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Review

Towards Treatment of Stargardt Disease: Workshop Organized and Sponsored by the Foundation Fighting Blindness

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Accumulation of fluorescent metabolic byproducts of the visual (retinoid) cycle is associated with photoreceptor and retinal pigment epithelial cell death in both Stargardt disease and atrophic (nonneovascular) age-related macular degeneration (AMD). As a consequence of this observation, small molecular inhibitors of enzymes in the visual cycle were recently tested in clinical trials as a strategy to protect the retina and retinal pigment epithelium in patients with atrophic AMD.

To address the clinical translational needs for therapies aimed at both diseases, a workshop organized by the Foundation Fighting Blindness was hosted by the Department of Pharmacology at Case Western Reserve University on February 17, 2017, at the Tinkham Veale University Center, Cleveland, OH, USA. Invited speakers highlighted recent advances in the understanding of the pathophysiology of Stargardt disease, in terms of its clinical characterization and the development of endpoints for clinical trials, and discussed the comparability of therapeutic strategies between atrophic age-related macular degeneration (AMD) and Stargardt disease. Investigators speculated that reducing the concentrations of visual cycle precursor substances and/or their byproducts may provide valid therapeutic options for the treatment of Stargardt disease. Here we review the workshop's presentations in the context of published literature to help shape the aims of ongoing research endeavors and aid the development of therapies for Stargardt disease.

translational vision science & technology

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Background on Stargardt Disease and Atrophic AMD

Stargardt disease type 1 (STGD1; OMIM 248200) is the most prevalent single-gene inherited retinal dystrophy in humans with an incidence of 1:8000 to 10,000.[1,2] It has an autosomal recessive mode of inheritance and may lead to legal blindness within the second or third decade of life. Allikmets et al.[3] identified mutations in the *ABCA4* gene as the cause for the disease, and there are currently over 1000 mutations in this gene that are associated with STGD1.[3,4] The large degree of genetic heterogeneity makes it difficult to associate specific phenotypic features with specific alleles. Although phenotypic heterogeneity also is substantial, there are predictable patterns of disease in all patients with STGD1. The pathology begins in the macula with involvement of the para-foveal and foveal regions and then progresses outward eventually to the peripheral retina. With time, there is degeneration of photoreceptors and of the retinal pigment epithelium (RPE) accompanied by progressive vision loss.

The *ABCA4* gene encodes the ATP-binding cassette, subfamily A, member number 4 (ABCA4) transporter and is expressed in retinal photoreceptors. ABCA4 is localized at the rim of rod and cone photoreceptor outer segment (OS) disc membranes and plays an important role in the visual cycle (Fig. 1). The visual cycle is a series of enzyme-catalyzed reactions that convert all-*trans*-retinal to 11-*cis*-retinal for the regeneration of visual pigments in photoreceptor cells. Produced by bleached visual pigments, all-*trans*-retinal is a reactive aldehyde that is reduced to all-*trans*-retinol. Acting as an importer, ABCA4 hydrolyzes ATP with its cytoplasmic ATPase domains to flip a molecule of the N-retinylidene-phosphatidylethanolamine (PE) Schiff base conjugate from the intradiscal space to the cytoplasm.[5] There this conjugate is hydrolyzed and the all-*trans*-retinal is reduced to the corresponding alcohol. Thus, ABCA4 is a critical component of photoreceptor homeostasis that transports all-*trans*-retinal to enzymes of the visual cycle.

AMD, a late-onset, multifactorial retinal degenerative disease, can display some phenotypic features similar to STGD1. AMD is the leading cause of vision loss in individuals older than 50 years of age in developed countries, affecting about 1.75 million people in the United States (National Eye Institute, National Institutes of Health, https://nei.nih.gov/health/maculardegen). With ageing, susceptible subjects develop drusen consisting of visible yellow acellular debris between the RPE and Bruch's membrane. The retina and RPE can develop choroidal neovascularization (CNV) and/or geographic atrophy (GA) manifesting as central blindness. GA represents an end-stage of this disease, characterized by the progressive degeneration of photoreceptors and the RPE. Much like STGD1, GA begins in the macula and progresses peripherally with associated vision loss. The foveal region can be spared early in the disease but typically is involved as symptoms worsen.

Foundation Fighting Blindness Workshop Discussion

A recent workshop sponsored by the Foundation Fighting Blindness reviewed the mechanistic basis of both current and possible future therapies for Stargardt disease. Also discussed was whether currently tested therapies for GA associated with AMD could be applied to Stargardt disease. The Department of Pharmacology at Case Western Reserve University School of Medicine hosted the meeting that involved presentations from an international group of scientists and clinicians. The scientific content of this meeting, selected by Hendrik Scholl from the University of Basel and the Foundation Fighting Blindness, is summarized below in conjunction with published research.

