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Rationale of Basic and Cellular Mechanisms Considered in Updating the Staging System for Diabetic Retinal Disease

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Purpose: Hyperglycemia is a major risk factor for early lesions of diabetic retinal disease (DRD). Updating the DRD staging system to incorporate relevant basic and cellular mechanisms pertinent to DRD is necessary to better address early disease, disease progression, the use of therapeutic interventions, and treatment effectiveness.

Design: We sought to review preclinical and clinical evidence on basic and cellular mechanisms potentially pertinent to DRD that might eventually be relevant to update the DRD staging system.

Participants: Not applicable.

Methods: The Basic and Cellular Mechanisms Working Group (BCM-WG) of the Mary Tyler Moore Vision Initiative carefully and extensively reviewed available preclinical and clinical evidence through multiple iterations and classified these.

Main Outcome Measures: Classification was made into evidence grids, level of supporting evidence, and anticipated future relevance to DRD.

Results: A total of 40 identified targets based on pathophysiology and other parameters for DRD were grouped into concepts or evaluated as specific candidates. VEGFA, peroxisome proliferator-activated receptor-alpha related pathways, plasma kallikrein, and angiopoietin 2 had strong agreement as promising for use as biomarkers in diagnostic, monitoring, predictive, prognostic, and pharmacodynamic responses as well as for susceptibility/risk biomarkers that could underlie new assessments and eventually be considered within an updated DRD staging system or treatment, based on the evidence and need for research that would fit within a 2-year timeline. The BCM-WG found there was strong reason also to pursue the following important concepts regarding scientific research of DRD acknowledging their regulation by hyperglycemia: inflammatory/cytokines, oxidative signaling, vasoprotection, neuroprotection, mitophagy, and nutrients/microbiome.

Conclusion: Promising targets that might eventually be considered within an updated DRD staging system or treatment were identified. Although the BCM-WG recognizes that at this stage little can be incorporated into a new DRD staging system, numerous potential targets and important concepts deserve continued support and research, as they may eventually serve as biomarkers and/or therapeutic targets with measurable benefits to patients with diabetes.

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An understanding of basic and cellular mechanisms underlying the onset and progression of diabetic retinopathy (DR) is important to the Diabetic Retinal Disease (DRD) Staging System effort for several reasons. Knowledge of pathophysiologic mechanisms can often be used to determine the response of selected patient subgroups to known therapeutic interventions. In addition, the mechanistic details provide opportunity for development of novel interventional approaches, therapeutic drug design, testing of specific targets, discovery and validation of biomarkers, and direction for investigation of next generation interventional approaches.

Prior to the advent of molecular biology approaches and rigorous clinical trial evaluations, clinical evidence was generally obtained based on careful observation of treated

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and untreated patients with the condition of interest. The Basic and Cellular Mechanisms Working Group (BCM-WG) reviewed the current literature on targets for DRD in various stages of preclinical and clinical evaluation and identified those thought most likely to inform future clinical research and guide new staging, screening, and/or clinical management efforts.[1] Specifically, evidence from preclinical and clinical data were analyzed to categorize candidates based on Biomarkers, EndpointS, and other Tools (BEST) as used by the United States Food and Drug Administration[2] (FDA) to assess diagnosis, monitoring, pharmacodynamic response, predictive potential, prognostic ability, surrogate end point validity, and safety.

Several of the targets have previously been studied in basic, translational, and clinical research. An example is VEGF and its role in the pathophysiology of retinal vascular leakage and intraocular neovascularization. Data have demonstrated that VEGFA is a key modulator of proliferative DR (PDR) and diabetic macular edema (DME) in many patients with diabetes.[3] However, as learned from clinical trials, not all patients with DME fully respond to VEGF inhibition and, therefore, greater understanding of predictive biomarkers for this target is needed to determine which individuals may become good versus suboptimal responders. Also important are potential interactions of VEGF and other mechanistic pathways and the interactions between different cell types. For example, angiopoietin 2 (Ang2), produced by pericytes, can antagonize angiopoietin 1 (Ang1)/Tie2 signaling, which normally stabilizes the vasculature.[4] Angiopoietin 2 appears to lead to increased permeability of vessels, and combined Ang2/VEGFA inhibition may lead to extended duration of action in DME and neovascular age-related macular degeneration as compared with VEGFA inhibition alone.[5–7]

A principal task of the BCM-WG was to review available preclinical and clinical evidence on basic and cellular mechanisms potentially pertinent to DRD that might eventually be relevant to update the DRD staging system. In addition, the BCM-WG addressed key questions regarding prioritization of targets and gaps in knowledge that would prevent these variables from being used as biomarkers and as targets for interventional therapy. After review of many candidates, the BCM-WG also identified several key concepts that deserved special attention and future research to facilitate discovery of biomarkers and new targets for therapeutic consideration over the next few years.

Methods

General

The BCM-WG was one of 6 areas of DRD studied as part of the DRD Staging System update, a project of the Mary Tyler Moore Vision Initiative, a joint effort of JDRF, the Caswell Diabetes Institute (at the University of Michigan) and the Kellogg Eye Center, honoring Ms. Moore's contributions to diabetes awareness and research. There were 61 participants from 12 countries worldwide included in this initiative (Appendix). Other working groups included: Vascular Retina, Neural Retina, Systemic Health, Visual Function, and Quality of Life (Appendix). A joint group virtual meeting reviewed the goals to define gaps in knowledge to update the DRD staging system and address key questions of priority variables, how they fit into a proposed interim staging system, and the gaps in knowledge and research that require additional study. The BCM-WG included 13 participants, all with internationally recognized expertise in basic and/or clinical research of diabetes including knowledge of basic target development, clinical trial methodology, biochemical mechanisms, therapeutic development, drug testing, and models used in evaluating pathophysiologic mechanisms of DRD. Participants included faculty from academic organizations, private practice, and industry.

No patient data were reviewed and, therefore, human subjects approval was not needed. Therefore, institutional review board approval was waived. The study adhered to the Declaration of Helsinki. We performed a narrative review from April 2021 through May 2022. A literature search of online resources using Pubmed and the internet was performed assessing potential mechanisms, targets, and therapeutic approaches pertinent to DRD using the following search terms individually or in combinations: basic mechanisms, biochemical mechanisms, biochemistry, blood levels, cellular mechanisms, center-involved diabetic macular edema, C-reactive protein (CRP), clinically significant diabetic macular edema, diabetes, DME, DR, biomarkers of DRD, DME, intercellular adhesion molecule 1, interleukin (IL) 1 beta, IL-6, mechanisms, nonproliferative diabetic retinopathy (NPDR), novel interventions, novel targets, novel therapies, novel treatments, NPDR, PDR, plasma kallikrein (PKa), potential interventions, potential targets, potential therapies, potential treatments, PDR, risk of DR, tumor necrosis factor, vascular adhesion molecule 1, VEGFA, retinol binding protein 3, Ang2. In addition, the participants were asked to supply targets and pathways based on their expert knowledge specific to basic and cellular mechanisms of DRD.

This study coincided with the global coronavirus disease 2019 pandemic. Therefore, workgroup meetings and workshops were conducted virtually, via videoconference and through email. The meetings reviewed and provided guidance to participants who reviewed and compiled data presented in this manuscript. The initial list of targets and pathways was expansive and honed down to 40 targets that met criteria of some clinical or research evidence involved in DRD (Table 1). These were then extensively reviewed through multiple iterations and classified into evidence grids, level of supporting evidence, and anticipated future relevance to DRD.

