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Potential Beneficial Effect of Low Dose Danazol in Combination With Renin Angiotensin Inhibitors in Diabetic Macular Edema: a 12-week Double Blind Randomized Controlled Trial

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DISCLOSURES:

Michael Singer has the following relationships to disclose: Ampio (G, L), Eyegate (G), Alimera (G), Aerpio (C, G), Genentech (L, G), Allergan (C, L, G), Regeneron (G, L, G), Santen (C, G), Acucela (C, G), Thrombogenics (C, G), Optos (G); G = Reseach Grant, C = Consultant, L = Lecturer

David Bar-Or (Chief Scientific Officer) and is an employee of Ampio Pharmaceuticals, Inc. and own shares and stock options in Ampio Pharmaceuticals, Inc.. David Bar-Or holds numerous patents on danazol and vascular permeability conditions. **Alessandro Orlando** is an independent contractor for, and holds stock options in, Ampio Pharmaceuticals, Inc.

INTRODUCTION:

In 2012, 29 million Americans had diabetes, and between 1.1 and 2 million had DME. If left untreated, DME can result in irreversible loss of vision, and result in a lowered quality of life.

The standard treatment modality for DME is intravitreal injections of anti-vascular endothelial growth factor (VEGF). One of the principal reasons for the use of anti-VEGF therapy for DME, stems from their ability to address one of the main culprits of vision loss, vascular permeability.

Danazol, a currently-approved synthetic steroid analog of 17-a-ethinyl testosterone, also has potential to be an effective treatment modality for DME due to is strengthening effects on vascular permeability at extremely low doses.

In 2007, we published the results of a study examining a tubulogenesis model, showing danazol decreasing endothelial cell proliferation and preventing capillary-like structures from forming. We recently discovered a biphasic effect of danazol on the retinal endothelial vascular barrier in-vitro. Danazol was discovered to amplify barrier function at nanomolar concentrations, and was associated with the reorganization of F-actin cytoskeleton to form of cortical actin rings, and increased retinal endothelial intercellular adhesion; these elements ultimately lowered vascular permeability.

Subsequent clinical trials supported the hypothesized beneficial effect of low-dose danazol, and highlighted a specific relationship with body mass index (BMI).

The objectives of the current study were to examine the data from the second Phase 2 clinical trial, and evaluate the efficacy and safety of low-dose danazol for the treatment of DME and to identify a potential target population for treatment with low-dose danazol for future confirmatory trials.

METHODS:

Study Design:

- Double-blind, randomized controlled trial.
- 21 ophthalmology clinics across the USA.
- Enrollment began February 26, 2013 and ended June 30, 2014.
- 12-week double-blind phase.
- The conduct of this trial was in accordance with the Declaration of Helsinki. The trial was prospectively approved by Liberty IRB and was retrospectively registered at ClinicalTrials.gov (NCT01821677).
- All enrolled subjects provided written informed consent.

Major Inclusion Criteria:

- Patient provided written informed consent.
- 18 years or older with DME.
- BCVA letter score 24–78; definite central retinal thickness ≥275µm on spectral-domain OCT due to DME.
- Focal photocoagulation can be deferred safely for 12 weeks.

Major Exclusion Criteria:

- Blood pressure >180/110 mmHg.
- HbA1c greater than 11%.
- History of treatment for DME at any time in the 8 weeks before randomization (such as focal/grid macular photocoagulation, intravitreal or peribulbar corticosteroids, anti-VEGF drugs, or any other treatment).
- Substantial cataract.
- Aphakia.

Treatment groups:

- 0.5mg danazol/BMI per day
- 1mg danazol/BMI per day
- Placebo

Outcome Measures:

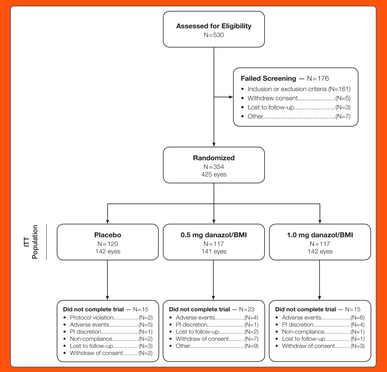
- The **primary outcome** of the trial was change in best corrected visual acuity (BCVA) from baseline to the end of the double-blind phase.
- The **secondary outcome** was change in central retinal thickness (CRT, measured by optical coherence tomography (OCT), µm) from baseline to the end of the double-blind phase.

