Response to Ranibizumab of Eyes With Pigment Epithelial Detachments, Including Eyes That Developed RPE Tears: Data From the HARBOR Study

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OBJECTIVE To evaluate the treatment outcomes of eyes with neovascular AMD and PED including eyes that developed on-study retinal pigment epithelial (RPE) tears or macular atrophy (MA) based on data from HARBOR.

PURPOSE Eyes with neovascular AMD with PEDs remain a difficult population to treat. In this analysis, we evaluate the visual and anatomic outcomes of such eyes treated with ranibizumab, including eyes with features that may contribute to potentially poorer outcomes, such as development of RPE tears or MA.

METHODS In the HARBOR study, 1097 study eyes were randomized to receive 3 monthly doses of intravitreal ranibizumab (RBZ) 0.5 mg or 2.0 mg. Eyes were then either continued on monthly therapy or were treated as needed (PRN) based on ≥5-letter decrease in best-corrected visual acuity (BCVA) or any evidence of disease activity on spectral-domain optical coherence tomography. In eyes with PED at baseline (BL), VA

and anatomic outcomes were evaluated over 24 months, including change in BCVA from BL, complete resolution of PED, reduction in PED height, development of MA, and development of RPE tear.

RESULTS 598 eyes had PEDs at BL with a height ranging from ~35-1400 μm. Eyes randomized to RBZ 0.5 mg and 2.0 mg PRN required 14.0 and 11.6 injections over 24 months, respectively. At 24 months, mean change in BCVA from BL was +9.0 letters in eyes randomized to RBZ 0.5 mg monthly; +8.4 letters to RBZ 0.5 mg PRN; +7.1 letters to RBZ 2.0 mg monthly; and +7.2 letters to RBZ 2.0 mg PRN. PED resolution occurred most often in eyes randomized to RBZ 2.0 mg monthly (70.5%) compared with RBZ 0.5 mg monthly (53.2%), RBZ 0.5 mg PRN (44.5%), and RBZ 2.0 mg PRN (57.3%). There was a significantly higher rate of MA development at month 24 in eyes with resolution of PED than eyes with PED present (44% vs 17%, $P<.0001$). Twenty-eight (5%) eyes developed a new RPE tear during the study, with 21 of these RPE tears in eyes with the largest PEDs (≥352 μm) at BL. Of eyes with a new RPE tear, the mean change in BCVA (LOCF) from BL was +0.6 letters (n=25) and 0 letters (n=28) at month 12 and 24 respectively.

CONCLUSION In the >50% of patients with PED at BL in HARBOR, a mean of 8–9 letters was gained from BL at month 24 with RBZ 0.5 mg PRN or monthly. While more PED resolution was seen with a higher RBZ dose, there was no additional VA benefit and PED resolution was associated with a higher rate of MA development. On average, patients experiencing on-study RPE tear avoided vision loss with continued RBZ therapy.

TAKE HOME MESSAGE In HARBOR patients, ranibizumab 0.5 mg monthly or PRN effectively managed pigment epithelial detachments and resulted in clinically significant visual acuity gains over 2 years.

HUMAN RESEARCH This study involves human research.
IRB Approval Status: Approved by institutional review board

Gene Expression Modifications in Anti-VEGF Treated Retinal Müller Cells

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OBJECTIVE Anti-VEGF treatment can affect pathways other than angiogenesis pathway in cultured retinal Müller cells.

PURPOSE Anti-vascular endothelial growth factor (VEGF) therapy is the most commonly used treatment for neovascular age-related macular degeneration, diabetic macular edema and macular edema due to vein occlusion. This study assesses the *in vitro* differences in gene expression of different pathways in cultured human retinal Müller cells (MIO-M1) treated with different concentrations of anti-VEGF drugs.

METHODS MIO-M1 cells were plated in 6-well plates and then treated with1x and 2x clinical dose of ranibizumab, bevacizumab, aflibercept or ziv-aflibercept for 24 hours. RNA was isolated, cDNA synthesized and quantitative polymerase chain reaction (Q-PCR) performed using primers for anti-oxidant genes: glutathione peroxidase 3(GPX3) and superoxide dismutase 2 (SOD2); pro apoptotic genes: B-cell lymphoma 2 like 13 (BCL2L13) and BCL2- associated X protein (BAX); and angiogenesis-related genes: VEGFA and placental growth factor (PlGF). Differences in cycle threshold values (delta deltaCT), a measure of gene expression level, were calculated, and folds were obtained with the formula 2^delta deltaCT.

