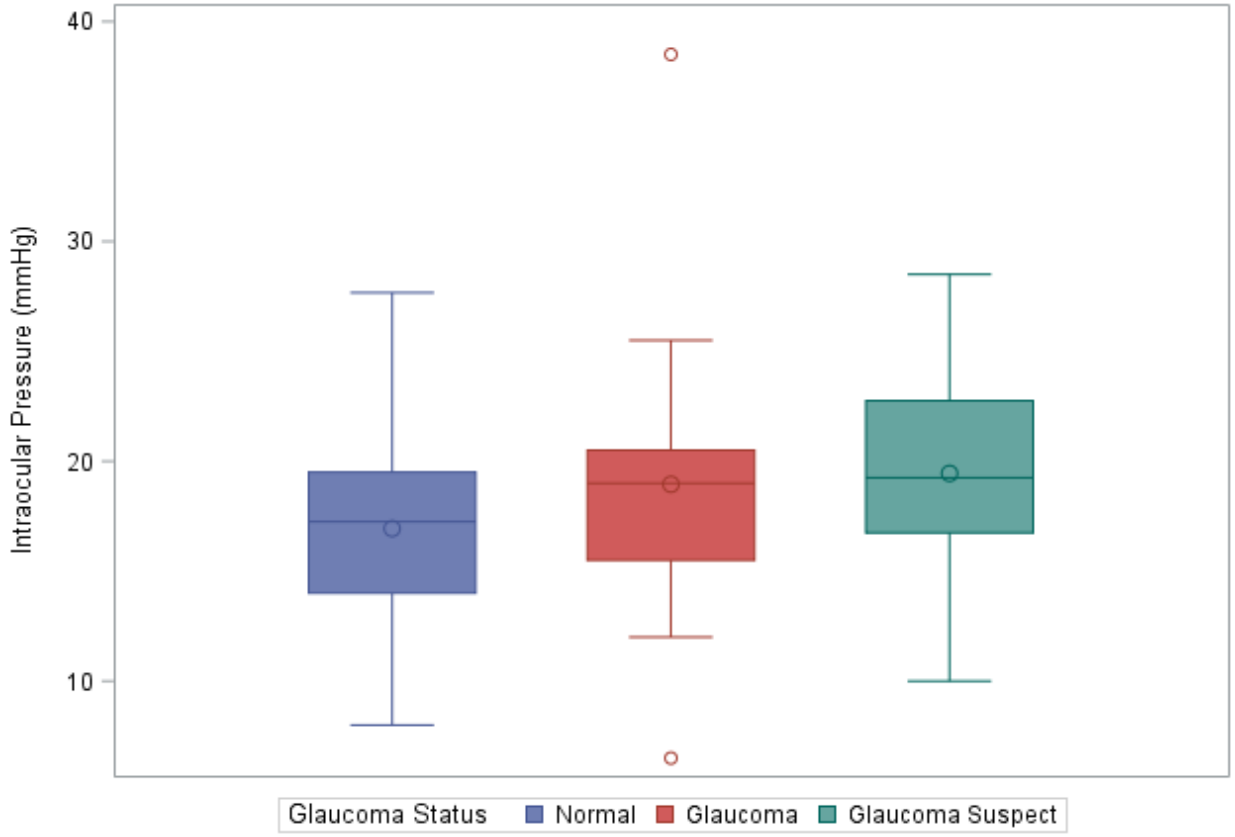
		Optic Nerve Head Grading Category			
Glaucoma Diagnosis by IATS Criteria		**Normal**	**Glaucoma Suspect**	**Glaucoma**	**Imaging Not Available or Inadequate**
Normal		58	1	1	7
Glaucoma Suspect		13	2	0	6
Glaucoma		11	4	4	5

eTable 6. Mean (Std. Deviation) Retinal Nerve Fiber Layer (RNFL) OCT Quality and Thickness Measurements, by Optic Nerve Head Grading Category