Statistical Analyses:

- We assessed efficacy with a repeated measures, mixed model analysis of covariance (ANCOVA), adjusting for baseline values of BCVA and CRT and utilizing a compound symmetry covariance structure. Adjustment for multiplicity was done using the Dunnett method.
- An ***a priori*** investigation into the interaction between treatment groups and mean double-blind BMI, was done using a repeated-measures analysis of covariance, adjusting for baseline CRT and BCVA, and including an interaction term for mean BMI quartile and treatment group.
- Furthermore, we explored the effect of concomitant use of RAS inhibitors (angiotensin receptor blockers [ARBs] or angiotensin converting enzyme [ACE] inhibitors) at baseline.
- A last observation carried forward approach was used for missing data. Outlying change from baseline in BCVA values were truncated to 3 standard deviations from the mean for each treatment group. Unlike the primary and secondary objectives, there were no adjustments for multiple comparisons in subgroup analyses as these were exploratory, aimed at identifying subgroups of patients who benefited from treatment, and for planning future confirmatory studies.
- An alpha level of 0.05 was used for all tests.

RESULTS:

A total of 354 subjects (425 eyes) were randomized to one of three treatment arms; 53 (15%) subjects did not complete the trial (Figure 1).



Overall, the treatment groups had very similar baseline characteristics, with 53% of subjects having a history of anti-VEGF therapy (Table 1).

There were no serious drug-related adverse events in the 354 subjects randomized to a treatment group.

The mean age was slightly over 60 years in all treatment groups, and previous anti-VEGF exposure and hemoglobin A1c levels were comparable among treatment groups.

There were no significant differences between the treatment groups in baseline BCVA (p=0.71) or CRT (p=0.50).

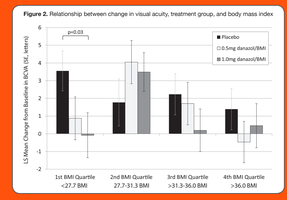
Overall, there was no significant difference between treatment groups in the least squares (LS) mean change in BCVA (p=0.41, Table 2). No significant differences between groups were seen when examining LS means changes in CRT (p=0.70).

Effect of Body Mass Index

Over 88% of subjects enrolled in this study were considered overweight or obese according to the World Health Organization (mean [SD] = 32.4 [6.90] kg/m2).

The effect of danazol on change in BCVA and CRT depended on BMI quartile (p-interactions=0.07 and 0.18). The best performing BMI quartile for both the 0.5mg and the 1mg danazol/BMI groups was the second BMI quartile (Figure 2).

Figure 2. Relationship between change in visual acuity, treatment group, and body mass index



Synergistic Effect of RAS Inhibitors

70% (75/107) of eyes in the second BMI quartile were on a concomitant RAS inhibitor. The effect of danazol on change in BCVA was shown to be dependent on whether the subject was being treated with RAS inhibitors (p-interaction=0.03).

The synergistic effect between danazol and RAS inhibitors over time is shown in Figure 3. In the 70% of eyes on a concomitant RAS inhibitor, significant increases from baseline in BCVA were observed at each visit in the 0.5mg danazol/BMI group (Figure 3). Furthermore, this group showed a significant 6.0-letter increase in BCVA in 12 weeks from baseline (p<0.001); this was significantly larger (p=0.01) than the 1.1-letter, non-significant increase from baseline to week 12, observed in the placebo group (Table 2).

In subjects on a concomitant RAS inhibitor, both the 0.5mg and 1mg danazol/BMI treatment groups showed significant decreases from baseline in CRT (-49.0µm, p=0.01; -39.3µm, p=0.03, Table 2), the decrease in the 0.5mg danazol/BMI group was significantly greater than the +10.2µm increase seen in the placebo group (p=0.03).

