OBJECTIVE To determine if metabolomics can provide an integrated biomarker signal for age-related macular degeneration.

PURPOSE To study the plasma metabolomic profile of patients with age related macular degeneration (AMD) at different stages, using nuclear magnetic resonance spectroscopy (NMR) and compare it to that of control subjects, in two different cohorts.

METHODS This cross-sectional, observational study prospectively recruited patients diagnosed with AMD, as well as controls with no evidence of AMD, all aged > 50 years. Metabolomic profiles for two separate cohorts (total = 369) from southern Europe and Northeastern US were studied. For each cohort, subjects were grouped by AMD stage...
(early, intermediate, or late) and multivariate analysis of plasma NMR spectra was performed, followed by signal integration and univariate analysis.

**RESULTS** Metabolomic analysis detected small changes in the levels of some amino acids, organic acids, creatine, dimethyl sulfone, and specific lipid moieties, providing some biochemical information on AMD. Potential confounding effects of gender, smoking history, body mass index, and age were assessed in each cohort and found to be minimal when compared to that of the disease. Interestingly, distinct AMD metabolite fingerprints were noted for the two cohorts studied, indicating the importance of nutritional and other lifestyle habits in determining AMD metabolic response and potential biomarker fingerprints. Notably, some of the metabolite changes detected were particularly important in differentiating controls from patients diagnosed with early AMD.

**CONCLUSION** This study is the first demonstration of metabolite changes in plasma analyzed with NMR of patients with AMD, as compared to controls. Regional variations were seen in the metabolomics profiles of AMD patients. Metabolomics has the potential to identify biomarkers for AMD, and further work in this area is warranted.

**TAKE HOME MESSAGE** Changes in plasma metabolomic profiles, as well as regional variations, occur in patients with AMD, as compared to controls. Metabolomics has the potential to identify biomarkers for AMD.

**HUMAN RESEARCH** This study involves human research.

IRB Approval Status: Approved by institutional review board
Reduced Risk of Choroidal Neovascularization Following Subthreshold Diode Micropulse Laser (SDM) Retinal Protective Therapy™ (RPT) For Dry AMD

OBJECTIVE To determine the effect of SDM RPT, performed in a program of functionally-guided (disease) management (FGM), on the long-term risk of new CNV in eyes with dry AMD.

PURPOSE To determine the incidence of new choroidal neovascularization (CNV) following panmacular subthreshold diode micropulse laser (SDM) as retinal protective therapy™ (RPT) in dry age-related macular degeneration (AMD).

METHODS In an observational retrospective cohort study, the electronic medical records database (EMR) records of all active patients in a private vitreoretinal practice having undergone SDM RPT for dry AMD were reviewed, classified by AREDS categories, and analyzed for the primary endpoint of new CNV after treatment.
RESULTS Treatment was offered to 305/326 (94%) treatment candidates during the study period, and elected by 296 (97%). Postoperative follow up (avg. 20 mos) was available for 290 (432 eyes) treated patients (98%). Risk factors for CNV included age (avg. 83 years, 68% over 80); high prevalence of reticular pseudodrusen (RPD) (158 eyes, 37%); high AREDS severity (77% category 3 and 4); and high prevalence fellow eye CNV (118 eyes, 27%). Without adjusting for these risk factors, the incidence of new CNV was 7/432 (1.6%, annualized rate 0.97%). For eyes completing 12 months follow up, there were 4 CNV per 4963 person-eye months (0.81 per 1,000 person-eye months). For eyes completing 24 months follow up, there were 6 CNV per 7635 total person-eye months (0.79 per 1000 person-eye months). RPD and fellow eye CNV increased the risk of CNV post SDM RPT (p=0.02, each). Systemic hypertension lowered the risk (p=0.048). Visually acuity (VA) was stabilized. There were no adverse treatment effects.

CONCLUSION In a high-risk population, SDM RPT markedly reduced the risk of new CNV in dry AMD and stabilized VA, without adverse treatment effects. SDM RPT may offer a new, safe, and highly effective method of reducing the risk of vision loss in patients with dry AMD.

TAKE HOME MESSAGE Panmacular SDM as retinal protective therapy markedly reduced the risk of CNV and stabilized visual acuity in eyes with intermediate, or "high-risk" dry AMD, without adverse treatment effects.

HUMAN RESEARCH This study involves human research.

IRB Approval Status: Approved by institutional review board
OBJECTIVE To identify clinical features and optical coherence tomography (OCT) biomarkers that predict progression from early or intermediate age-related macular degeneration (AMD) to advanced disease.

PURPOSE We evaluated specific OCT features to determine if they predict progression from early or intermediate AMD to advanced disease, geographic atrophy (GA) and neovascular disease (NV).