RNFL Parameter[a]	Glaucomatous Subjects (n=3)	Glaucoma Suspect Subjects (n=5)	Neither (n=60)
Image Quality (1-40) (p=0.5)	27.0 (8.2)	29.2 (4.1)	26.2 (5.8)
Overall (µm) (p=0.2)	91.0 (16.4)	90.6 (24.9)	102.3 (15.0)
Nasal (µm) (p=0.7)	78.0 (10.1)	69.6 (14.9)	77.2 (21.2)
Nasal Inferior (µm) (p=0.3)	86.3 (27.9)	100.6 (29.2)	115.6 (38.9)
Nasal Superior (µm) (p=0.2)	92.0 (30.4)	92.2 (34.8)	112.5 (29.1)
Temporal (µm) (p=0.8)	74.7 (17.2)	76.6 (16.5)	79.2 (15.7)
Temporal Inferior (µm) (p=0.3)	121.7 (46.5)	137.6 (48.7)	145.5 (22.7)
Temporal Superior (µm) (p=0.05)	124.7 (8.5)	103.2 (52.9)	133.9 (23.3)

a. P-value for F-test for equality of means across the three glaucoma status groups.

eFigure 1. 10-year Intraocular Pressure (mm Hg) vs. Glaucoma Status



Additional statistical results:

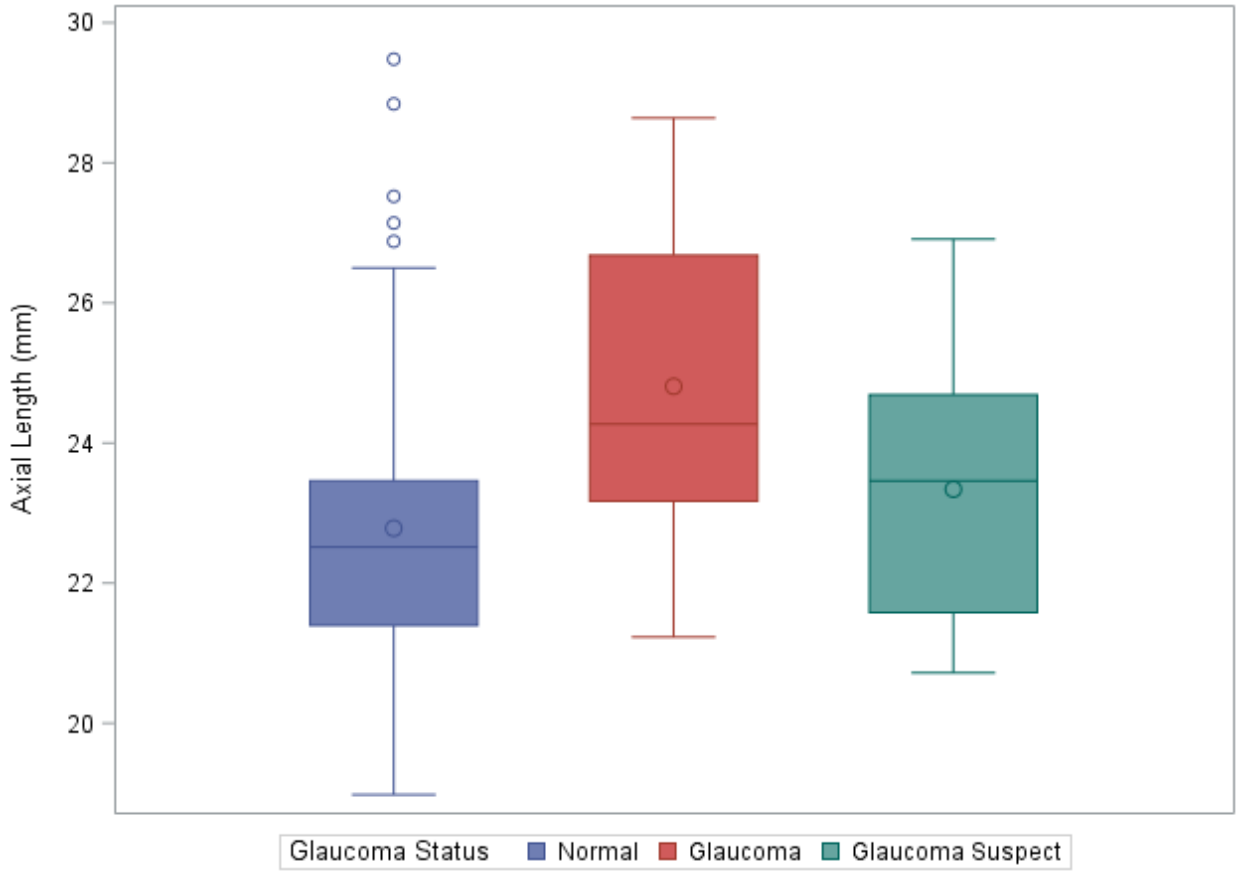
Mean ± standard deviation intra-ocular pressure (IOP):