RESULTS Significant upregulation for the anti-oxidant GPX3 gene was found for 1x and 2x doses of ranibizumab, bevacizumab and aflibercept. Significant downregulation of

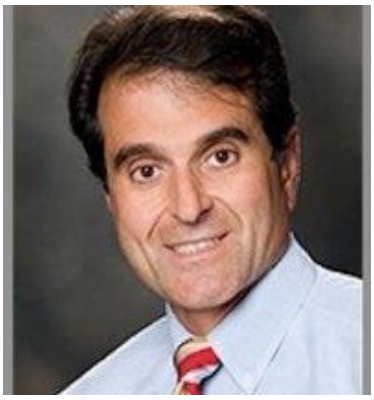
the anti-oxidant SOD2 gene was found for both concentrations in all drugs studied. The pro-apoptotic BCL213 gene was significantly upregulated for ranibizumab 1x, bevacizumab 1x/2x, aflibercept 1x/2x, and ziv-aflibercept 1x. The pro-apoptotic BAX gene was upregulated for bevacizumab 1x and ziv-aflibercept 1x/2x. VEGFA was significantly down regulated for both concentrations of all drugs studied except for ziv-aflibercept 2x. PlGF was significantly upregulated for 1x/2x bevacizumab and aflibercept, and significantly downregulated for ziv-aflibercept 1x/2x. (Table 1)

CONCLUSION Anti-VEGF drugs in MIO-M1 cells can change the gene expression of pathways distinct from VEGF, including anti- oxidant and pro-apoptotic genes. Bevacizumab as well as aflibercept can upregulate PlGF. Ziv-aflibercept appears to be less efficient in regulating VEGF and PlGF. These changes in non-VEGF gene expression pathways may be associated with the progressive atrophy observed with anti-VEGF use.

TAKE HOME MESSAGE Anti-VEGF drug treatment of *in vitro* MIO-M1 cells can change the non-VEGF gene expression pathways which may be associated with the progressive atrophy observed with chronic anti-VEGF use.

	RANIBIZUMAB				BEVACIZUMAB				AFLIBERCEPT				ZIV-AFLIBERCEPT			
	1x		2x		1x		2x		1x		2x		1x		2x	
Gene	Fold	p	Fold	p	Fold	p	Fold	p	Fold	p	Fold	p	Fold	p	Fold	p
GPX3	2.31	0.0001	1.96	0.0001	2.13	0.0011	2.22	0.0006	2.18	0.0002	1.67	0.001	0.95	0.457	1.17	0.156
SOD2	0.58	0.002	0.3	0.0006	0.69	0.0402	0.41	0.0013	0.22	<0.0001	0.44	0.0014	0.22	<0.0001	0.24	<0.0001
BAX	1.06	0.58	0.93	0.56	1.22	0.0114	1.01	0.82	0.98	0.75	1.08	0.19	1.85	0.0009	1.46	0.0005
BCL213	1.39	0.040	1.12	0.055	1.38	0.0015	1.60	0.0007	1.35	0.0094	1.21	0.0122	2.65	0.0443	2.06	0.094
VEGFA	0.66	0.029	0.41	0.0008	0.65	0.0016	0.41	0.0107	0.55	0.0057	0.58	0.0136	0.88	0.014	0.92	0.618
PlGF	1.11	0.55	1.41	0.066	1.79	0.0122	1.49	0.0260	2.20	0.0147	1.84	0.0108	0.70	0.0245	0.53	0.0063

“Real World” U.S. Outcomes of Anti-VEGF Therapy in Neovascular AMD: Risk of Vision Loss is Greatest in Patients with Better Baseline Visual Acuity



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- Keith Westby, MS, MBA
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OBJECTIVE To assess "real world" visual acuity (VA) outcomes of anti-VEGF therapy for neovascular AMD (nAMD) in a demographically diverse sample of U.S. retina specialists electronic medical records (EMR).

PURPOSE European studies of anti-VEGF therapy for nAMD show that "real world" visual outcomes do not achieve those in randomized clinical trials (RCT). Patient characteristics and under treatment related to discontinuous treatment regimens are implicated. We explored this issue by analyzing a large database of aggregated, longitudinal EMR from a demographically diverse sample of U.S. retina specialists.

METHODS The HIPAA-compliant Vestrum Health Retina Database was used retrospectively. Inclusion required at least 3 monthly anti-VEGF injections for naive nAMD patients in the first 4 months between January 2011 and July 2013 with availability of 6 to 24 months of follow-up data, ending July 2015 (n=2,213). The eyes were divided into 3 cohorts: those with records including VA measurements up to and

including 6-months but not beyond (“6-month cohort”, n=97), up to 12-months but not beyond ("12-month cohort”, n=195), and up to and including 24 months (“24-month cohort”, n=1,921), with each cohort being mutually exclusive. VA outcomes were assessed on each cohort and also stratified by baseline VA.