Evidence Grids

The group populated evidence grids that were based on relevance of targets to disease stage, level of evidence currently available, and reasonable anticipated future evidence based on BEST.[2] Biomarkers were characterized as to their potential value for diagnosis, monitoring of disease,

Table 1. Relevance of Variables to Disease Stage

	Ready (For Current Use or within the Next 1−2 years)	Promising (Unmet, but Defined Research Needs That Can Be Accomplished Within Next 5 Years)	Potential (Unmet Research Needs That Will Need >5 Years to Accomplish)
Subclinical DRD (not clinically visible or evident)*		*Inflammation/proinflammatory cytokines*, *neuroprotection*, erythropoietin derivatives	RBP3, miR200, *mitochondrial/mitophagy*, sICAM1, sVCAM1, CRP
Early-stage clinical DRD†	Fenofibrate/lipid	VE-PTP, TNFα, *inflammation/proinflammatory cytokines*, *neuroprotection*, erythropoietin derivatives	RBP3, soluble epoxide enolase, IL-6, IL-1b, sICAM1, sVCAM1, CRP, erythropoietin derivatives, *mitochondrial/mitophagy*
Midstage clinical DRD‡	Fenofibrate/lipid, VEGF-A, plasma kallikrein	VEGF-R2, TNFα, *inflammation/proinflammatory cytokines*, *neuroprotection*, erythropoietin derivatives	RBP3, DLL4, soluble epoxide enolase, IL-6, LRG1, Egln1, IL-1b, sICAM1, sVCAM1, CRP, erythropoietin derivatives, *vascular protection/regeneration*, *mitochondrial/mitophagy*, TNFα, CRP, sVCAM1
Late-stage clinical DRD§	Ang2/Tie2, VEGF-A	CCL2/MCP-1, CCR2/CCR5, VEGF-R2, IL-6, TNFα	Norrin, Notch-3, LRG1, Egln1, CRP, IL-1b, sICAM1, sVCAM1, *vascular protection/regeneration*, *mitochondrial/mitophagy*,TNFα, sICAM1, sVCAM1

Ang2 = angiopoietin 2; CCL2/MCP-1 = chemokine ligand 2/monocyte chemoattractant protein-1; CCR2/CCR5 = chemokine receptor 2/chemokine receptor 5; CIDME = center-involved diabetic macular edema; CRP = C-reactive protein; DLL4 = notch ligand delta like ligand 4; DR = diabetic retinopathy; DRD = diabetic retinal disease; Egln1 = Egl-9 family hypoxia-inducible factor 1; IL = interleukin; LRG1 = leucine rich alpha-2-glycoprotein 1; miR200 = microRNA-200; RBP3 = retinol binding protein 3; sICAM1 = soluble intercellular adhesion molecule-1; sVCAM1 = soluble vascular cell adhesion molecule-1; TNFα = tumor necrosis factor alpha; VEGF-R2 = VEGF receptor 2; VE-PTP = vascular endothelial protein tyrosine phosphatase. A Summary of Target and *Concept* Priorities Based on Stage of DRD. Concept priorities in italic.
*Not clinically visible or clinically evident. Requires nonclinical exam approaches to detect.
†DR severity encompassed by current standard ETDRS severity levels 14 to 35, inclusive (questionable-mild NPDR).
‡DR severity encompassed by current standard ETDRS severity levels 43 to 53E, inclusive (moderate-severe NPDR).
§DR severity encompassed by current standard ETDRS severity levels 60 and higher, inclusive (PDR levels and ungradable) or CIDME.

prediction of outcome, prognosis, safety, pharmacodynamic response, and susceptibility/risk. Each member was asked to individually populate 4 different tables that characterized the 40 targets of interest.

A summary of the group's research of candidates relevant to research needs based on stage of DRD is presented in Table 1. Within each level of DRD, candidates were sorted as "ready" (currently in use or anticipated use within 2 years), "promising" (requiring research but believed to achieve use within 5 years), or "potential" (important and unmet need but probably requires >5 years of additional research). It was recognized by the group that research would be valuable regardless of whether outcomes supported the further use of the candidate. Levels of DRD were: subclinical or not clinically visible or clinically evident, requiring nonclinical exam approaches to detect; early-stage clinical DRD, DR severity encompassed by current standard ETDRS severity levels 14 to 35, inclusive from questionable DRD to mild NPDR; midstage clinical DRD, DR severity encompassed by current standard ETDRS severity levels 43 to 53E, inclusive of moderate to severe NPDR; and late-stage clinical DRD, DR severity encompassed by current standard ETDRS severity levels ≥60, inclusive of PDR levels and ungradable, or center-involved DME.

In Table 2, the BCM-WG categorized the level of evidence available for target candidates as I to V.[8] In Table 3, targets were characterized by their anticipated future relevance as biomarkers based on the FDA-National Institutes of Health Biomarker Working Group BEST criteria.[2] Targets were then finally characterized by level of evidence available in the literature as whether they were low, middle, or top candidates for eventual usefulness, or whether they were at too early a stage to be considered a candidate now but were important conceptually. The BCM-WG members were asked to participate actively for final conclusions, gap analysis and writing of the manuscript, especially within their areas of expertise and interest. All members reviewed the entirety of the manuscript and data and provided comments, edits, and revisions.

Selection of Top Candidates, Gap Analysis, and Final Conclusions

Group review of the tables was used to obtain consensus on the targets that had sufficient evidence to be most likely relevant within the time frames indicated. Due to the large number of diverse potential targets overall, only the selected targets were included for detailed discussion in the Results section of this manuscript. It was also clearly identified by the group that there were important conceptual areas, not just specific targets, which were highly promising and deserved discussion. These concepts identified as important for future study were inflammation/cytokines, oxidative signaling, vasoprotection, neuroprotection, mitophagy, and nutrients/microbiome.

Table 2. Level of Evidence Currently Available for Variables

	Level of Evidence				If Level I–III: What is Needed to Address Current Unmet Research Needs to Definitely Show whether This Variable is Worthwhile or Not Worthwhile for Use in a DRD Staging System?
	I	*II*	*III*	*IV-V*	
7-ketocholesterol				X	7-ketocholesterol, which increases in cardiovascular disease and in aging. Intersection of aging and DR.
Ang2	X	X			Need data ideally from human studies aimed at clearly separating the value of VEGF-A inhibition ± Ang2 inhibition. Tie2 activation directly is another approach to consider.
Angptl4				X	
CCL2/MCP-1			X	X	Potential target, could be targeted using trispecific antibodies (VEGF, Ang2, and CCL2) similar to current bispecific drugs in trials. Strong animal data, human data need to be generated in cross-sectional and longitudinal studies.
CCN2/CTGF				X	
CCR2/CCR5			X	X	Strong animal data; only 1 trial (Pfizer) inconclusive; needs further trials for confirmation. Human data need to be generated in cross-sectional and longitudinal studies.
CRP			X	X	Based on cross-sectional and limited longitudinal data in plasma/serum. Utility as biomarker, not as target.
DLL4				X	
Epoxide hydrolase soluble			X	X	Animal data with soluble epoxide hydrolase inhibitor available. Need studies on human retina samples. Data have shown the products of these enzymes are beneficial to many organs and are major products of brown fat.
Erythropoietin derivatives				X	
Fenofibrate/PPARα	X	X			Needs trial with eye outcome as primary end point. DRCR study underway should provide it.
IL-1b				X	Based on cross-sectional data only in plasma/serum. With more human data available it could quickly turn into level III because of the systemic evidence in diabetes.
IL-6			X	X	Vitreous levels are elevated in patients with DME, plasma level of IL-6 is an indicator for predicting DME. Preclinical studies have shown IL-6 targeting can be effective in animal models. READ-4: A clinical trial with IV infusion of tocilizumab (IL-6 inhibitor) started in DME. Tocilizumab is effective in uveitis-induced ME. FDA has approved it for GCA. Based on cross-sectional data only in plasma/serum/vitreous humor.
IL-8				X	
Inflammation				X	Markers of monocytes/leukocytes infiltration into the eye are of interest. Interaction between local and systemic levels of inflammatory mediators is of great interest. Requires novel ways of biomarker assessment (e.g., leukostasis) in the clinics.
Kynurenic acid/Egln1				X	Enzyme Egln1, which increases hepatic production of kynurenic acid, may protect against ischemia. Elevated in plasma of insulin resistance even without T2D but not elevated in T1D.
Lipid metabolism (retinal-specific effects of fatty acids oxidation products, cholesterol, ceramide, lipid independent effects of PPARα activation)				X	See soluble epoxide hydrolase; more research needed probably at basic level.
LRG1			X		Comparison of LRG1 blockade with current clinical used anti-angiogenic therapies.
MiR200				X	Medalist study: Patients with T1D w/o DR had low levels, those with had high levels.
Mitochondrial targeting				X	More research needed probably at basic level. 1) mtDNA mutations have clinical relevance in Leber hereditary optic neuropathy, and are now actively being applied to glaucoma. Experimental models and donor samples have clearly confirmed mtDNA damage in DR. We need additional experimentation to investigate the mutations in mtDNA. 2) Impaired removal of damaged mitochondrial by mitophagy in DR. Animal data in other models show promise for activators of mitophagy (chaperone-mediated autophagy).
Neuroprotection				X	
Norrin				X	Genetic data reveal this cytokine induces barrier genesis and formation of the blood-retinal barrier. Norrin treatment effectively reverses VEGF or diabetes-induced permeability in animal models.
Notch-3				X	
Nutrients				X	Experimental models have provided encouraging results. Microbiome of the gut. Nutritional approaches vs. therapeutic approaches should be differentiated.
Oxidized cholesterols				X	7-ketocholesterol which increases in cardiovascular disease and in aging. So the intersection of aging and DR may be a link.
PIGF				X	
Plasma kallikrein		X			Intraviteal PK inhibitors, completed phase II study with KVD001 and ongoing phase II study with THR-149. Multiple oral PK inhibitors in early-stage clinical development. Not better than Ang2 and fenofibrate.
Proinflammatory cytokines				X	See comment above for "inflammation." IL-1b and IL6 of great interest.
RBP3				X	Needs additional basic work and correlation with human data, and then interventional studies.
Semaphorin 3A				X	