normal (n=60)	16.9 ± 3.7 mm Hg
glaucoma (n=25)	19.0 ± 5.9 mm Hg
glaucoma suspect (n=20)	19.5 ± 4.2 mm Hg

No significant pairwise differences were found between the group means; 95% Tukey-Kramer confidence intervals for pairwise differences in mean IOP between the groups were:

Glaucoma vs. Glaucoma Suspect:	(-3.6, 2.6)
Glaucoma vs. Normal:	(-0.5, 4.5)
Glaucoma Suspect vs. Normal:	(-0.2, 5.2)

eFigure 2. 10-year Axial Length (mm) vs. Glaucoma Status



Additional statistical results:

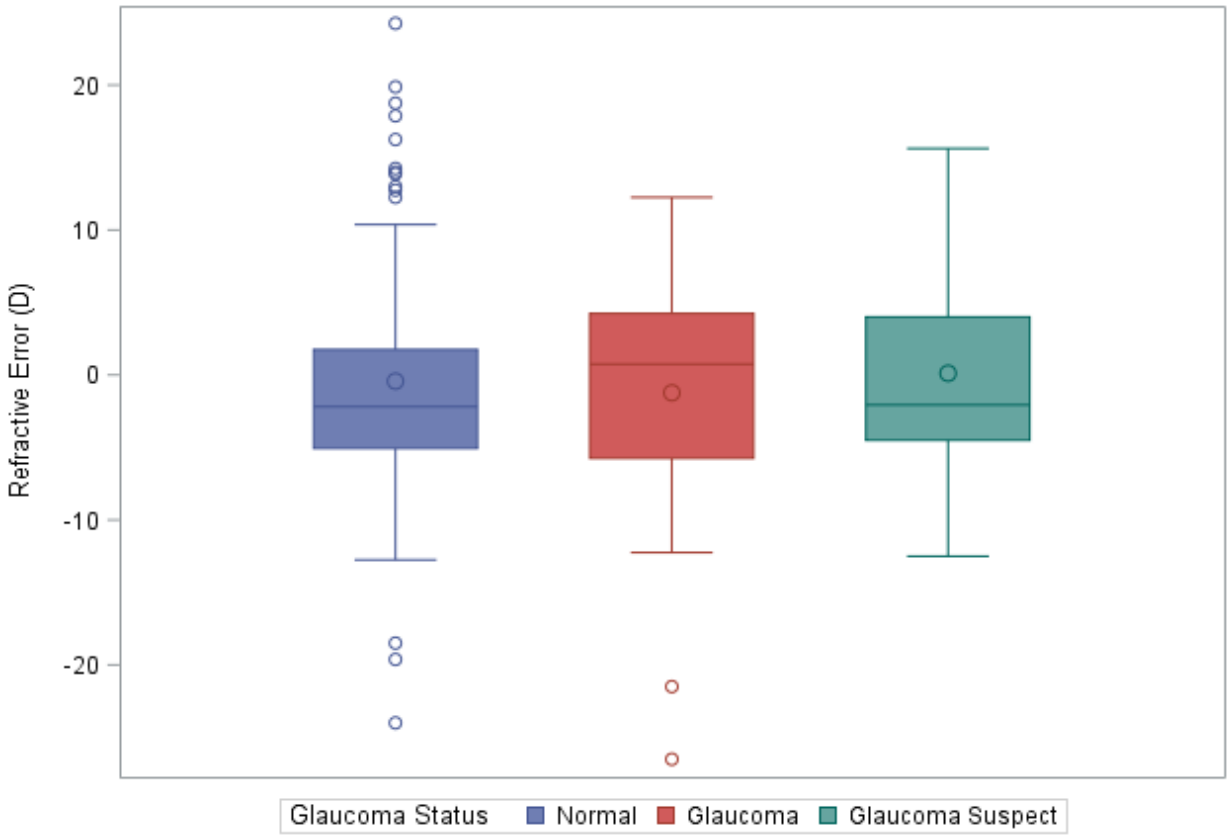
Mean ± standard deviation Axial Length:

normal (n=60)	22.8 ± 2.2 mm
glaucoma (n=22)	24.8 ± 2.4 mm
glaucoma suspect (n=19)	23.3 ± 1.9 mm

The mean length for the glaucoma group was significantly different from that of the normal group. 95% Tukey-Kramer confidence intervals for pairwise differences in mean axial length between the groups were:

Glaucoma vs. Glaucoma Suspect:	(-0.1, 3.1)
Glaucoma vs. Normal:	(0.7, 3.3)
Glaucoma Suspect vs. Normal:	(-0.8,1.9)

eFigure 3. 10-year Refractive Error (D) vs. Glaucoma Status



Additional Statistical Results:

Mean ± standard deviation refractive error:

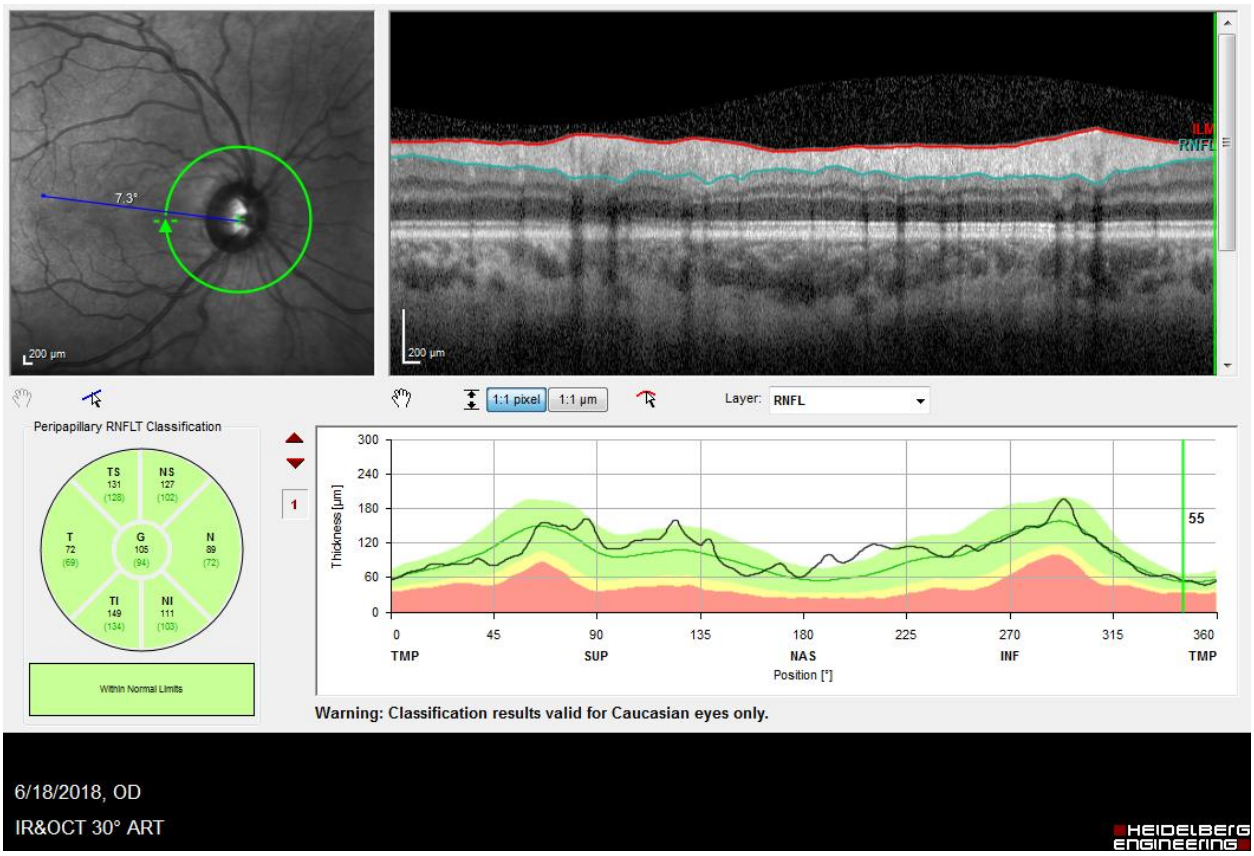
normal (n=60)	-0.4 ± 9.6 D
glaucoma (n=23)	-1.2 ± 9.8 D
glaucoma suspect (n=20)	0.1 ± 8.1 D