RESULTS The mean age at initial presentation was 82 years. Overall, VA outcomes correlated with the duration of treatment, best in the 24-month cohort (1.5 injections per quarter), followed by the 12-month (1.8 injections per quarter) and finally the 6-month cohorts (2.7 injections per quarter). When stratified by baseline VA of 20/200 or worse, 20/70-20/200, 20/40-20/70, and 20/40 or better, the final mean change in number of letters gained or lost in the 24-month cohort was +19.9, +2.6, -1.2, and -5.2 letters respectively, in the 12-month cohort +8.9, -1.3, -5.6, and -4.5 letters respectively, and in the 6-month cohort +8.8, -4.6, -1.9, and -5.2 letters respectively. Eyes with better baseline visual acuity experienced worse visual outcomes compared to those with worse baseline visual acuity, irrespective of the number of injections received, suggesting a ceiling effect.

CONCLUSION Visual outcomes following anti-VEGF therapy for nAMD in the “real world” do not achieve those seen in RCT. Eyes with better baseline VA are disproportionately affected. Duration of treatment and/or follow-up appear to correlate with best visual outcome. More intensive therapy and treatment compliance in nAMD is warranted, especially those patients with better baseline VA.

TAKE HOME MESSAGE "Real world" visual outcomes following anti-VEGF therapy for neovascular AMD do not achieve those seen in randomized clinical trials. Eyes with better baseline VA are disproportionately affected.

HUMAN RESEARCH This study involves human research.
IRB Approval Status: Exempt from approval

Long Term Results of Pro Re Nata Regimen of Aflibercept Treatment in Recurrent or Persistent Neovascular Age-Related Macular Degeneration

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OBJECTIVE To show the efficacy of PRN regimen of aflibercept treatment in persistant cases.

PURPOSE To determine the 24-month results of patients who had pro-re-nata (PRN) aflibercept treatment due to recurrent or resistant neovascular macular degeneration.

METHODS Eighty-one eyes of 78 patients with persistent or multiple recurrences of intraretinal or subretinal fluid while receiving bevacizumab or ranibizumab injections every 4 weeks (Q4W) and switched to every 8 weeks (Q8W) aflibercept treatment were included. Anatomic outcomes including maximum retinal thickness, central retinal thickness, maximum pigment epithelial detachment height and maximum fluid height and visual acuity (VA) were assessed.

RESULTS All anatomic end points significantly improved following 3 consecutive Q8W aflibercept injections, which was maintained through 24-months ($p<0.05$ for all end points at all visits). Thirty-seven eyes (45.6%) required escalation to Q4W injections at a median of 37 weeks (interquartile range, 30-62) to adequately treat the retinal fluid. Seventy-one of 81 eyes (87.7%) became completely dry on at least one follow-up visit during the study. The best-corrected visual acuity remained stable following either Q8W or Q4W aflibercept injections.

CONCLUSION Every 8 weeks aflibercept injectons with a PRN regimen was effective in many eyes previously treated with Q4W bevacizumab or ranibizumab injections that had persistent or recurrent fluid. Despite significant improvement in anatomic outcomes, vision remained stable throughout the 2-year follow-up.

TAKE HOME MESSAGE PRN regimen of aflibercept injections every 8 weeks was effective at improving most of the anatomic outcomes, and vision remained stable throughout the 24 month follow-up.

Interim Results and Key Learnings from an Ongoing Phase 2 Study of Encapsulated Cell Therapy Compared to Aflibercept in Patients With Wet AMD

- Szilard Kiss, MD

OBJECTIVE Interim results from an ongoing Phase 2 study comparing ECT to aflibercept in treatment-experienced patients with wet AMD will be presented, along with key learnings of the optimal ECT patient.

PURPOSE Encapsulated cell therapy (ECT), an intravitreal implant that continuously releases a soluble anti-VEGF receptor antagonist (NT-503), may maintain vision long-term in patients with wet AMD without the need for frequent intravitreal injections. The Phase 2 study will determine if NT-503 ECT can maintain vision in comparison to injections of aflibercept in treatment-experienced patients.