Earliest Detectable Abnormalities in *ABCA4*-Associated Retinal Disease

Artur V. Cideciyan, PhD, started the scientific section of the workshop with a description of the natural history of STGD1 associated with mutations of the *ABCA4* gene. Current hypotheses of human pathophysiology are based on a combination of experimental results from in vitro studies, animal models, and noninvasive measurements in patients. There are two potential early disease triggers that lead to well-accepted late disease consequences of photoreceptor degeneration and vision loss in STGD1. Earliest disease may be triggered by accumulation of bisretinoid adducts of all-*trans*-retinal in the OS or in the RPE, or both (Fig. 2).[6–10] Bisretinoid adducts in the RPE are thought to form a subset of lipofuscin pigments that can be detected noninvasively with autofluorescence imaging using

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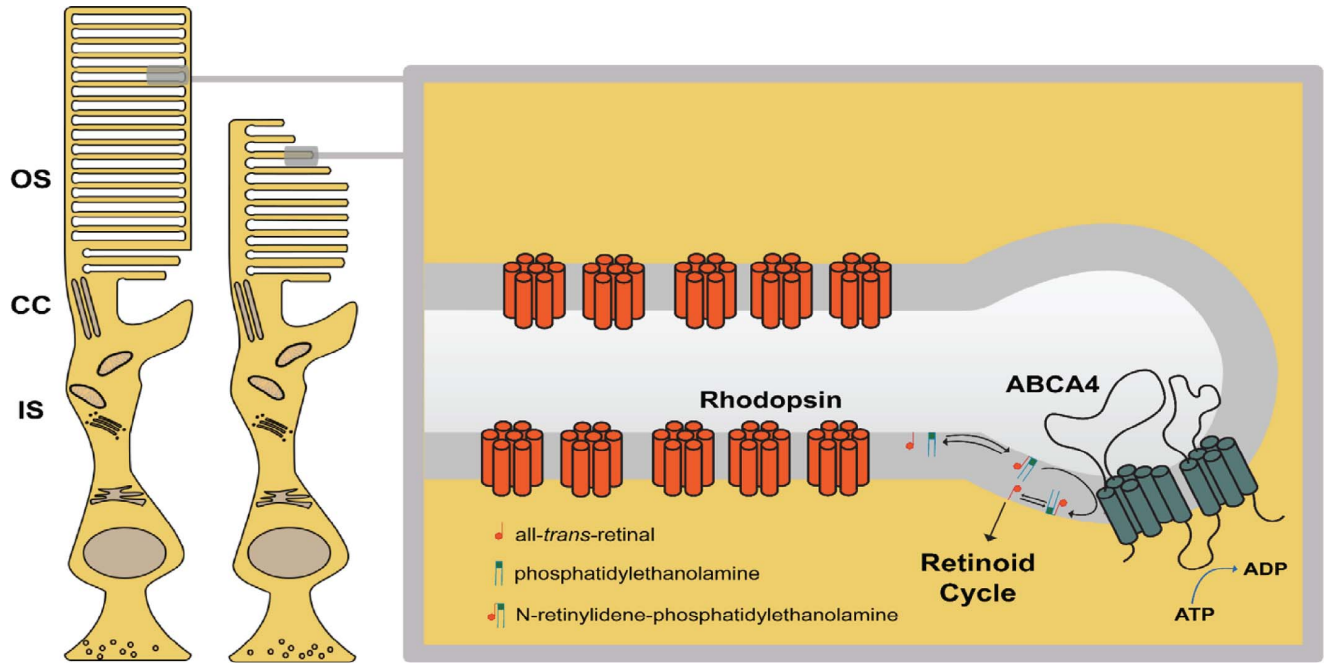

Figure 1. Rod and cone photoreceptors and ABCA4 mechanism in OS disc membranes. In both rod and cone cells, the light-sensitive OS is attached to the inner segment (IS) by a connecting cilium (CC). Rhodopsin, the visual pigment in rod cells, comprises the protein opsin bound to the chromophore 11-*cis*-retinal, which is isomerized to all-*trans*-retinal upon photon absorption. Once released from opsin, all-*trans*-retinal then reacts with PE to form a Schiff base. ABCA4 then "flips" all-*trans*-retinal to the outer portion of the disc membrane to access the visual cycle.

short-wavelength excitation light. The other major RPE pigment, melanin, can be detected with autofluorescence imaging using near-infrared excitation light. Melanin is not known to be related to bisretinoid adducts.