Table 2. (Continued.)

	Level of Evidence				If Level I–III: What is Needed to Address Current Unmet Research Needs to Definitely Show whether This Variable is Worthwhile or Not Worthwhile for Use in a DRD Staging System?
	I	*II*	*III*	*IV-V*	
sICAM1			X	X	Based on cross-sectional data in plasma/serum. Utility as biomarker, not as target.
sVCAM1				X	Based on cross-sectional data only in plasma/serum/vitreous humor. Utility as biomarker, not as target.
TNFα				X	Based on cross-sectional data in plasma/serum.
Vascular Protection				X	
VEGFA	X				In order to understand what contributes to DR beyond VEGFA, we need to understand VEGFA itself better. Limitations in ocular sample availability from patients with NPDR.
VEGF-R2				X	
VE-PTP			X		Animal and clinical data available for DKD. Needed for DR.

Ang2 = angiopoietin 2; CCL2/MCP-1 = chemokine ligand 2/monocyte chemoattractant protein-1; CCN2/CTGF = cellular communication network factor 2/connective tissue growth factor; CCR2/CCR5 = chemokine receptor 2/chemokine receptor 5; CRP = C-reactive protein; DKD = diabetic kidney disease; DLL4 = notch ligand delta like ligand 4; DME = diabetic macular edema; DR = diabetic retinopathy; DRCR = diabetic retinopathy clinical network; DRD = diabetic retinal disease; Egln1 = Egl-9 family hypoxia-inducible factor 1; FDA = Food and Drug Administration; GCA = giant cell arteritis; IL = interleukin; IV = intravenous; LRG1 = leucine rich alpha-2-glycoprotein 1; ME = macular edema; miR200 = microRNA-200; mtDNA = mitochondrial DNA; NPDR = nonproliferative diabetic retinopathy; PIGF = phosphatidylinositol glycan anchor biosynthesis class F; PK = plasma kallikrein; PPARα = peroxisome proliferator-activated receptor alpha; RBP3 = retinol binding protein 3; READ-4 = Ranibizumab for Edema of the mAcula in Diabetes: Protocol 4 with Tocilizumab; sICAM1 = soluble intercellular adhesion molecule-1; sVCAM1 = soluble vascular cell adhesion molecule-1; T1D = type 1 diabetes; T2D = type 2 diabetes; TNFα = tumor necrosis factor alpha; VEGF-R2 = VEGF receptor 2; VE-PTP = vascular endothelial protein tyrosine phosphatase.
Level of evidence available for target candidates.

Results

Targets were grouped into concepts or evaluated as specific candidates/targets. In some cases, participants in the BCM-WG had different views regarding the designation of targets as top, middle, or low candidates. Given the extensive target list and the group's diverse expertise, this is not surprising. Differences were resolved by consensus. If it was agreed that targets were substantively promising but required substantially more research, the targets were moved into "concepts." For example, several candidates, i.e., IL-6, chemokine ligand 2/monocyte chemoattractant protein-1 (CCL2/MCP-1), or other cytokines, were included in the "inflammation/cytokines" concept, recognizing the important relationship of hyperglycemia, a major cause of early stages of DRD, to these the targets and concepts.[9]

Candidates reviewed were (Tables 2 and 3): 7-ketocholesterol, Ang2, angiopoietin like 4, CCL2/MCP-1, cellular communication network factor 2, chemokine receptor 2 (CCR2)/chemokine receptor 5, CRP, connective tissue growth factor, notch ligand delta like ligand 4, soluble epoxide hydrolase, erythropoietin (EPO) derivatives, fenofibrate/lipid/peroxisome proliferator-activated receptor-alpha (PPARα), IL-1b, IL-6, IL-8, inflammation, kynurenic acid/endoglin (Egl-9 family hypoxia-inducible factor 1), lipid metabolism (retinal-specific effects of fatty acids oxidation products, cholesterol, ceramide, lipid independent effects of PPARα), leucine rich alpha-2-glycoprotein 1, microRNA-200, norrin, notch-3, PKa, phosphatidylinositol glycan anchor biosynthesis class F, retinol binding protein 3, semaphorin 3A, soluble intercellular adhesion molecule-1, tumor necrosis factor alpha (TNFα), VEGFA, VEGF receptor 2, vascular endothelial protein tyrosine phosphatase, and soluble vascular cell adhesion molecule-1. In addition to these specific candidates, the following broad concepts were thought to be highly relevant to DRD: inflammation/cytokines, oxidative signaling, vasoprotection, neuroprotection, mitophagy, and nutrients/microbiome.

Following this process, 4 targets had strong agreement as "promising" within 2 years of study (Table 1) and by evidence (Table 2). Table 3 identifies the value of each biomarker based on BEST analysis. These targets were: VEGFA, PPARα-related pathways (fenofibrate therapy), PKa, and Ang2/Tie2. The 4 targets were further characterized by scientific rationale, performance expectations, analytic aspects, and gap analysis as per below. However, it is important to note that many targets in the promising and potential categories are strongly recommended for ongoing research.

Targets

VEGFA

Rationale and General Summary of Studies. VEGFA (VEGF) is an angiogenic and vasopermeability factor that was deemed to be relevant for mid and late stage DRD (Table 1). Strong and extensive preclinical and clinical level I evidence (Table 2) exists that ocular levels of VEGF play a substantial role in NPDR progression, DME onset, and PDR development. Inhibition of VEGF bioactivity can inhibit proliferative retinopathy and reduce macular edema to some extent in >80% of patients and does so nearly completely in about 40% of patients.[10,11] VEGF is upregulated in hypoxia, and its transcription is mediated by DNA-binding hypoxia-inducible factors that are stabilized in hypoxia. Hypoxia and ischemia occur in the retina as part of the pathophysiology of DR when capillaries are lost over time resulting in areas of retinal nonperfusion and subsequent hypoxia.[12] Other mechanisms such as reactive

Table 3. Potential Relevance of Variables to Biomarkers, EndpointS, and Other Tools Categories