Figure 3. Synergistic relationship between 0.5mg danazol/BMI and RAS inhibitors

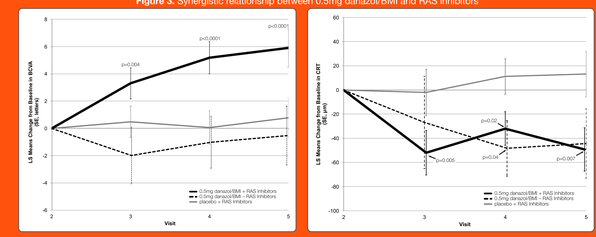


Table 1. Baseline demographic differences between treatment groups

Subjects	Placebo N=120	0.5mg danazol/BMI N=117	1mg danazol/BMI N=117	P
Age, mean (SD)	62.4 (10.62)	64.2 (11.21)	62.7 (8.78)	0.36
Male, no.(%)	52 (43%)	59 (50%)	66 (56%)	0.13
Caucasian, no.(%)	78 (66%)	72 (62%)	77 (66%)	0.80
Body Mass Index, mean (SD)	32.4 (6.73)	32.3 (7.10)	32.4 (6.91)	0.99
Intraocular pressure, mean (SD), mmHg	15.3 (2.61)	14.9 (2.49)	15.0 (2.98)	0.60
Mean arterial pressure, mean (SD)	98.6 (9.63)	97.7 (8.96)	98.0 (8.75)	0.77
Length of DME, no.(%)[a]				0.36
0 to ≤1 year	44 (38%)	43 (39%)	43 (37%)	
>1 to ≤2 years	18 (15%)	10 (9%)	24 (21%)	
2 to ≤3 years	16 (14%)	17 (15%)	14 (12%)	
>3 years	39 (33%)	41 (37%)	34 (30%)	
Anti-VEGF history, no.(%)	65 (54%)	60 (51%)	62 (53%)	0.90
Concomitant use of RAS inhibitor, no.(%)	81 (68%)	85 (73%)	75 (64%)	0.37
Labs, median (IQR)				
Hemoglobin A1c, mean (SD), %	7.9 (1.35)	7.9 (1.54)	7.9 (1.51)	>0.99
Sex hormone binding globulin, nmol/L	51 (32.6, 72.3)	51.1 (38.9, 72.1)	46.7 (36.8, 72.7)	0.69
Total cholesterol, mmol/L	162 (135, 191)	158 (139, 189)	164 (141, 197)	0.32
Fructosamine, µmol/L	291 (253, 337)	273 (253.5, 320)	286 (251, 326)	0.65
Total testosterone, nmol/L	1.3 (0.7, 8.3)	4.0 (0.8, 10.7)	5.5 (0.9, 9.6)	0.11
Free androgen index	0.03 (0.01, 0.2)	0.1 (0.01, 0.2)	0.1 (0.02, 0.2)	0.26

[a] numbers do not add to total group size due to missing data.
BMI, body mass index; DME, diabetic macular edema; VEGF, vascular endothelial growth factor; RAS, renin-angiotensin system; IQR, interquartile range

Table 3. Changes in visual acuity and retinal thickness by treatment group and RAS inhibitor use in subjects in the second BMI quartile (27.7-31.3)

Eyes	Placebo	0.5mg danazol/BMI	1mg danazol/BMI	P
RAS Inhibitor Use	**N=22**	**N=25**	**N=28**	
Loss ≥5 letters, no. (%)	4 (18%)	0	0	0.01
Gain ≥5 letters, no. (%)[a]	6 (27%)	15 (60%)	10 (36%)	0.04
Reduction of 20% in CRT, no. (%)	0	5 (20%)	5 (18%)	0.06
No RAS Inhibitor Use	**N=6**	**N=11**	**N=15**	
Loss ≥5 letters, no. (%)	0	2 (18%)	3 (20%)	0.68
Gain ≥5 letters, no. (%)	2 (33%)	2 (18%)	6 (40%)	0.54
Reduction of 20% in CRT, no. (%)	1 (17%)	2 (18%)	1 (7%)	0.51

[a] p-value shown is comparing 0.5mg danazol/BMI to placebo.
Outlying values were truncated to three SD of mean for each treatment group. RAS, renin-angiotensin system; CRT, central retinal thickness; BMI, body mass index.

Table 2. Changes in visual acuity and central retinal thickness by treatment group and RAS inhibitor use