Most patients with *ABCA4*-STGD1 demonstrate characteristic spatially homogeneous increases in RPE lipofuscin signals in retinal regions with otherwise normal photoreceptor function.[7] In addition, there are colocalized increases in RPE melanin signals. Measurable changes to photoreceptor structure and function appear to temporally follow or spatially trail the RPE lipofuscin and melanin abnormalities.[11] However, it is important to note that there are no current noninvasive methods that can detect bisretinoid adducts in the OS of *ABCA4*-STGD1 patients. Future work evaluating earliest disease and its progression should be designed to minimize the iatrogenic potential of light that can be absorbed by opsins and accumulated bisretinoid. Reduced-illuminance autofluorescence imaging (RAFI) methods were developed for this purpose.[12–14] Better understanding of disease initiation and progression will allow design of outcome measures appropriate for treatment trials directed at different severity stages of *ABCA4*-STGD1.

Various Pharmacological Approaches to Treat Stargardt Disease

Paul Bernstein, MD, PhD, summarized the various pharmaceutical approaches to decreasing production of di-retinoid-pyridinium-ethanolamine (A2E) to slow retinal cell death, many of which are discussed in sections to follow. He expanded on the concept that the fluorophores in ABCA4-mediated disease are lipofuscinlike and composed of Schiff base forms of A2E.[15] As noted above, A2E is one of many autofluorescent bisretinoids that can cause light-induced free radical production, lysosomal dysfunction, and complement activation.[16] Therefore, many therapies under investigation are aimed at restricting all-*trans*-retinal and consequently the production of A2E. These include restricting all-*trans*-retinal production by limiting light exposure and reducing vitamin A (all-*trans*-retinol) intake, increasing vitamin A excretion, and using inhibitors to prevent vitamin A transport to the eye. Other tested strategies include

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direct inhibitors of the visual cycle, such as Accutane (Roche, Basel, Switzerland) and fomepizole (X-gen, Horseheads, NY) which inhibit oxidation of atROL to all-*trans*-retinal. Methods of reducing bisretinoid formation include the use of a deuterated form of vitamin A (ALK-001, C20-D3-vitamin A, Alkeus Pharmaceuticals, Boston, MA), which slows the rate of all-*trans*-retinal dimerization, A2E formation, and lipofuscinogenesis.[17] Remofuscin (Katairo, Kusterdingen, Germany) is a compound linked to the reduction of lipofuscin in RPE cells after its oral administration. All mechanisms theoretically decrease A2E formation, potentially slowing the progression of Stargardt disease. PE is the other component of A2E, but its reduction is not practical due to its more general role as a major constituent of cellular membranes.

Bernstein also demonstrated that A2E concentration is inversely related to the concentration of carotenoids. These yellow to orange macula pigments are derived from plants, and studies of animal eyes and cadaver eyes of patients with Stargardt disease indicate that high levels of these carotenoids reduce A2E levels.[18] Bernstein noted that carotenoids can reduce the amount of lipofuscin by half.[19] Finally, although light restriction could decrease the nonenzymatic formation of A2E, Bernstein underscored the limited practicality of this therapeutic approach.

Visual Cycle Modulators and Aldehyde Traps: Results from Animal Models of Stargardt Disease

Attendees at the meeting next discussed the importance of determining additional molecular details of the visual cycle to further establish the mechanisms underlying ABCA4-mediated retinal dystrophy. Rhodopsin is the initiating component of visual transduction.[20] In the dark, opsin proteins with bound chromophore exist at high concentrations (3–5 mM) in the outer retina, and are fully regenerated with the chromophore 11-*cis*-retinal after the absorption of light. The visual cycle, consisting of a cascade of enzymes and binding proteins in both the photoreceptor and RPE cell layers,[21] is required for this process. The potentially toxic aldehyde moiety in all-*trans*-retinal is formed following the isomerization from the 11-*cis* to all-*trans* configuration upon absorption of a photon (Fig. 2). ABCA4 uses an energy-dependent process to promote the clearance of all-*trans*-retinal from the OS disc membranes after its conjugation to PE. That A2E accumulation could be alleviated either by increased clearance or decreased production of the all-*trans*-retinal substrate offers researchers a testable strategy for developing new therapies. Thus, inhibiting enzymes of the visual cycle could be one such possibility. The RPE-specific 65 kDa retinoid isomerase (RPE65) is the rate-limiting enzyme of the visual cycle that converts all-*trans*-retinyl esters to 11-*cis*-retinol, before the active chromophore is formed by subsequent oxidation and recombination with opsin. Solving the molecular structure of RPE65 enabled the development of specific inhibitors, which slow the visual cycle, and thereby the formation of all-*trans*-retinal as demonstrated both in vitro and in vivo.[22–24] Most notably, the visual cycle inhibitor, emixustat, is a primary amine and a structural mimetic of 11-*cis*-retinol. Emixustat binds to the catalytic site in RPE65 with high affinity. This inhibitor is transported from the serum into the eye after its absorption in the digestive tract, providing a safe route for therapeutic delivery. However, a problem associated with the inhibition of RPE65 stems from the concomitant reduction of visual responses and transient reductions of light sensitivity arising from the decrease of visual chromophore. Thus, new therapies that do not directly target visual cycle inhibition are likely to be required to prevent an unwanted, although reversible, side effect.