	BEST Categories*						
	Diagnostic	*Monitoring*	*Predictive*	*Prognostic*	*Pharmacodynamic/Response*	*Safety*	*Susceptibility/Risk*
7-ketocholesterol			X				
Ang2	X	X	X	X	X		X
ANGPTL4							
CCL2/MCP-1	X	X	X	X	X		X
CCN2/CTGF	X						
CCR2/CCR5	X	X		X			X
CRP	X	X		X		X	X
DLL4							X
Epoxide hydrolase - soluble	X	X	X	X	X		
Erythropoietin derivatives					X		
Fenofibrate/lipid/PPARα		X	X	X	X		X
IL-1b	X	X		X			X
IL-6	X	X	X	X	X		X
IL-8	X						
Inflammation	X	X	X	X	X		X
Kynurenic acid and Egln1			X	X			
lLpid metabolism (retinal-specific effects of fatty acids oxidation products, cholesterol, ceramide, lipid independent effects of PPARα activation)		X	X	X	X		X
LRG1	X	X	X	X	X		X
MiR200	X		X	X			X
Mitochondrial targeting (mitophagy)	X	X	X		X		X
Neuroprotection	X	X	X	X	X		X
Norrin					X	X	X
Notch-3							X
Nutrients	X	X		X	X		
Oxidized cholesterols			X	X			X
Plasma kallikrein		X	X	X	X		X
PIGF	X	X	X	X	X		X
Proinflammatory cytokines	X	X	X	X	X		X
RPB3	X	X	X	X	X		X
Semaphorin 3A	X		X	X	X		X
sICAM1	X	X		X			
sVCAM1	X	X		X			
TNFα	X	X	X	X	X		
VEGF-A	X	X	X	X	X		X
VEGF-R2	X	X		X	X		
VE-PTP	X	X	X	X	X		X

Ang2 = angiopoietin 2; ANGPTL4 = angiopoietin like 4; BEST = Biomarkers, EndpointS, and other Tools; CCL2/MCP-1 = chemokine ligand 2/monocyte chemoattractant protein-1; CCN2/CTGF = cellular communication network factor 2/connective tissue growth factor; CCR2/CCR5 = chemokine receptor 2/chemokine receptor 5; CRP = C-reactive protein; DLL4 = notch ligand delta like ligand 4; Egln1 = Egl-9 family hypoxia-inducible factor 1; IL = interleukin; LRG1 = leucine rich alpha-2-glycoprotein 1; miR200 = microRNA-200; PIGF = phosphatidylinositol glycan anchor biosynthesis class F; PPARα = peroxisome proliferator-activated receptor alpha; RBP3 = retinol binding protein 3; sICAM1 = soluble intercellular adhesion molecule-1; sVCAM1 = soluble vascular cell adhesion molecule-1; TNFα = tumor necrosis factor alpha; VEGF-R2 = VEGF receptor 2; VE-PTP = vascular endothelial protein tyrosine phosphatase.
Summary of the value of each biomarker based on BEST analysis.
*Definitions see FDA-NIH Biomarker Working Group.[2]

oxygen species and inflammatory cytokines are also involved in the regulation of VEGF.[13]

Performance Expectations. As a biomarker, VEGF may have value across categories (Table 3). Additional research is needed. VEGF is also clearly an established therapeutic target.

Analytic Aspects. VEGF measurements can vary substantially among plasma, serum aqueous, and vitreous. It is known that platelets sequester high levels of VEGF, thus accounting for much of the plasma/serum differences observed, depending on the collection process and state of degranulation of platelets. Association of eye disease with blood VEGF is generally not reliable to date, presumably due to low concentrations and the very short half-life of VEGF in the circulation. Measurement of VEGF in vitreous or aqueous could be very useful, but efficient, noninvasive measurement approaches will need to be developed. The correlation between aqueous humor and vitreous levels of VEGF requires additional work to fully study their bioresponses.[14]

Gap Analysis. From the level 1 clinical trial data, it is clear VEGF is involved in NPDR progression, DME, and PDR. Measuring these levels in a manner useful for clinical management remains an unmet need. Several studies associated serum VEGF levels with DRD and severity of DR[15,16] (Table 2). In a meta-analysis, serum, but not plasma VEGF, was associated with DR and severity.[15] Population based studies and confirmation of findings are warranted. A recent report showed that vitreous VEGF levels correlated to severity of DR, but plasma VEGF levels of the same individuals did not correlate to DR severity.[17] Direct comparison of vitreous and serum/plasma levels in the same individual from a group of many subjects is needed. Aqueous VEGF was correlated to vitreous levels in patients with PDR[18,19] and was associated with central subfield thickness (CST) by OCT in association with worsening DR and especially DME.[20]

Therefore, it remains necessary to determine the best methods to analyze systemic (plasma or serum) or ocular (aqueous, vitreous) VEGFA, and if the measurements correlate adequately with DRD severity and bioresponse to be clinically useful. It will also be important to determine intraindividual variability of measurements as well as variability in systemic versus ocular measurements within the same individual. This will require prospective studies to determine the variability and potential correlation between measured VEGFA and level of severity of DRD pretreatment and posttreatment. If variability is too great within the sampling approaches to be useful as a biomarker, then alternative approaches will be considered.

PPARα-Related Pathways (e.g., Fenofibrate Therapy)

Rationale and General Summary of Studies. Hyperglycemia is considered the major contributor to DR, but other systemic factors including elevated blood pressure and lipids may also contribute to its development.[21] A cross-sectional study, conducted >4 decades ago, showed that diabetic patients with retinopathy had higher fasting serum triglyceride and cholesterol measurements than those without retinopathy.[22] In addition, experimental models of DR have suggested that hyperlipidemia plays a role in DR, and many clinical trials and epidemiological studies have documented associations between hyperlipidemia and DR.[23] However, epidemiologic studies have not produced consistent results to show a strong association of dyslipidemia treated with statins and DR.[24] In addition, patients with dyslipidemia alone without diabetes do not develop significant DRD.

Fenofibrate, a safe and inexpensive orally administered fibric acid derivative, is routinely used to treat dyslipidemia. Oral fenofibrate treatment in 2 large randomized studies, Action to Control Cardiovascular Risk in Diabetes and Fenofibrate Intervention and Event Lowering in Diabetes, showed reduced progression of retinopathy and less need for laser treatment for macular edema in type 2 diabetic patients treated with fenofibrate. These beneficial effects were observed to be independent of serum lipid levels.[24–27] A recent multicenter cohort study using data for >150 000 patients from United States insurers found an association between fenofibrate use and decreased risk of PDR, and vision threatening DR.[28]

Although the beneficial effects of fenofibrate in DR progression have been shown by the Action to Control Cardiovascular Risk in Diabetes study, its role in DME has not been fully investigated and thus still needs to be established. Also, the beneficial effect on DR was gender-specific as the major benefit was observed in men only with little to no effect in women.[21] The Diabetic Retinopathy Clinical Research Retina Network has a trial currently underway to address the benefit of oral fenofibrate therapy. It is the first large randomized clinical study of fenofibrate with DR progression as the primary end point. If a benefit is observed, fenofibrate would likely be ready for clinical use for this indication and could have major societal impact by providing an oral therapy that reduces DR progression in a broad patient population.

In addition to ameliorating dyslipidemia, fenofibrate has other potential beneficial effects including acting as an agonist of PPARα. Peroxisome proliferator-activated receptor alpha is downregulated in the retina in diabetes (a condition associated with increased oxidative stress, reduced mitochondrial oxygen consumption, and increased apoptosis),[29–31] and these metabolic abnormalities closely associate with the development of DR.[32,33] Thus, fenofibrate as an agonist of PPARα could be beneficial in inhibiting the development and/or progression of DR via lipid-independent pathways and be another potential mechanism of benefit.

Performance Expectations. If clinical efficacy is shown, fenofibrate given as an oral pill could substantially reduce progression of NPDR to the more sight threatening stages of the disease. This noninvasive therapy would be applicable to a large at-risk population worldwide with dramatic potential reductions of DRD complications and reduction in the therapeutic efforts and costs needed to address them.

Understanding the mechanisms of fenofibrate action could provide a biomarker for risk of DR progression at the early stages of DR. The identified biomarker could then not only be used to predict risk of future worsening but also be used to determine the need and appropriateness of initiating fenofibrate or related therapies.