No significant pairwise differences were found between the group means; 95% Tukey-Kramer confidence intervals for pairwise differences in mean refractive error between the groups were:

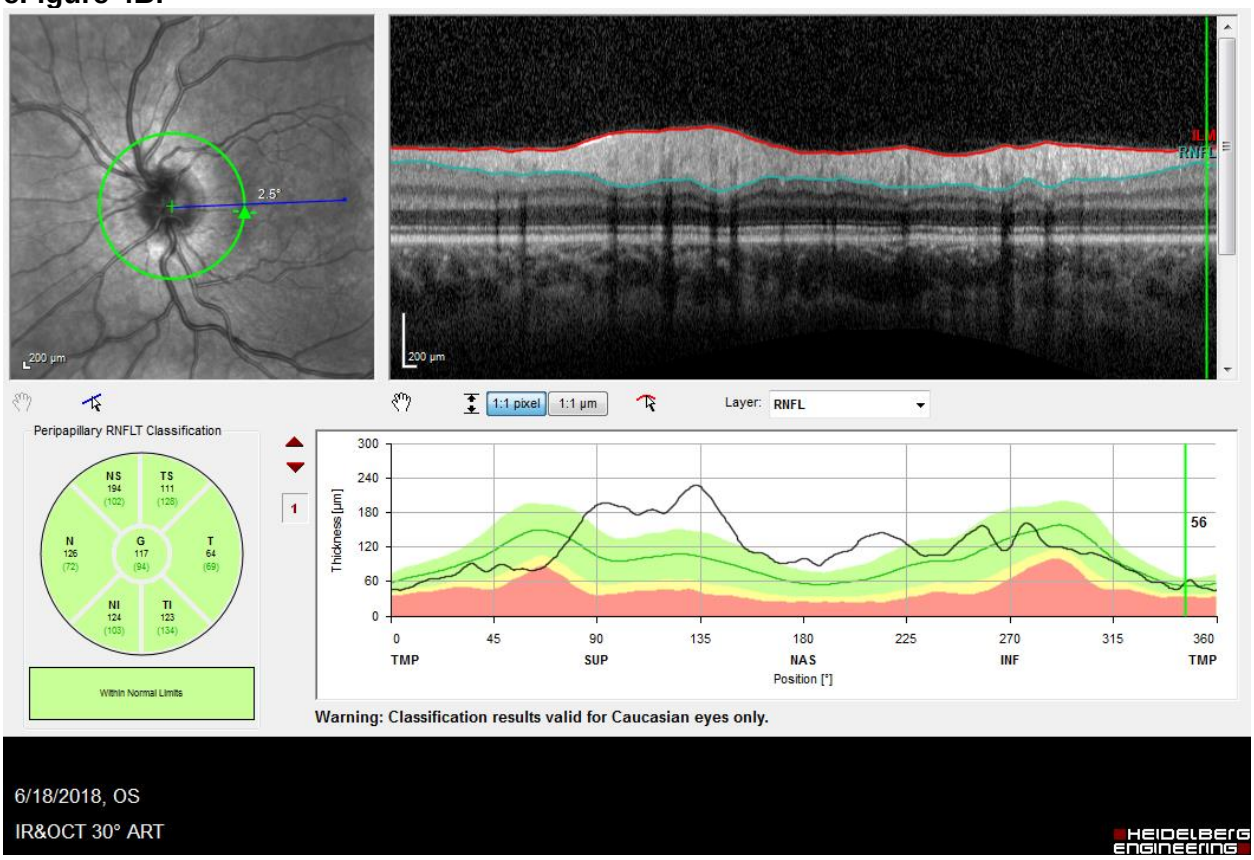
Glaucoma vs. Glaucoma Suspect:	(-8.1, 5.5)
Glaucoma vs. Normal:	(-6.3, 4.7)
Glaucoma Suspect vs. Normal:	(-5.2, 6.3)