The value of a novel double knockout (KO) mouse ($Rdh8^{-/-}Abca4^{-/-}$) as a model for the development of novel therapeutics for Stargardt disease was next discussed.[25] This model displays an acute light-induced retinal degeneration that recapitulates many aspects of Stargardt disease, even though it is not an exact model. The mouse model has been used to demonstrate that primary amines can create a protonated Schiff base adduct with free all-*trans*-retinal and thereby alleviate light-induced pathology potentially providing an alternative strategy for the treatment of retinal dystrophies.[26] For example, *VM200* can trap all-*trans*-retinal without inhibiting the visual cycle. Retinoid-mimetics without a primary amine can be effective inhibitors of RPE65, but they cannot react directly with all-*trans*-retinal and, therefore, they are less effective than the primary amines at preventing retinal degeneration in the $Rdh8^{-/-}Abca4^{-/-}$ mouse model. These findings support the testing of primary amines that act as Schiff base traps but do not inhibit RPE65 to treat Stargardt

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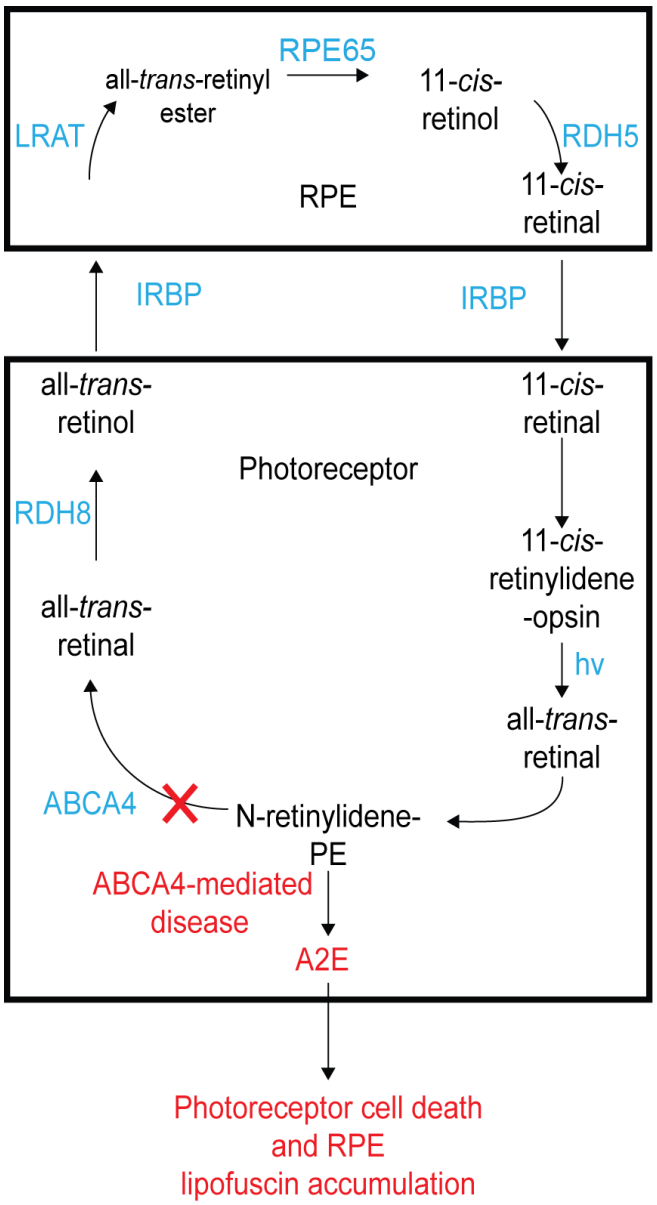