Gap Analysis. Anticipated findings from ongoing prospective randomized clinical trials are expected to yield data regarding the level of efficacy and the benefits to quality of life. Studies to define the mechanisms of fenofibrate action are needed so this promising therapy can help identify a biomarker for risk of DR progression at the early stages of DR.

PKa

Rationale and General Summary of Studies. Plasma kallikrein is a serine protease that cleaves high molecular weight kininogen to generate bradykinin; a potent vasoactive peptide that increases vascular permeability. Plasma kallikrein has been implicated as a VEGF-independent mediator of DME.[34,35] Concentrations of PKa are increased in the vitreous of eyes with DME.[34] In preclinical studies, intravitreal injection of PKa was shown to induce retinal edema.[36] Plasma prekallikrein deficiency and PKa inhibition reduced

retinal vascular hyperpermeability and retinal edema in diabetic rodents.[35,36] In addition, preclinical studies have shown that PKa inhibitors are partially protective against VEGF stimulated neuroretinal dysfunction and retinal edema.[36,37]

Intravitreally administered PKa inhibitors have been investigated in phase Ib and phase II studies in patients with DME who were previously treated with anti-VEGF and continued to experience impaired visual acuity. Phase Ib open label studies with the PKa inhibitors KVD001 and THR-149 showed 4.1 and 6.4 letter gains compared with baseline, respectively, at 3 months.[38,39] A phase IIa sham-controlled clinical study randomized (1:1:1) sham, 3 μg, and 6 μg KVD001 (Q4W) was performed in 129 patients with DME previously treated with anti-VEGF whose baseline best-corrected visual acuity was 23-73 letters (NCT03466099). Although this study did not meet its primary end point there appeared to be a trend for reduced vision loss in patients receiving KVD001 compared with sham (NCT03466099).

Plasma kallikrein inhibitors, including ecallantide, lanadelumab, and berotralstat, are FDA-approved for the treatment of hereditary angioedema.[40] Systemically administered PKa inhibitors may provide additional opportunities to investigate the role of the kallikrein kinin system in DME. An orally available PKa inhibitor, RZ402, is currently in a phase II clinical study for DME.[41]

Performance Expectations. Oral PKa inhibitors may provide an opportunity as a noninvasive treatment in early DME at risk for vision loss. Since PKa has a VEGF-independent mechanism, combination treatment regimens with PKa inhibitors and anti-VEGF may provide additive therapeutic benefit.

Gap Analysis. Gap analysis includes the ability to evaluate the extent of PKa mechanistic engagement in visual function for an individual which could be helpful to identify patients who are most likely to be responsive to PKa inhibition. The effects of DME duration and severity of vision loss at baseline on treatment responses to PKa inhibitors require further investigation as well. Additional studies are needed to determine whether early interventions with PKa inhibitors are associated with better responses compared with delayed treatments,[42] as suggested with ranibizumab in the RIDE and RISE trials.[43]

If the clinical effect is demonstrated, then PKa could be a useful biomarker in several ways. Plasma kallikrein measurement in plasma could determine the extent of PKa activation and may correlate with risk of DME. If not possible, then aqueous or vitreous measurement may more accurately reflect the risk within an eye. Such measurements could not only assess risk but also determine when PKa inhibitor treatment is indicated. In addition, PKa activity might be used as a marker to determine relative likelihood of anti-VEGF response since high PKa activation would suggest a need for more than anti-VEGF therapy alone.

Ang2/Tie2

Rationale and General Summary of Studies. The angiopoietin-tyrosine kinase with immunoglobulin like domains (Tie) signaling pathway plays a critical role in vascular development and is a master regulator of vascular stability. The Tie2 receptor on endothelial cells is activated by phosphorylation that triggers intracellular signaling to promote vascular stability; Ang1 is a strong agonist and binds the Tie2 extracellular domain to activate the pathway. In comparison, Ang2 is a partial agonist which binds to the Tie2 extracellular domain but does not trigger downstream signaling. While Ang1 has historically been considered to be constitutively expressed under normal and diseased states, Ang2 is released by local inflammatory events and hypoxia associated with retinal vascular diseases, including DME and DR. When Ang2 increases, Ang1 is displaced from Tie2 and the intracellular signaling cascade maintaining vascular stability is interrupted, leading to blood-retinal barrier (BRB) compromise.[44] Angiopoietin 2 has been shown to be upregulated in retinas of an animal model of DR, and elevated Ang2 levels lead to increased retinal vascular permeability. Furthermore, Ang2 and VEGFA may have synergistic effects on endothelial barrier dysfunction and neovascularization.[4,45] Vitreous Ang2 levels are also increased in patients with DR. As such, neutralization of both VEGFA and Ang2 may lead to greater vascular stability, less vascular permeability, and potentially greater sustained treatment efficacy.

Faricimab (VABYSMO, also known as RG7716; Roche) is a bispecific monoclonal antibody targeting VEGFA and Ang2. VABYSMO has just been approved by the FDA for treating neovascular age-related macular degeneration, DME, and retinal vein occlusion. Faricimab selectively binds and neutralizes Ang2 and VEGFA, both independently and simultaneously without steric hindrance.[45] Faricimab binds VEGFA isoforms with equilibrium dissociation constant (KD) comparable to that of ranibizumab (3 nM), and Ang2 with a KD of 22 nM.[4] Given the molecular weights of faricimab (150 kDa), aflibercept (115 kDa), and ranibizumab (48 kDa), the molar anti-VEGF binding capacity of 6 mg faricimab is approximately 2.3 times >2 mg aflibercept, 3.8 times >0.5 mg ranibizumab, and 6.4 times >0.3 mg ranibizumab.

Performance Expectations. Based on phase III DME trials, a majority of patients treated with faricimab achieved every 12-week or longer dosing at year 2 (78%), and >60% of patients achieved every 16-week dosing. At the same time, within these DME trials, many of the OCT-based, anatomic outcomes assessing fluid status appeared to favor faricimab through both 1 and 2 years, including mean change in CST measured on OCT, the proportion of patients achieving CST <325 μm, and the proportion of patients achieving absence of intraretinal fluid (IRF). These improved anatomic outcomes were more pronounced in the every 8-week dosing faricimab arms, but were also evident in the personalized treatment interval arms, which received fewer doses on average compared with the other arms. The combination of improved outcomes in subretinal and IRF with fewer doses on average than observed in other DME trials suggests an increased clinical usefulness.[5–7]

Analytic Aspects. OCT parameters (IRF, CST) and time from injection into the eye and reduction in IRF or cysts.

Gap Analysis. Although it remains unknown if faricimab will increase duration of time between injections to a greater

extent than aflibercept when a treat or extend method is permitted for aflibercept, the results of the faricimab trials suggest that assessing Ang2 and VEGF pathway activation and their interactions may eventually prove useful in assessing risk in an updated staging system.

Concepts

Several targets of interest in DRD showed strong scientific evidence but only in cultured cells and/or animal models, or only in human studies without evidence for mechanism, or with potential off-target effects and/or concerns for long-term safety. The integration of data from hypothesis-driven robust science from in vitro molecular biology and animal models with human studies is optimal for target identification. For many target areas, there was strong evidence from $\geq$1 published studies that the area was worth further investigation. The BCM-WG believed there was strong reason to pursue the following concepts regarding scientific research of DRD.

Inflammation/Cytokines

Rationale and General Summary of Studies. Many features of inflammation like leukostasis, microglial activation and macrophage infiltration, cytokine upregulation, increased vascular permeability and blood flow, and tissue edema have been described in animal models of DR as well as in human DR.[46,47] Increased leukostasis, an early event in DR, results in upregulation of retinal intercellular adhesion molecule 1 and alteration of the BRB. Several cytokines like TNFα, ILs (IL-1b, IL-6, IL-8), proteases, and chemokines (e.g., CCL2) have been implicated in DME and have been targeted in animal models. Small clinical trials have been initiated in patients with DME using inhibitors of these molecules (except ILs).[48] Larger clinical trials are needed to further assess the efficacy of these inhibitors in DME and possibly early-stage patients with DR. In addition, inflammatory cytokines are elevated in many retinal and systemic diseases that don't manifest DRD unless hyperglycemia or diabetes is present. Therefore, it is important to understand the additive effects of inflammatory cytokines to those of hyperglycemia.