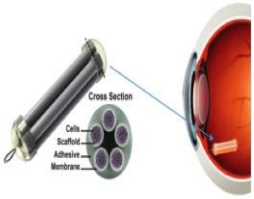
METHODS In this phase 2 multicenter, active-controlled, masked safety and efficacy study, 90 subjects with active subfoveal CNV secondary to AMD will be randomized to a single intravitreal NT-503 ECT implant or intravitreal injections of aflibercept every 8 weeks. All eligible subjects will have had at least 3 previous anti-VEGF injections, with the last injection no more than 4 months prior to enrollment. All subjects will have demonstrated a clinical response to aflibercept, and have a BCVA between 80 letters

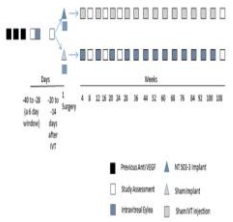
(20/25) and 35 letters (20/200) at screening and baseline. The primary outcome is visual function non-inferiority compared to aflibercept as assessed at 12 months.

RESULTS As of February 1, 2016, a total of 28 subjects, with mean age of 76.5 years, were randomized. Upon enrollment, the average time since diagnosis of wet AMD was 3.4 years. At screening, mean best-corrected visual acuity was 64.5 letters (ETDRS) and mean central retinal thickness (sdOCT) was 290.8 µ. Following induction with aflibercept, the mean change in central retinal thickness was -55.8 µ and vision remained stable at 66.3 letters. To date, 19 patients have been implanted with NT-503 ECT (based on 2:1 randomization) with no serious or severe implant nor surgery related adverse events. Additional demographic information and available safety and efficacy data for a larger patient population will be presented.

CONCLUSION Interim results indicate that the NT-503 ECT implant, along with the surgical procedure, appear in this study to be safe and well tolerated in patients with wet AMD. Complete one-year safety and efficacy results from the Phase 2 study are anticipated in 2017.

TAKE HOME MESSAGE Interim results from the ongoing Phase 2 clinical trial indicate that the NT-503 ECT implant, along with the surgical procedure, appears to be safe and well tolerated in patients with wet AMD.





HUMAN RESEARCH This study involves human research.
IRB Approval Status: Approved by institutional review board

Treatment Response to Antioxidant Supplementation Based on *CFH* and *ARMS2* Genetic Risk Allele Number in AREDS Patients With No AMD at Baseline

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OBJECTIVE To investigate the interaction of AMD genetic risk and antioxidant supplementation on progression to intermediate AMD in AREDS patients with no AMD at baseline.

PURPOSE The AREDS reported no reduction in progression to advanced AMD for patients with minimal AMD. Using data and DNA from 554 AREDS patients with no AMD, we analyzed the progression to intermediate AMD. We performed an outcomes analysis of antioxidant treatment as a function of pre-specified genetic risk groups based on *CFH* and *ARMS2* risk allele number.

METHODS We performed Cox regression analysis of AREDS data and DNA from 554 patients with no AMD, with progression to intermediate AMD in either eye by 12 years as an end point. In AREDS, patients with no AMD were randomized to treatment with antioxidants or placebo, and not to any zinc-containing regimen. We calculated overall progression to intermediate AMD for this group of patients, and progression in each of 4 pre-specified genetic groups based on total number of *ARMS2* and *CFH* risk alleles. We

calculated the impact of antioxidant treatment on progression to intermediate AMD for patients in each group.

RESULTS Overall, 81 (14.6%) of 554 patients with no AMD at baseline progressed to intermediate AMD after a mean of 8.57 years of follow up. Number of risk alleles (RA) in each group, the number of patients in each group, and the number and (%) who progressed to intermediate AMD were as follows: RA zero: 109, 8 (7.3%); RA 1: 242, 31 (12.8%); RA 2: 175, 31 (17.7%); RA3/4: 28, 11 (39.3%). Treatment groups were balanced with respect to non-genetic risk factors (age, smoking hx, BMI, education). The hazard ratios and (p-values) for progression with antioxidant therapy for each genetic group were: RA zero: 4.27 (0.003), RA 1: 1.72 (0.05), RA2: 0.69(0.18) and RA3/4: 0.28 (0.008). Antioxidants and risk allele number interacted statistically (Cox) with a p-value of <0.05.

CONCLUSION Progression from no AMD to intermediate AMD increases with genetic risk. Reducing progression should result in fewer cases of advanced AMD. Patients with no AMD and with high genetic risk may benefit from antioxidant treatment. The increased risk of progression for patients with no AMD and with low genetic risk suggests that antioxidant treatment should be actively discouraged in these patients.

TAKE HOME MESSAGE Antioxidant supplements increased the risk of AMD progression in AREDS patients with no AMD and with low genetic risk, but significantly decreased progression in patients with high genetic risk.

HUMAN RESEARCH This study involves human research.
IRB Approval Status: Approved by institutional review board