Figure 2. The visual (retinoid) cycle in ABCA4 mediated disease. The retinoid cycle, supporting both rod and cone photoreceptor cells, displaying the enzymatic conversion of all-*trans*-retinal to 11-*cis*-retinal. Absorption of a photon of light (hv) by the visual pigment (11-*cis*-retinylidene-opsin) is isomerized to the active state of rhodopsin. Hydrolysis of the Schiff base linkage then releases free all-*trans*-retinal, which conjugates to PE within the disc membrane to form N-retinylidene-PE. Acting as an importer, ABCA4 hydrolyzes ATP by its cytoplasmic ATPase domains to flip a molecule of the N-retinylidene-PE Schiff base conjugate from the intradiscal space to the cytoplasm, where this conjugate is hydrolyzed and the all-*trans*-retinal is reduced to the nontoxic all-*trans*-retinol. The molecule is transported across the interphotoreceptor matrix by interphotoreceptor retinoid binding protein (IRBP) to the RPE where it is esterified by LRAT and subsequently isomerized by RPE65 to form the 11-*cis*-retinal molecule, which is able to bind opsin to form the visual chromophore after transport back to photoreceptors by IRBP. In the absence of functional ABCA4, all-*trans*-retinal will accumulate in OS membranes, which leads to the accumulation of bisretinoid and lipofuscin in both photoreceptors and the RPE, a hallmark of ABCA4 mediated disease, and ultimately cell death.

disease. This strategy would eliminate the undesired side effects on visual performance while retaining retinal specificity.

Studies utilizing the double KO mouse model also provide evidence that the reactive aldehyde is indeed toxic, because the absence of retinol dehydrogenase 8 (RDH8) prevents the reduction of all-*trans*-retinal to the corresponding alcohol. The double KO mouse is unable to clear or reduce all-*trans*-retinal, which eventually accumulates leading to retinoid deposition and cell death. Together, these studies help define a strategy for protecting the macula by reducing the level of reactive aldehydes derived from the visual cycle. In addition, a missense mouse mutant, L541P;A1038V *Abca4*$^{-/-}$, was discussed as a possible model for Stargardt disease as it genetically resembles the human disease in addition to its pattern of progressive retinal degeneration and increased levels of autofluorescence and all-*trans*-retinal accumulation.[10]

Palczewski also described a systems pharmacology approach to identify convergent pathways that could provide targets for pharmacological protection of photoreceptors and RPE cells at relatively safe drug concentrations. Systems pharmacology initially involves the identification of multiple signaling pathways that culminate in a combined molecular response. Thus, instead of using high and potentially toxic doses of an individual drug to affect the desired response, a combination of two or more drugs, each at its own subtherapeutic safe dose, could be employed to achieve the desired synergistic pharmacological effect. For example, in addition to being the targets for over half of all Food and Drug Administration (FDA)-approved drugs, different G protein-coupled receptors (GPCRs) can target the same convergent intracellular pathway. In this manner, known and FDA-approved pharmaceutical ligands targeting differing receptors can be combined to selectively modulate these convergent pathways. Receptors can be identified by genomic and proteomic approaches, such as RNA sequencing, and compounds known to target them can be repurposed for the treatment of diseases unrelated to the original

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disorders for which they were developed. Palczewski's group has recently validated this approach to show protection against light-induced deposition of lipofuscin and bisretinoid in the *Rdh8*$^{-/-}$*Abca4*$^{-/-}$ mouse model.[27]

ALK-001 (Deuterated Vitamin A) Corrects the Aberrant Dimerization of Vitamin A and Prevents Loss of Visual Function in Mouse Models

The drug ALK-001, under development by Alkeus Pharmaceuticals, is currently being evaluated in a double-masked, placebo-controlled, clinical trial in Stargardt disease (Tolerability and Effects of ALK-001 on Stargardt Disease, TEASE, clinicaltrials.gov identifier: NCT02402660). Ilyas Washington, PhD, summarized this effort. ALK-001 is a chemically modified form of vitamin A, in which three hydrogen atoms have been replaced with deuterium atoms (heavy hydrogen). This replacement mitigates the abnormal dimerization of vitamin A,[28,29] which is thought to lead to retinal degeneration, yet preserves the necessary functions of vitamin A.

ALK-001 is unique in that it acts as a replacement for vitamin A. ALK-001 can mitigate the formation of bisretinoids and other oligomers of vitamin A without depriving the photoreceptors of vitamin A or modifying the concentration of ocular retinaldehyde. As such, ALK-001 is not a visual cycle modulator. Vision and the survival of the retina are dependent on a steady supply of vitamin A.[30–33] For example, genetic mutations, such as *RBP4*,[34] *LRAT*, and *RPE65*,[35] which result in the impaired delivery of vitamin A to retinal photoreceptors cells, can lead to visual impairment and retinal degeneration.