METHODS Participants were enrolled in our prospective epidemiologic studies of AMD. Those who transitioned to advanced disease (either GA or NV) were classified as progressors (n=40 eyes), and were matched on baseline AMD grade and duration of follow up to participants who did not develop advanced AMD (nonprogressors; n=40 eyes). Mean follow up time was 5.4 years. Morphological OCT features of the neurosensory retina, retinal pigment epithelium (RPE) and choroid were systematically...
evaluated by two independent graders. Logistic regression was used to evaluate the association between these OCT features and progression to advanced AMD.

RESULTS Neurosensory retinal thickness abnormalities were significantly associated with higher odds of progression to advanced AMD (either GA or NV forms), as well as to GA and NV independently (odds ratios [ORs]: 19.2 to 72.6; P<0.001). Disruption of the ellipsoid zone was also associated with progression to advanced AMD and NV (ORs: 17.9 and 30.6; P<0.001), with similar trends for GA. Disruption of the photoreceptor outer segments and interdigitation zone had non-significant trends in the same direction for each endpoint. Drusenoid pigment epithelial detachment and RPE thickening were also associated with higher odds of progression to advanced AMD (ORs: 5.6 and 5.0; P<0.001) and NV (ORs: 17.2 and 10.8; P<0.001, P=0.003). Presence of pigmentary hyperreflective material was associated with progression to each outcome (ORs: 7.5 to 12.8; P<0.01). Choroidal abnormalities were also investigated, and irregularity of these features was associated with progression.

CONCLUSION Systematic assessment of OCT features in early or intermediate AMD showed that abnormalities at the level of photoreceptors, RPE and choroid are associated with progression to both GA and NV. These findings provide insights into mechanisms of AMD progression, and may be helpful when identifying earlier endpoints for clinical studies.

TAKE HOME MESSAGE OCT Features Precede Advanced AMD and Differ Between Progressors and Non-Progressors with the Same Baseline Severity of AMD.

HUMAN RESEARCH This study involves human research.
IRB Approval Status: Approved by institutional review board
OCT Angiography (OCTA) and Exudative Age-Related Macular Degeneration (ARMD): Diagnosis, Treatment, and Correlation to IVFA and ICG Videoimaging

- Mark H. Nelson, MD, MBA

**OBJECTIVE** Is OCTA a valuable imaging modality in the diagnosis and treatment of Type 1 Exudative ARMD?

**PURPOSE** The effective diagnosis and treatment of Type 1 Exudative ARMD requires multimodality imaging in order to determine the most efficient therapy. ICG videoangiography frequently shows the presence of arteriolarized neovascularization, the presence of which might indicate possible resistance to anti-VEGF monotherapy. OCTA reveal these vessels with higher resolution and anatomic localization.

**METHODS** Retrospective, non-randomized study of 100 patients with treatment-naive and treated Type 1 Exudative ARMD who were sequentially evaluated by OCTA (Heidelberg Beta Version). Exclusion criteria involved concurrent vasculopathies, optic neuropathies, and/or the presence of previous vitrectomy. Preoperative VA, OCT, IOP, IVFA/ICG Videoangiography and OCTA were determined. Correlation to VA, degrees of
subRPE, subretinal and intraretinal exudation, heme, and changes in OCTA were determined to evaluate new biomarkers for threshold treatment and for retreatment/rescue when persistence was present.

**RESULTS** 57 of the 100 patients with Type 1 Exudative ARMD had distinct CNV noted on OCTA. These lesions were less apparent on ICG videoangiography and not appreciated at all on IVFA. 6 patients had CNV that were not being treated (subthreshold for anti-VEGF therapy), 10 patients had CNV that were being treated with anti-VEGF monotherapy (varying degrees of persistent CNV), 19 patients had CNV that were new targets for OCTA Directed PDT Triple Therapy, 16 patients had CNV without leakage s/p ICG-Directed PDT Triple Therapy (recurrent CNV subthreshold for repeat combination therapy), and 6 patients had thick (pachychoroid) choroid with CNV. 37 of the remaining patients had an pattern of perichoroidal activity that did not represent high flow, however, correlated to areas of ICG hyperflourescence and which evolved into frank CNV in 3 patients.

**CONCLUSION** OCTA provides a higher quality image of CNV present compared to ICG Videoangiography but has limitations of blood flow and leakage that still require OCT and IVFA modalities. Imaging CNV and how it responds to anti-VEGF monotherapy will give further insight into the pathophysiology of non-responsive lesions and provide a refined target for OCTA-Directed PDT Triple Therapy.