CCL2. Gene expression array studies in retinas of diabetic animals show that CCL2 (also known as MCP-1) is significantly upregulated (almost 16-fold) compared with other angiogenic factors like VEGF, Ang2, and TNFα.[49] Similar increases of CCL2 levels have been described in vitreous samples of patients with DME. The increased monocyte/macrophages trafficking into the retinas of diabetic animals is believed to be regulated by the increased CCL2 levels.[50] A significant reduction of monocytes/macrophages with inactivated microglia with long ramifying processes has been described in the CCL2 knockout mice. Activated monocytes that differentiate into macrophages along with activated microglia in diabetes secrete cytokines and growth factors including VEGF, Ang2, TNFα and ILs, matrix metalloproteinase (MMP) 2 and MMP 9, all of which have been described to alter the BRB. Targeting the chemokine pathway appears to be a novel therapeutic strategy in the management of DME as the dual inhibitor of CCR2/chemokine receptor 5 significantly decreased retinal vascular permeability in diabetic animals with a significant reduction in macrophage/microglia infiltration in retinas.[51] A phase II randomized trial using an oral chemokine CCR2/5 receptor antagonist (PF-04634817, Pfizer) has shown a modest improvement in best-corrected vision, but the study did not meet the predefined non-inferiority criteria compared with intravitreal ranibizumab injections.[52]

Gap Analysis. The reasons for the modest improvement with the CCR2/5 receptor antagonist (PF-04634817) could be short duration of treatment, route of administration (oral vs. intravitreal), and lack of bioavailability of the drug in the retina. More study is needed, including to determine if CCL2 levels might be a biomarker.

TNFα. Tumor necrosis factor alpha, an important mediator of retinal leukostasis, has been implicated in many inflammatory diseases like rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis, plaque psoriasis, and Crohn's disease, insulin resistance alone and with type 2 diabetes. In animal models of DR, TNFα has been shown to cause chemoattraction of monocytes, leukostasis, and BRB breakdown.[53] In diabetic rats, a TNFα inhibitor, etanercept, has been reported to reduce leukostasis, retinal intercellular adhesion molecule 1 expression, and to decrease BRB breakdown. Interestingly, TNFα inhibition does not affect VEGF levels in retinas, suggesting an interaction between VEGF and TNFα-mediated pathways that act independently in diabetes. A double-masked, randomized, placebo-controlled, clinical trial in refractory patients with DME resulted in significantly improved vision and decreased retinal thickness with intravenous infliximab.[54] Intravitreal administration of TNFα inhibitors in patients with refractory DME showed instead limited or no efficacy but local adverse events and inflammation, advocating for a systemic rather than local role of TNFα in DRD.[55,56] This is in line with the fact that aqueous or vitreous TNFα data did either not qualify for inclusion into a recent quantitative meta-analysis in patients with DME[57] or did not show a relationship with DRD or DME stages.[58]

Gap Analysis. Larger sample sizes of patients with DME with longer duration of treatment are required to assess the efficacy of systemic anti-TNFα drugs, as well as the long-term safety. These findings will also help determine if TNFα can be useful as a biomarker in staging DRD.

ILs. Interleukins are well known for activation and differentiation of immune cells and have both proinflammatory and anti-inflammatory properties. Elevated levels of IL-1b, IL-6, and IL-8 have been reported in the vitreous[59,60] and in retinas of diabetic rats.[61] While bioanalytical detection of IL-1b in humans represents a major challenge due to its low levels in body fluids,[62] there is consistent evidence of increased ocular[57,58] and systemic[63] IL-6 as well as ocular IL-8[57] in patients with DRD. Intravitreal administration of an antibody against human IL-6 (EBI-029) (Eleven Biotherapeutics) potently inhibits IL-6 cis and trans-signaling and has been effective in an animal model of choroidal neovascularization.[64] Interleukin-6 inhibition in uveitis-induced macular edema

improved visual acuity and retinal thickness in the phase I multicenter, non-randomized, open-label, multiple ascending dose study that investigates the safety, tolerability, efficacy, and PK/PD profile of RG6179 in both diabetic macular edema (DME) and uveitic macular edema (UME) patients (DOVETAIL) trial (Association for Research in Vision and Ophthalmology abstract presented June 2023); however, the translation from inflammation-driven macular edema to DME remains to be proven. Systemic IL-1β inhibition in diabetic patients has a beneficial effect on glucose control and development of macrovascular complications.[40,65,66] A small interventional trial also tested systemic inhibition of IL-1β by administering canakinumab, an approved anti-IL-1β used in rheumatoid arthritis and auto-inflammatory diseases, in patients with proliferative DRD: although not conclusive due to the small sample size, promising effects were seen on reducing DME.[40]

Proteinases. The role of MMPs in the maintenance of systemic vessel integrity and remodeling has been well documented in animal models of angiogenesis. The MMPs are zinc-dependent proteinases that are capable of degrading numerous structural components of the extracellular matrix and a variety of nonextracellular matrix proteins. In an animal model of diabetes, both MMP 2 and MMP 9 are elevated in the retinas, and the increased retinal vascular permeability could be inhibited with an MMP inhibitor (BB-94, British Biotech).[67] These preclinical studies suggest a possible mechanism by which diabetes contributes to BRB breakdown through the proteolytic degradation of vascular endothelial-cadherin, and a potential role of MMP inhibitors in DME. The role of MMPs in the maintenance of systemic vessel integrity and remodeling has been well documented in animal models of angiogenesis.

CRP. C-reactive protein is an acute-phase protein that is synthesized in the liver or adipose tissue in response to an injury or infection. In clinical practice, the levels of CRP in blood have been measured for diagnosis of inflammatory diseases. A recent meta-analysis has shown that the blood CRP levels in patients with PDR are significantly higher than those in patients with NPDR.[68] However, the significance of using CRP as a biomarker is limited in part because of not being specific to DR. In combination with other biomarkers to assess general inflammation and which patient subgroup could benefit from a targeted anti-inflammatory drug, CRP and high-reactive CRP, potentially in combination with other factors, might have value.

Gap Analysis. Many of the described factors are being considered as targets for treatment. Some are general indicators of inflammation, such as CRP. However, further work into systemic predictive, prognostic, or diagnostic biomarkers for greater inflammation associated with DRD, and related treatment responses or their combinations, may be valuable. In addition, aqueous samples have associated VEGF, several inflammatory markers, MCP-1, IL-6, and IL-8, with DME or PDR and suggested aqueous factors as predictors of DME development.[69] Additional studies into systemic and local inflammation biomarkers are warranted including earlier disease stages of DRD. Another aspect for consideration is the bioanalytical capabilities to reliably measure the above-mentioned analytes with the required level of validation and compliance in clinical studies and as diagnostic tests.

Oxidative Signaling

Rationale and General Summary of Studies. Hypothesis-testing and evidence support a role for serum or plasma oxidized end-products and/or reduced concentrations of antioxidant enzymes[70] as biomarkers for DR or severity of retinopathy.[70] This is based on evidence that diabetes increases oxidative stress in the retina, and oxidative stress plays a pivotal role in the development of DR. The possible sources of oxidative stress include auto-oxidation of glucose, high glucose-induced metabolic abnormalities, decreased concentrations of antioxidants, and impaired activities of antioxidant defense enzymes.[32] The diabetic environment also facilitates interactions of glucose with amino acids in proteins, lipids, and nucleic acids, and via nonenzymatic reactions, formation of Schiff's base and Amadori products, and a complex cascade of reactions, which ultimately results in the conversion of Amadori products into the formation of advanced glycation end products.[71] In addition to tissue and cells, these advanced glycation end products can also be analyzed in the body fluids (serum), but patient-based studies in patients with DR have revealed that the levels of advanced glycation end products, including carboxymethyl lysine, pentosidine, or hydroimidazolone, in serum have not produced consistent results.[72]

Oxidative stress can be exacerbated by hyperlipidemia, and in lipid-rich oxidative environment, lipid peroxidation reactions can form advanced lipoxidation end products.[73] The retina is rich in polyunsaturated fatty acids and has the highest oxygen uptake,[74] and accumulation of lipid aldehydes and advanced lipoxidation end products is associated with the development of DR,[75,76] but how they contribute in the development of DR is unclear. The BCM-WG believed that although the area of research was important, additional study into understanding their role as a biomarker and as potential drug targets would likely extend beyond 5 years.