ALK-001 has been evaluated by multiple groups, in long-term preclinical studies, lasting between 9 and 18 months, using three different mouse models of Stargardt disease—two single *Abca4* KO models and the double KO mouse model discussed during the meeting.[17,36] In preclinical studies, ALK-001 reduced signature changes associated with retinal degeneration, including the accumulation of lipofuscin, age-related declines in electroretinography (ERG) amplitudes, delayed dark-adaptation, and RPE pathology. Furthermore, since ALK-001 does not modulate the concentration of retinaldehyde, but prevents the aforementioned retinal pathology, preclinical data indicate that retinaldehyde does not contribute to retinal degeneration in Stargardt disease.[37]

Preclinical and clinical studies demonstrated that ALK-001 can prevent the dimerization of vitamin A by approximately 4- to 5-fold, within 4 weeks, of its once-a-day dosing. In the ongoing phase 2 study, TEASE, the longest treatment duration to date has been about 21 months. There has been no report of vision-related side effects, such as delayed dark adaptation or nyctalopia. Because its safety profile is expected to be identical to that of nondeuterated vitamin A, ALK-001 may be safely administered to elderly patients and to children, as a strategy to mitigate the aberrant dimerization of vitamin A, well before vision loss begins, and with the potential to prevent vision loss and retinal degeneration due to Stargardt disease.

Removal of Lipofuscin As an Approach to Treat Stargardt Disease

Ulrich Schraermeyer, PhD, discussed the removal of lipofuscin as an alternative approach to decreasing the amount of deleterious fluorescent material. He described the difference between fluorescent signals in pigmented versus albino mice that harbor either the ABCA4$^{-/-}$ KO or the ABCA4$^{-/-}$/RDH8$^{-/-}$ double KO. Pigmented mice display a granular distribution of fluorescent material within melanolipofuscin granules versus a diffuse distribution throughout the cytoplasm of photoreceptors in albino mice. This suggests that melanosomes, a form of lysosomes, might be important mediators of lipofuscin formation, detoxification, and/or clearance.[38,39] He presented transmission-electron microscopy (TEM) images of RPE after photodynamic therapy, intravitreal horseradish peroxidase injection, and blue light exposure, indicating that removal of lipofuscin from the RPE is possible without damaging the RPE cell layer. Remofuscin (Katairo), a small molecule granted orphan status in Europe, stimulated the exocytosis of lipofuscin from the RPE. In primates, 1 year of dosing of Remofuscin at 24 mg/kg/day caused the reduction of lipofuscin with no adverse effects when quantified by TEM.[40] In ABCA4$^{-/-}$ KO mice, there was greater than a 2-fold trend in reduction in lipofuscin granules after intravitreal injection of Remofuscin, as measured by TEM. In ABCA4$^{-/-}$/

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RDH8$^{-/-}$ double KO mice, treatment was associated with an increase in the survival of photoreceptor nuclei (Fang Y, et al. *IOVS*. 2017; ARVO E-Abstract B0287).[32]

Lessons Learned from the Natural History of the Progression of Atrophy Secondary to Stargardt Disease (ProgSTAR)

SriniVas Sadda, MD, reported the interim imaging results of ProgSTAR (funded by the Foundation Fighting Blindness), a combined retrospective and prospective natural history study of Stargardt disease using fundus autofluorescence (FAF) to quantify the progression of Stargardt disease as the primary outcome measure. This was combined with secondary assessments of visual acuity, microperimetry, and spectral-domain optical coherence tomography (SD-OCT) to analyze the progression of retinal disease.[41,42] Due to the variability of FAF abnormalities in Stargardt patients, two main categories were adopted for the study: (1) definitely decreased autofluorescence (DDAF) and (2) questionably decreased autofluorescence (QDAF) that is either poorly demarcated QDAF (PD-QDAF) or well-demarcated QDAF (WD-QDAF). Using this FAF classification approach, Doheny Image Reading Center researchers found near equal distribution of PD-QDAF, DDAF, and PD-QDAF with a similar distribution of DDAF and WD-QDAF. Sadda concluded that Stargardt disease comprises heterogeneous phenotypes of atrophy with variable natural histories and progression rates. Of importance, specific associated features on FAF imaging in a heterogeneous background appeared to be associated with a more rapid progression of disease based on FAF and SD-OCT. The findings may enable improved patient selection for clinical trials that must be appropriately designed to determine safety and efficacy.

Similarities between the Pathophysiology of Recessive Stargardt Disease and Atrophic AMD

Janet R. Sparrow, PhD, presented her findings that lipofuscin is formed by condensation of retinaldehyde and amines. The resulting bisretinoid products formed in photoreceptors with accumulation in the RPE being correlated to atrophy and blindness. Her studies on lipofuscin granules indicate that the content of RPE lipofuscin housed in lysosomal storage bodies includes less than 2% amino acids derived mostly from the membranous coat of storage organelles.[43] While genotype and phenotype correlations are difficult to establish in ABCA4-mediated disease, analysis by quantitative AF (qAF) revealed that STGD1 patients carrying the complex allele [L541P:A1038V]R1640W have, at an early age, 4- to 5-fold increased qAF as compared to the disease-causing G1961E mutation.[44] Also, carriers of ABCA4-mutations (heterozygous) exhibit qAF levels within the range of healthy eyes.[45] As a model of STGD1, *Abca4*$^{-/-}$ KO mice have a 3- to 4-fold increase in bisretinoid measured by HPLC or qAF relative to control mice, and photoreceptor loss is detected by 8 months of age as a reduction in outer nuclear layer thickness.[46–48] Bisretinoids may damage the retina because they are photoreactive and degrade into smaller reactive aldehydes and carbonyls in the presence of light and oxygen. Bisretinoid photodegradation has been demonstrated by comparing cyclic-light versus dark-reared mice, comparing albino and black mice and by studying mice treated with vitamin E.[49]