eFigures 4A and 4B: Example of Retinal Nerve Fiber Layer (RNFL) Optical Coherence Tomography (OCT) Images for the treated eye (4A) and fellow eye (4B) of an IATS study subject. [Example of Optic Nerve Head (ONH) Images for the treated and fellow eye of the same IATS subject are shown in manuscript Figures 4A and 4B, respectively.] The treated eye was diagnosed with glaucoma based both on the IATS criteria (eTable 1) and ONH imaging, while the fellow eye was graded as 'Normal' (neither glaucomatous nor glaucoma suspect).

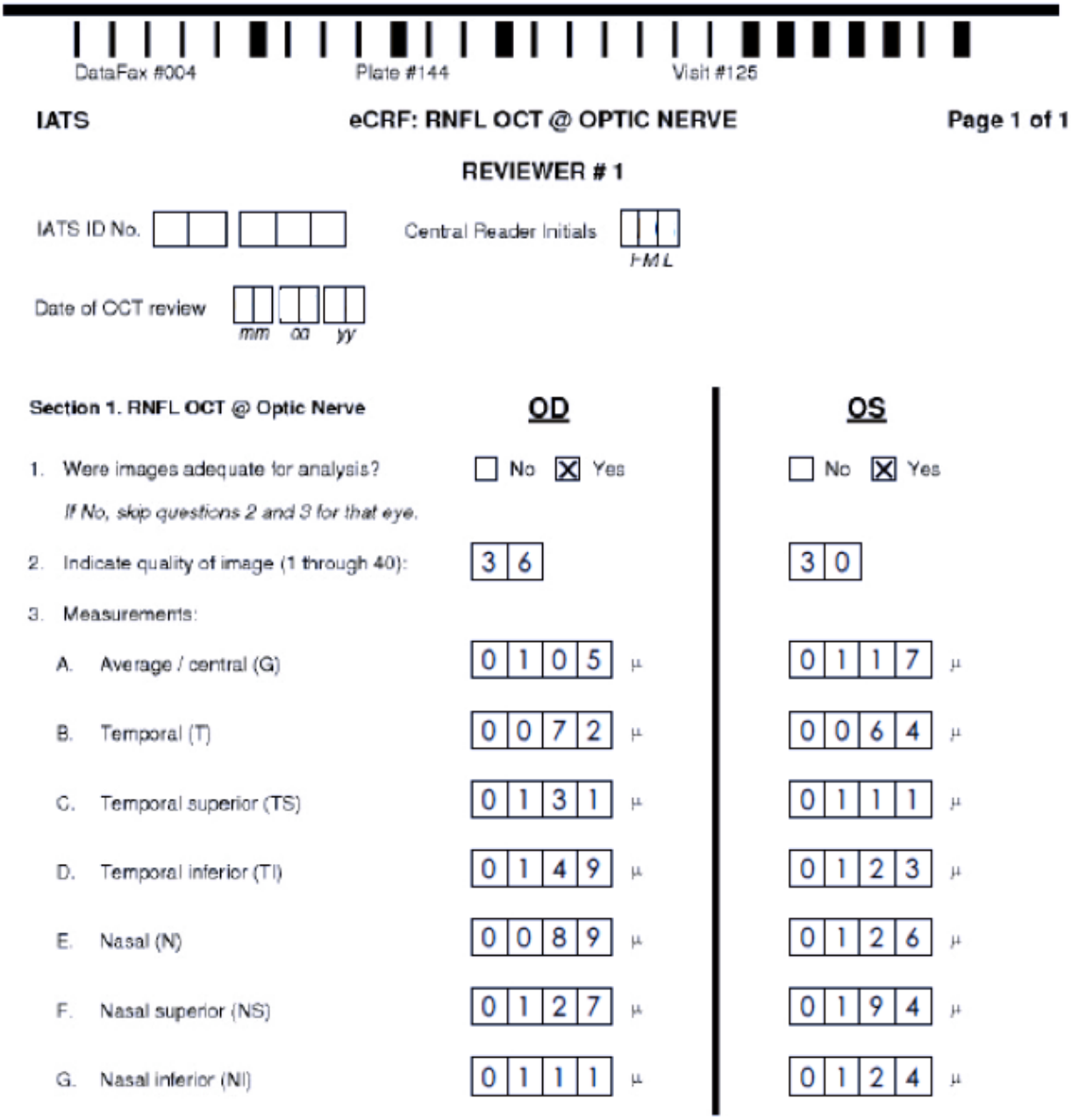
eFigure 4A:



eFigure 4B:



eFigure 5: Retinal Nerve Fiber Layer (RNFL) by Optical Coherence Tomography (OCT): Study Case Report Form



DataFax #004 Plate #144 Visit #125

IATS eCRF: RNFL OCT @ OPTIC NERVE Page 1 of 1

REVIEWER # 1

IATS ID No. ☐☐ ☐☐☐ Central Reader Initials ☐☐☐ FML

Date of OCT review ☐☐ mm ☐☐ dd ☐☐ yy

Section 1. RNFL OCT @ Optic Nerve	OD	OS
1. Were images adequate for analysis? *If No, skip questions 2 and 3 for that eye.*	☐ No ☒ Yes	☐ No ☒ Yes
2. Indicate quality of image (1 through 40):	36	30
3. Measurements:		
A. Average / central (G)	0105 µ	0117 µ
B. Temporal (T)	0072 µ	0064 µ
C. Temporal superior (TS)	0131 µ	0111 µ
D. Temporal inferior (TI)	0149 µ	0123 µ
E. Nasal (N)	0089 µ	0126 µ
F. Nasal superior (NS)	0127 µ	0194 µ
G. Nasal inferior (NI)	0111 µ	0124 µ

eFigure 6: Optic Nerve Head Photography: Study Case Report Form

IATS **eCRF: OPTIC DISC PHOTO REVIEW** **Page 1 of 1**

REVIEWER # 1

IATS ID No. [ ] [ ] Central Reader Initials [ ] *FML*

Date of OCT review [ ][ ][ ] *mm dd yy*

	OD	OS
1. Were photos submitted adequate for analysis? *If No, skip questions 2 and 3 for that eye.*	☐ No ☒ Yes	☐ No ☒ Yes
2. Indicate cup-disc ratio:	0.6	0.2
3. Indicate glaucoma status *(mark one):*	☐ Normal ☐ Suspicious ☒ Glaucoma	☒ Normal ☐ Suspicious ☐ Glaucoma
4. Was cup-disc asymmetry of ≥ + 0.2 indicated?	☐ No ☒ Yes	

5. Provide any comments that you feel will be helpful to the adjudication process:

References

1. Beck AD, Freedman SF, Lynn MJ, Bothun E, Neely DE, Lambert SR. Glaucoma-related adverse events in the Infant Aphakia Treatment Study: 1-year results. *Arch Ophthalmol.* 2012;130(3):300-305.
2. Freedman SF, Lynn MJ, Beck AD, Bothun ED, Orge FH, Lambert SR. Glaucoma-Related Adverse Events in the First 5 Years After Unilateral Cataract Removal in the Infant Aphakia Treatment Study. *JAMA Ophthalmol.* 2015;133(8):907-914.