Vasoprotection

Rationale and General Summary of Studies. Several mechanisms, such as activated signaling pathways or endothelial progenitor cells, can protect the vasculature during pathologic stresses.[77] Additional study was deemed worthy. One area of interest with potential of usefulness within 5 years involved EPO signaling.

EPO Signaling. Erythropoietin is a hematopoietic hormone that has been more recently recognized as having neuro and vasoprotective effects.[78] Erythropoietin binds the EPO receptor (EPOR), which can form a dimer or heterodimer with VEGF receptor 2 or the beta common receptor (CD131). When EPOR forms a heterodimer with CD131, it is believed to lead to tissue repair.[79,80] Evidence supports EPO signaling in neurovascular protection, including vascular stability from high oxygen induced capillary dropout in murine model of oxygen-induced retinopathy[81,82] as well as support of retinal

function and electroretinography (ERG) responses following vascular occlusion in wild type compared with transgenic humanized hypoactive EPOR mouse models exposed to vascular injury.[83] Difficulties studying the signaling pathways of EPO include that it is a hormone and affects multiple tissues and that antibodies are not reliably consistent in all tissue types making it difficult to measure presence or activation of the receptors. In addition, EPO can trigger signaling through several receptors and downstream pathways. Methods to improve the study of EPO signaling include the use of humanized models of EPOR,[82–85] including in retinal diseases. In addition, human studies have found associations in EPO gene polymorphisms and DR[86] and a potential role of exogenous EPO in protection against DR. Although a recent clinical trial failed to show efficacy in visual acuity end points for exogenous EPO and DME,[87] there was improved patient-reported visual function. This clinical study along with strong preclinical data support additional study to test the neuro- and vascular protective effects of EPO signaling. If found, then EPO/EPOR signaling activity may prove to be useful in staging DRD.

Analytic Aspects. The clinical and preclinical parameters of outcomes, including OCT examination, ERGs, and oscillatory potentials, are being assessed. Erythropoietin receptor or its activation of signaling is not reliable through antibody testing in tissues. However, EPO and its other receptors can be measured in experimental studies. It remains to be determined if such measurement can be used as a reliable biomarker for DR risk.

Performance Expectations. More research is needed to identify safe and targeted protective effects of EPO and determine potential outcome variables for human studies. Better clarity of the signaling effects of EPO through its many receptors in various tissues is needed to determine the usefulness of potential signaling effectors as biomarkers to assess relative DR risk.

Gap Analysis. Evidence supports EPO signaling in angiogenesis and neuroprotection. Angiogenesis in DRD can be beneficial to support an ischemic or hypoxic retina if the damaged retina can accept intraretinal vascularization. However, the stimuli for angiogenesis can be detrimental if the retina is unable to support intraretinal angiogenesis and intravitreal angiogenesis, as occurs in PDR. Additional studies are needed perhaps at earlier stages and with neural outcomes, such as ERG or other sensitive parameters for visual function. In addition, EPO derivatives that target tissue protective effects and minimize potential risks from hematopoietic effects of EPO should be studied. Finally, the portions of the activation pathway that can be measured must be evaluated to determine if such assessment can be used as a reliable biomarker for DR risk.

Neuroprotection

Rationale and General Summary of Studies. Mounting evidence indicates the presence of retinal neuronal dysfunction and neurodegeneration relatively early in diabetes.[88] However, studies of people with very long durations of type 1 diabetes (>50 years of diseases) showed protection of both neuro- and vascular retinal morphology in the presence of hyperglycemia in these individuals.[17,89] Studies of postmortem human retinas demonstrated apoptosis of retinal neuronal elements,[90] similar to diabetic rodent models.[88] Additional manifestations of neurodegeneration include thinning of the inner retina on OCT,[88,91] retinal electrophysiological abnormalities measured by ERG, and abnormalities in psychophysical testing parameters such as contrast sensitivity and dark adaptation.[92] Neuronal dysfunction and neurodegeneration can be detected in patients without observable microvascular alterations. It remains unclear whether earlier microvascular changes occur that are currently undetectable with existing methods. A study of individuals with diabetes and 2 diabetic mouse models reported progressive thinning of the inner retina as measured by OCT before detectable microvascular damage, including from either reduction in retinal capillary density or increase in capillary degeneration.[91] Studies of diabetic rodent models indicate that neuronal damage could directly contribute to vascular damage, either by promoting the neuroinflammatory environment[93] or by promoting retinal vascular hyperpermeability.[94] Conversely, preserving retinal vascular permeability by genetic manipulation specifically in vascular endothelial cells prevented loss of visual acuity and contrast sensitivity in a mouse model of diabetes.[95] While the relationship of vascular and neuronal alterations remains an area of active research, ERG changes have been demonstrated to be highly predictive of development of clinical DR.[96]

These findings have led to the concept that neuroprotective therapies could prevent or slow DR early on in disease. Several topical drugs have been tested in clinical trials of individuals with early DR.[92] Of these, the largest study for which the greatest amount of data is available is the European Consortium for the Early Treatment of Diabetic Retinopathy (EUROCONDOR) trial.[97]

Performance Expectations. The EUROCONDOR trial, with 449 patients, studied whether topical treatment of individuals with type 2 diabetes and early DR with 2 neuroprotective drugs, somatostatin/SST and brimonidine, could slow the progression of retinal neurodysfunction.[98] In addition, the effect of treatment on microvascular changes was examined. The trial was a 96-week prospective phase II to III study. At baseline and completion of study, multifocal ERG and spectral-domain OCT were obtained to find evidence for neuroretinal changes. Retinal vessel caliber analyses were performed to detect retinal vascular changes.[99,100]

Analytic Aspects. As measured by multifocal ERG, only a subset of study participants (34.7%) exhibited abnormalities at baseline.[97,101] Neurodegeneration was not found in around 30% of patients with early microvascular changes of DR. At study completion, topical neuroprotective treatment did not provide a significant effect in prevention of neuronal dysfunction or progression of microvascular disease.[101] In the subset of patients with neuronal dysfunction at baseline, treatment did delay progression of changes on multifocal ERG. In addition, the study

demonstrated that treatment resulted in retinal arteriolar and venular dilatation in patients with preexisting DR,[99] suggesting a link between neuroprotection and retinal vascular changes. Of note, this trial had a short follow-up period of 2 years, a high proportion of the study population had no or very mild DR, and participants exhibited excellent metabolic control. These factors could have impacted on the ability to detect improvement of these parameters by treatment.[101]

Gap Analysis. It remains unknown whether neuroprotection with other agents or over longer periods of time could prevent the development of neurodegeneration or prevent or slow microvascular dysfunction characterizing clinical DR. It has been suggested that ideal treatments might involve drugs that have dual neuroprotective and vasoprotective effects.[98] Future studies can also be done to determine if OCT markers or other biomarkers of neural function may be useful as biomarkers in DR.