**TAKE HOME MESSAGE** OCTA of Type 1 Exudative ARMD reveals neovascular vessels with excellent detail and provide information that is critical to treating Exudative ARMD which is resistant to anti-VEGF monotherapy.

**HUMAN RESEARCH** This study involves human research.
IRB Approval Status: Exempt from approval
OBJECTIVE Discuss the challenges of studying surgical treatment (subretinal delivery of RGX-314 gene therapy) of a medical disease (nAMD).

PURPOSE RGX-314 (an AAV8 vector delivering an anti-VEGF fab protein) is currently being developed for the treatment of nAMD. An automated subretinal delivery process was developed by study investigators to deliver the gene therapy construct. Trial design, and the difficulty of designing a surgical therapy for a medical disease, as well as trial experience will be discussed.

METHODS RGX-314 subretinal delivery procedure was developed by study investigators for the Phase 1 RGX-314 clinical trial. An animal wetlab procedure was developed in which all investigators participated prior to trial initiation. The Phase 1 study will explore 3 doses of RGX-314 in a dose escalation with 6 subjects per arm. Patients enrolled in the trial demonstrated a treatment response and frequent need of anti-VEGF therapy. Subjects will receive gene therapy and be followed for 24 months to evaluate safety and evidence of a biologic effect.
RESULTS  RGX-314 gene therapy is delivered via an automated subretinal delivery process via the vitrectomy machine using a MedOne Microdose Injector syringe. The technique, approach, training and initial results have been standardized and will be presented. The Phase 1 study design, the challenges of designing a surgical therapy for a medical disease, and early surgical results will be presented as well as an update on study status.

CONCLUSION  RGX-314 gene therapy subretinal delivery utilizes an automated technique that was the result of a collaborative development which will have future potential for gene therapy delivery. The challenges of standardizing a surgical approach to retinal treatment, as well as designing a viable study protocol that can evaluate surgery for the management of a medical retinal disease will be discussed.

TAKE HOME MESSAGE  Study design of a surgical approach to a medical disease is challenging. RGX-314 gene therapy has potential to minimize the treatment burden of frequent intravitreal injections for nAMD.

HUMAN RESEARCH  This study involves human research.
IRB Approval Status: Approved by institutional review board
OBJECTIVE Stem cell-based therapies and gene therapy vectors delivered into subretinal (SR) space are increasingly promising surgical options for atrophic ARMD and other retinal conditions.

PURPOSE The SR stem cell or gene therapy surgical delivery techniques and visualization are currently evolving. Treatment success is dependent on accurate SR therapy delivery and intraoperative tissue depth assessment is challenging. We investigate the utility of prototype, swept-source, 4-dimensional (volumes over time) microscope-integrated optical coherence tomography (4D MIOCT) to guide SR surgery.

METHODS A prototype, swept-source 4D-MIOCT device was used to image ex-vivo surgical maneuvers in freshly enucleated porcine eyes. These surgical maneuvers include: 1) SR injection of fluid, 2) SR injection of retinal pigment epithelium (RPE) cell
suspension, 3) retinotomy creation, 4) SR insertion of polyester membranes with an RPE cell monolayer and 5) SR delivery of biodegradable RPE cell scaffold. The 4D-MIOCT scanner allowed live OCT imaging of surgical maneuvers, and the surgeon controlled the en face images, B-scans, and near real-time volumes displayed within surgical oculars and on a high-resolution monitor with a joystick foot pedal.

RESULTS High-quality 4D-MIOCT en face images, B-scans, and volumes were successfully acquired live and were visible to the surgeon while performing the 5 maneuvers. There was a clear advantage to the 4D-MIOCT guided subretinal surgery: the extent of the induced retinal detachment (bleb) was assessed; the cannula tip was guided in the SR space and any efflux of RPE cells into the vitreous cavity was immediately identified; SR scissors and open/closed maneuvers with respect to native RPE were clearly visualized; RPE scaffold was delivered precisely and carefully without damage to it or damage to surrounding tissues such as Bruch’s membrane or choroid. 4D MIOCT views allowed direct visualization and direct adjustment by the surgeon to avoid complications such as scissors touching RPE, or scaffold related RPE and choroid damage.

CONCLUSION 4D-MIOCT provided continuous high-resolution, cross-sectional visualization and volumetric data to guide SR surgery. Such near real-time visualization may enhance various current and under development SR delivery techniques for stem-cell therapies by guiding the precise delivery location and implant positioning and by decreasing the chances of known complications such as Bruch’s membrane rupture.

TAKE HOME MESSAGE Intraoperative 4D MI-OCT can contribute to the advancement of subretinal therapy delivery.

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