RPE atrophy is a significant feature of both STGD1 and GA in AMD. In STGD1, the extent of photoreceptor ellipsoid zone loss in SD-OCT correlated with RPE atrophy visualized as greatly reduced or absent near infrared (NIR)-AF signal generated primarily from RPE melanin.[50] Thus, in most cases RPE atrophy probably precedes photoreceptor cell degeneration. Additionally, since the dicarbonyls released by bisretinoid photodegradation are capable of cross-linking proteins, bisretinoid photodegradation may be linked to age-related changes in Bruch's membrane. Proteins modified by these dicarbonyls are also detected in drusen.[51] The photo-oxidative processes initiated by RPE bisretinoid could explain suggested links to light exposure in both STGD1 and AMD.[52–55] In a study of fundus flecks in STGD1, it was found that the NIR-AF signal generated largely by RPE melanin is absent at positions of flecks visible in photoreceptor-attributable bands of SD-OCT images. Thus, Sparrow suggested that the short-wavelength autofluorescence signal of flecks originates in the augmented bisretinoid of impaired photoreceptor cells.[56]

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Comparison of RPE Atrophy Progression in Late-Onset Stargardt Disease and AMD

Carel Hoyng, PhD, made the case that there are similarities between AMD and STGD1, but thinks this may be the consequence of some late-onset Stargardt patients being misdiagnosed as AMD patients. He described a form of late-onset Stargardt disease that is remarkably similar to AMD but has a few noticeable differences. Primarily, foveal sparing occurs in both diseases, but patients with late-onset Stargardt disease have longer survival of foveal regions. Secondly, RPE atrophy progresses slower in late-onset Stargardt disease. Other notable differences in late-onset Stargardt disease that are often overlooked is the presence of flecks, not drusen, and the absence of CNV. Hoyng made clear that foveal sparing with parafoveal atrophy is a common finding in both Stargardt and AMD and implies subfoveal RPE survival. A noninterventional prospective study is underway to assess the *Abca4* genotype in AMD-like patients with a Stargardt-like phenotype, which will help clarify treatments between the overlapping phenotypes and possible overlying disease.

Lipofuscin-Related Quantitative AF: Findings and Relevance in AMD and Stargardt Disease

Peter Charbel Issa, MD, PhD, refined the lessons learned from qAF imaging. Cideciyan, Sparrow, and others noted the increase in FAF with age and disease in the peripheral macula and retina, yet Sadda described an FAF biomarker that relies on decreased central AF. Charbel Issa highlighted previous references and personal observations that an increase in lipofuscin-related autofluorescence intensity measured by qAF precedes a relevant decline of retinal function, particularly outside the fovea where qAF measurements are more reliable.[7] This concept is confirmed in the *Abca4*$^{-/-}$ mouse in which lipofuscin-related autofluorescence intensity increases to a ceiling level before significant photoreceptor damage and functional deterioration is observed.[39] Although qAF thus may be a good parameter to monitor disease progression in early disease, potential light toxicity of this imaging method needs to be considered.

Charbel Issa also noted that in contrast to Stargardt disease, direct evidence is missing for an overall abnormally increased lipofuscin load in patients with AMD. In fact, a recent study indicated that patients with early and intermediate AMD have normal-low lipofuscin-levels when measured by qAF,[57] a finding supported by histological studies.[58,59] This finding is an additional parameter distinguishing early and intermediate AMD from nonatrophic Stargardt disease in which increased qAF is a rather consistent phenotype. Although limitations of the study need to be considered, this qAF study may influence the understanding of AMD pathogenesis and may have an effect on upcoming treatment trials that aim to modify lipofuscin accumulation.