Mitophagy

Rationale and General Summary of Studies. Mitochondria play an important role in the development of diabetes and complications, and increased generation of mitochondrial reactive oxygen species is believed to affect major metabolic abnormalities associated with the development of DR.[32,102,103] In the initial stages of the disease, overproduction of cytosolic reactive oxygen species damage mitochondrial membranes, impairing their structural and functional stability. As the disease progresses, mitochondrial DNA (mtDNA) is damaged and its transcription is reduced, compromising the electron transport chain. This is further complicated by a compromised defense mechanism to scavenge free radicals, and the damaged mitochondria continue to fuel a vicious cycle of free radical generation. In rodent models of DR, mitochondrial damage is detectable before the development of background retinopathy. Furthermore, mtDNA biogenesis is also impaired, leading to decreased mitochondrial copy numbers.[103,104] Although not as widely studied as in the retinal vasculature, mitochondria in other retinal cells, including ganglion cells, glia, and photoreceptors, are also damaged in diabetes.[93,105,106]

Damaged mitochondria are removed by an autophagic-lysosomal degradation pathway (mitophagy). Mitophagy controls metabolic homeostasis by removing the damaged or unnecessary mitochondria, which prevents further mitochondrial dysfunction and subsequent molecular events that can lead to oxidative stress.[107] In disease states, mitophagy can be beneficial by partially compensating for other deficits, but when mitochondrial activity is compromised, mitophagy can play a detrimental role.[108] In addition, mitophagy itself is regulated by various mitochondrial and extramitochondrial factors, including mitochondrial morphology, oxidative stress, and DNA damage.[109] As indicated above, oxidative stress is increased and mtDNA is damaged in DR. Experimental models of DR have documented altered mitophagy in retinal vascular and nonvascular cells, and in retinal samples from human donors with DR.[110]

Thus, mitochondrial dysfunction could play a central role in the pathogenesis of DR and other complications of diabetes, and this exciting area of research has tremendous potential, but using mitochondria damage as a disease biomarker is still in infancy. Peripheral blood samples from patients with DR have increased mtDNA damage and decreased transcription of mtDNA compared with their age-matched nondiabetic counterparts. In addition, these measured outcomes are minimally affected in diabetic patients without retinopathy in some studies, suggesting a close association between peripheral blood mtDNA damage and DR.[111] However, there are significant associations of mtDNA changes with diabetic nephropathy as well, which could make the measurements of mtDNA changes as diagnostic markers for DRD more difficult.[110]

Furthermore, methylation of mtDNA is associated intimately with gene suppression. Methylation of mtDNA is greater in the peripheral blood of diabetic patients compared with age-matched nondiabetic individuals. Also, compared with diabetic patients without retinopathy, patients with proliferative retinopathy have significantly higher mtDNA methylation and significantly lower transcription of mtDNA-encoded genes.[112]

Significant reduction in the specific autophagic markers (autophagy protein 5) and mitophagy markers (Parkin protein) is seen in the serum of patients with neurodegenerative diseases that have impaired autophagy-mitophagy, including Alzheimer's disease, mixed dementia, or mild cognitive impairment.[113] In addition, an mtDNA point mutation is associated with Leber's hereditary optic neuropathy disease.[114] A strong relationship between these biomarkers and neurodegenerative diseases supports the possibility of using autophagy-mitophagy markers as biomarkers for DR. In addition, many studies are ongoing in rodents using therapeutic agents targeting the regeneration of mitochondria in several diseases, including in pancreatic beta cells, skeletal muscle, and diabetic nephropathy. These agents could potentially be repurposed for DR.

Thus, the logic to use mitochondrial biomarkers for DR is very strong, but more research is needed to directly correlate changes of DNA methylation of the retina and mtDNA damage in circulating peripheral blood mononuclear cells, recognizing potential confounding by the presence of other diabetic complications, such as nephropathy, before commercial testing in patients can be expected.

Performance Expectations. Mitochondrial homeostasis is essential for cell functioning, and measurement of mtDNA damage, copy number, methylation, and mtDNA-encoded genes might be considered biomarkers for DR with future study.

Gap Analysis. Most markers can be easily analyzed in the body fluids, such as serum or plasma, and are stable to ease their analysis. Research of the possibility of measuring them in aqueous or vitreous and the association with serum or plasma measurements may be studied in the future. Since biomarkers may be related to RNA and DNAs, studies of circulating exosomes may be helpful if they are specifically related to retinal proteins. Considering the importance of mitochondria in cellular function, the enthusiasm is high,

but more studies are needed before moving toward gathering patient information for clinical trials.

Nutrients/Microbiome

Although the group recognized the exciting area of study regarding nutrition and the gut microbiome,[115–117] the evidence to date was believed to require additional studies beyond 5 years. Therefore, this concept was not a focus of the current manuscript.

Conclusions

The BCM-WG identified 4 factors currently that could underlie new assessments and eventually be considered within an updated DRD staging system or treatment, based on the evidence and need for research believed to fit within a 2-year timeline (VEGFA, PPARα related pathways, PKa, and Ang2). VEGFA, PKa, and Ang2 should be evaluated for use as biomarkers in diagnostic, monitoring, predictive, prognostic, and pharmacodynamic responses as well as for susceptibility/risk biomarkers. Their activation, or activation of portions of their signaling pathways, including as related to hyperglycemia, might be used as an assessment of risk as well as to help determine timing of treatment and selection of therapeutic agents. Measurement approaches will need to be defined since some may require vitreous sample measurement or other methodology in cases where aqueous measurement correlates with vitreous activity or if the variability in blood measurements is not found valuable. None of the 4 targets was considered likely as a safety biomarker at this time.

Given that the area of Basic and Cellular Mechanisms is primarily focused prior to clinical work and is a mechanism for target identification, it is not surprising that there were many promising and potential targets, and yet few that were ready for use at the current time. Many of these targets could be grouped into research areas and thus the identification of key important concepts was presented. The BCM-WG strongly supported the need for ongoing research in all the conceptual areas. There were limitations to this group's evaluation approach. The BCM-WG members had recognized expertise within their own areas of research, but perhaps less in other areas. However, all members were established acknowledged scientific experts and were capable of carefully reviewing and vetting the literature and participating in discourse with other scientists. In addition, ongoing research may not have been captured in the period of time the BCM-WG met and prepared the manuscript. It is clearly expected that targets will continually evolve as more data and more mechanistic understanding is realized over time.

In summary, the BCM-WG of the Mary Tyler Moore Vision Initiative reviewed the literature and evidence for identified targets based on pathophysiology and other parameters for DRD. Candidates were populated into 4 different evidence grids, ranking their importance to level of severity of DRD and readiness as a target, level of evidence from the literature, value as a biomarker based on BEST system, and as to whether the candidate was believed to be top, middle, or low. Four candidates (VEGFA, PPARα-related pathways, PKa, and Ang2/Tie2) were considered to be very promising for potential inclusion in the DRD classification effort within 2 years as required by the DRD Staging Update Project of the Mary Tyler Moore Vision Initiative. Other candidates that were not deemed to have sufficient evidence at the present time were clustered into important concepts. Although the BCM-WG recognized that at this stage little can be incorporated into a new DRD staging system, these numerous exciting potential targets and important concepts deserve continued support and research, as they may eventually be moved into the clinic as biomarkers and/or therapeutic targets. A classic example of this development process is well known—that of targeting the VEGFA signaling pathway. The future of research in the evolution of DR care remains an exciting one.

Footnotes and Disclosures

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Abbreviations and Acronyms:
Ang1 = angiopoietin 1; **BCM-WG** = The Basic and Cellular Mechanisms Working Group; **BEST** = Biomarkers, EndpointS, and other Tools; **BRB** = blood-retinal barrier; **CCL2** = chemokine ligand 2; **CCR2** = chemokine receptor 2; **CRP** = C-reactive protein; **CST** = central subfield thickness; **DME** = diabetic macular edema; **DR** = diabetic retinopathy; **DRD** = diabetic retinal disease; **EPO** = erythropoietin; **EPOR** = erythropoietin receptor; **ERG** = electroretinography; **FDA** = Food and Drug Administration; **IL** = interleukin; **IRF** = intraretinal fluid; **KD** = equilibrium dissociation constant; **MCP-1** = monocyte chemoattractant protein-1; **MMP** = matrix metalloproteinase; **mtDNA** = mitochondrial DNA; **NPDR** = nonproliferative diabetic retinopathy; **PDR** = proliferative diabetic retinopathy; **PKA** = plasma kallikrein; **PPARα** = peroxisome proliferator-activated receptor alpha; **TNFα** = tumor necrosis factor alpha.

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