Eyes	Placebo	0.5mg danazol/BMI	1mg danazol/BMI
Overall (all BMI ranges)	**N=140**	**N=140**	**N=141**
Baseline BCVA, mean (SD), letters	62.7 (11.70)	63.7 (11.23)	63.7 (10.18)
End of study BCVA, mean (SD), letters	65.0 (12.72)	65.2 (12.96)	65.0 (12.31)
LS mean change from baseline (95%CI), letters[a]	2.3 (1.1, 3.5)	1.5 (0.3, 2.7)	1.2 (-0.3, 2.4)
p-value, change from baseline	<0.001	0.02	0.06
p-value, difference[b]	Ref.	0.55	0.32
Baseline CRT, mean (SD), µm	426.9 (126.79)	440.0 (135.48)	422.0 (120.19)
End of study CRT, mean (SD), µm	409.2 (108.72)	428.4 (146.29)	412.1 (132.93)
LS mean change from baseline (95%CI), µm[c]	-18.4 (-33.7, -3.2)	-10.0 (-25.3, 5.3)	-11.2 (-26.5, 4.0)
p-value, change from baseline	0.02	0.20	0.15
p-value, difference[b]	Ref.	0.67	0.59
Overall (second BMI quartile, 27.72-31.31)	**N=28**	**N=36**	**N=43**
Baseline BCVA, mean (SD), letters	66.0 (8.17)	62.6 (10.53)	63.5 (9.87)
End of study BCVA, mean (SD), letters	67.5 (8.26)	66.9 (12.74)	67.1 (9.87)
LS mean change from baseline (95%CI), letters[a]	2.0 (-0.8, 4.8)	3.9 (1.3, 6.5)	3.4 (1.1, 5.8)
p-value, change from baseline	0.16	0.005	0.01
p-value, difference	Ref.	0.31	0.42
Baseline CRT, mean (SD), µm	410.1 (96.30)	435.7 (118.72)	425.0 (122.85)
End of study CRT, mean (SD), µm	412.8 (105.33)	388.17 (120.88)	408.6 (140.04)
LS mean change from baseline (95%CI), µm[c]	-0.44 (-34.8, 33.9)	-45.2 (-75.6, -14.7)	-16.3 (-44.0, 11.5)
p-value, change from baseline	0.97	0.01	0.24
p-value, difference	Ref.	0.06	0.46
RAS Inhibitor Use (second BMI quartile)	**N=22**	**N=25**	**N=28**
Baseline BCVA, mean (SD), letters	66.7 (6.47)	65.7 (9.08)	62.5 (10.50)
End of study BCVA, mean (SD), letters	67.2 (8.59)	71.7 (10.08)	66.5 (10.45)
LS mean change from baseline (95%CI), letters[a]	1.1 (-2.0, 4.1)	6.0 (3.1, 9.0)	4.0 (1.2, 6.7)
p-value, change from baseline	0.48	<0.001	0.01
p-value, difference	Ref.	0.02	0.16
Baseline CRT, mean (SD), µm	407.7 (97.98)	426.9 (88.76)	428.8 (106.46)
End of study CRT, mean (SD), µm	421.3 (108.67)	378.0 (76.70)	388.9 (99.94)
LS mean change from baseline (95%CI), µm[c]	10.2 (-27.9, 48.4)	-49.0 (-85.1, -12.9)	-39.3 (-73.2, -5.5)
p-value, change from baseline	0.58	0.01	0.03
p-value, difference	Ref.	0.03	0.06
No RAS Inhibitor Use (second BMI quartile)	**N=6**	**N=11**	**N=15**
Baseline BCVA, mean (SD), letters	63.5 (13.23)	55.6 (10.60)	65.4 (8.61)
End of study BCVA, mean (SD), letters	68.8 (7.49)	56.1 (11.78)	68.2 (11.54)
LS mean change from baseline (95%CI), letters[a]	5.7 (-0.2, 11.5)	-1.1 (-5.7, 3.5)	2.4 (-1.5, 6.3)
p-value, change from baseline	0.06	0.62	0.21
p-value, difference	Ref.	0.07	0.35
Baseline CRT, mean (SD), µm	419.2 (98.18)	455.6 (172.60)	418.1 (152.78)
End of study CRT, mean (SD), µm	381.5 (84.05)	411.2 (190.22)	445.5 (193.23)
LS mean change from baseline (95%CI), µm[c]	-39.6 (-112.7, 33.5)	-36.5 (-91.3, 18.3)	27.9 (-18.9, 74.6)
p-value, change from baseline	0.27	0.18	0.23
p-value, difference	Ref.	0.94	0.12

[a] adjusted for baseline BCVA and CRT.
[b] p-values are adjusted for multiple comparisons using the Dunnett method.
[c] adjusted for baseline CRT.
Outlying values were truncated to 3 SD of the mean for each treatment group.
BCVA, best corrected visual acuity; CRT, central retinal thickness; RAS, renin-angiotensin system; BMI, body mass index; SD, standard deviation; LS, least squares; SE, standard error

CONCLUSIONS:

The combination of oral RAS inhibitors and oral low dose danazol might be a painless, safe, and efficacious therapy for a subset of patients suffering from DME. Confirmatory studies of these observations are required to confirm the clinical utility of low dose danazol, and to construct a dosing algorithm that makes treatment with low dose danazol effective for all BMI groups, not just a narrow range.