Emixustat Hydrochloride for Macular Atrophy in AMD and Stargardt Disease: Lessons Learned from a Phase 2 Efficacy Trial of GA in AMD

Philip J. Rosenfeld, MD, PhD, next communicated the results of a study involving emixustat hydrochloride (SEATTLE, clinicaltrials.gov identifier: NCT01802866), the oral small molecule primary amine that is a reversible antagonist of RPE-65 (see above). In the study, 508 patients with GA secondary to nonexudative AMD participated in a dose/response versus placebo study. Subjects were randomized to one of four types of treatment; placebo or 2.5, 5, or 10 mg of emixustat taken orally each evening for 24 months. Thirty days after completion of the study drug, the patients underwent an exit visit. The primary endpoint was mean change in total area of GA in the study eye after 2 years. Means ranged from 1.69 mm^2/year to 1.84 mm^2/year, and there existed no statistical differences between placebo and any dose of emixustat. Importantly, ERG rod response at month 3 showed a dose-dependent reduction in b-wave amplitude pre- and postbleaching. Rosenfeld reported that emixustat failed to meet its primary goal in the phase 2 study because it did not prevent the growth of GA and vision loss. However, the growth of GA in AMD may involve different pathways in addition to the potential pathway of RPE death as a result of A2E accumulation. If the goal was to slow down the visual cycle by inhibiting RPE65,

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then the outcomes from the emixustat study strongly suggest that the goal of visual cycle inhibition was achieved. There was a high rate of patient dropout due to diminished vision because of inhibiting the visual cycle. In fact, by 3 months, emixustat had caused a 40% and 90% reduction of the b-wave amplitudes after patients received oral daily doses of 2.5 mg and 10 mg, respectively. This study provided the first demonstration in a prospectively, randomized trial that an orally administered drug could selectively and reversibly interfere with the visual cycle in a dose-dependent manner. Based on the safety and visual cycle efficacy of emixustat, a small pharmacodynamics study of emixustat in Stargardt disease is underway (NCT03033108).

Summary

The workshop organized and sponsored by the Foundation Fighting Blindness highlighted the critical role that the reactive aldehyde all-*trans*-retinal plays in bisretinoid production and the importance of bisretinoid accumulation in the pathophysiology of Stargardt disease. The impaired activity or expression of ABCA4 hinders the transfer of conjugated all-*trans*-retinal from the inner to the outer surface of disc membranes where it can be reduced and enter the visual cycle for further processing. Instead, retinaldehyde forms complexes with PE (bisretinoids such as A2E) that are autofluorescent and toxic. This process is an indicator of the metabolic cooperativity between the RPE and photoreceptors, because it reflects an inherent photoreceptor defect that ultimately compromises the RPE. There are two phases to the development of autofluorescence in the RPE: an early disease stage in which the severity positively correlates with an increase in AF, and a late disease stage characterized by a decrease in AF, which reflects RPE cell death.

Do bisretinoids such as A2E cause the death of the RPE? There is ample evidence that bisretinoids are harmful to cells in culture and to the RPE in *Abca4*$^{-/-}$ and *Rdh8*$^{-/-}$*Abca4*$^{-/-}$ KO mice. The deposition of bisretinoids within lipofuscin in the RPE eventually results in reduced ERG signals and atrophy of the RPE/retina. Do these lipofuscin deposits uncouple the metabolic cooperativity between the RPE and retina? Do they destroy the lysosomal activity of the RPE? Are they targets of the immune system? The exact mechanisms by which bisretinoids induce cell death are yet to be determined.

Is there a good rationale for slowing the production of bisretinoids through visual cycle inhibition to limit production of the reactive aldehyde in recessive Stargardt disease and/or AMD? The clinical data regarding the use of emixustat for nonexudative AMD suggest that this strategy has clear limitations. First, half the patients did not complete the study owing to side effects limiting vision. Second, treatment had no demonstrable effect on the progression of GA. However, it is possible that younger Stargardt patients may exhibit greater tolerance toward emixustat and demonstrate some efficacy relative to AMD patients, and, indeed, a clinical study has recently been initiated for Stargardt disease. Results from the SEATTLE study on GA indicate that by blocking RPE65, emixustat may be a victim of its own success. A slower visual cycle may indeed promote cell survival, but the depletion of the visual chromophore impairs vision to an extent potentially comparable to that observed in Leber congenital amaurosis. ALK-001, which does not inhibit the visual cycle, may be better suited to elucidate the role of bisretinoids in Stargardt disease. Perhaps emixustat and other visual cycle inhibitors might have a role in a low dose combination therapy with drugs targeting synergistic pathways. Future research involves pharmaceutical or genetic means to quench or improve the clearance of retinaldehyde in addition to further investigation into inhibiting the visual cycle.

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relating to Remofuscin, which have been licensed to Katairo (P); **J.R. Sparrow,** None; **I. Washington** is an inventor on patents relating to ALK-001, which have been licensed to Alkeus Pharmaceuticals (F); **H.P.N. Scholl** is on the scientific advisory board of Vision Medicines and lead PI of Alkeus Pharmaceuticals' TEASE study (E)

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