Molecular Biology of Diabetic Retinopathy: *How to Best Preseve Vision in these Patients*

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BY LLOYD PAUL AIELLO, MD, PhD

STATEMENT OF NEED

Diabetic retinopathy, including proliferative diabetic retinopathy (PDR) and diabetic macular edema (DME), are leading causes of diminished vision and blindess in the United States. Their impact will continue to be felt as the diabetes epidemic grows. Diabetic retinopathy is the most common diabetic microvascular complication, and all health care providers have a role to play in its prevention and treatment.

Efforts aimed at improving the prevention, early detection and treatment of PDR and DME must incorporate research into the underlying molecular mechanisms with clinical strategies aimed at optimizing control and blood glucose and other systemic factors.

TARGET AUDIENCE

Ophthalmologists, specifically medical and surgical retinologists.

LEARNING OBJECTIVES

Upon successful completion of this learning program, the participant should be able to:

- describe the diagnosis, classification, and manifestations of diabetic retinopathy and DME and its underlying molecular biology;
- evaluate currently available therapeutic strategies for intervention; and
- discuss the DRCR.net initiatives and the role of the diabetic team in interacting with each other and the patient toward providing optimum treatment.

METHOD OF INSTRUCTION

Participants should read the learning objectives and CME program in their entirety. After reviewing the material, they must complete the self-assessment test, which consists of a series of multiple-choice questions.

Participants have a choice of completing this activity online by visiting www.RetinaToday.com; getting real-time results at www.CMEToday.net; or by using the print forms following this activity.

Upon completion of the activity and achieving a passing score of ≥70% on the self-assessment test, participants will receive a CME credit letter awarding

AMA PRA Category 1 Credit™ 4 weeks after the registration and evaluation materials are received. The estimated time to complete this activity is 1 hour.

ACCREDITATION

This activity has been planned and implemented in accordance with the essentials and standards of the Accreditation Council for Continuing Medical Education (ACCME) through the joint sponsorship of The Dulaney Foundation and *Retina Today*. The Dulaney Foundation designates this educational activity for a maximum of 1 *AMA PRA Category 1 Credit.*™ Physicians should only claim credit commensurate with the extent of their participation in the activity.

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FACULTY DISCLOSURE

Lloyd Paul Aiello, MD, PhD, serves as a consultant to and receives honoraria from Eli Lilly and Company (Indianapolis) and OSI/Eyetech (New York, NY and Pfizer, New York, NY). Conni Koury, Editor, Retina Today, has no commercial relationships to disclose. C. Ling, MD, Medical Reviewer, has no commercial relationships to disclose.

FACULTY CREDENTIALS

Dr. Aiello is associate professor of ophthalmology at Harvard Medical School, director of the Beetham Eye Institute and head of the section on eye research at Joslin Diabetes Center, Boston. He may be reached at LloydPaul.Aiello@joslin.harvard.edu.

INTRODUCTION

According to the American Diabetes Association (ADA), the United States is experiencing an epidemic of diabetes, with 20.8 million Americans currently suffering from the condition. This represents 7% of the population, and most of these have type 2 diabetes. Diabetic retinopathy exists across a spectrum of stages and severity, and DME can occur at any point across the spectrum. Diabetic retinopathy is the most frequent late complication of type 1 diabetes; it occurs in 100% of those with type 1 and 60% of those with type 2. Among patients with diabetes who are aged ≥40 years, 40.3% have diabetic retinopathy and 8.2% have vision-threatening retinopathy, according to the *Archives of Ophthalmology.*

HYPERGLYCEMIA IS THE KEY

Even before diabetic retinopathy is evident, biochemical changes begin.[1] Hyperglycemia plays a role with regard to cellular dysfunction and damage, with a host of complex processes that come into play.

> Controlling of blood pressure and other systemic factors also reduces the risk of developing microvascular disease.

Hyperglycemia is the principal underlying cause of the complications of diabetes. It is important that patients recognize that every incremental decrease in HbA1c signficantly reduces the risk of microvascular complications. Controlling blood pressure and other systemic factors also reduces the risk of developing microvascular disease.

Ophthalmologists should be prepared to recongize the early stages of diabetic retinopathy in their patients, identify risk factors for the development and progression of diabetic retinopathy and formulate strategies for the prevention and early detection of diabetic retinopathy.

Patients with no evidence of diabetic retinopathy should visit an ophthalmologist at least yearly. If they have type 2 diabetes or evidence of the disease, then they should be seen more often.

DETERMINING DIABETIC RETINOPATHY SEVERITY

The levels of diabetic retinopathy are: none, NPDR, which can be mild, moderate, severe or very severe; and PDR, which can be less than high risk and high risk.

Risk factors for diabetic retinopathy include diabetes duration, age at diagnosis, blood glucose con-

trol, hypertension, dyslipidemia, renal disease, pregnancy, anemia and body mass index.

There is no apparent retinopathy if no abnormalities are found during a dilated ophthalmoscopy exam. Microaneurysms only indicate mild NPDR. Moderate NPDR is indicated by the finding of more than just microaneurysms but less than severe NPDR.

Severe NPDR includes any of the following: >20 intraretinal hemorrhages in each of the four quadrants, definite venous beading in two or more quadrants, prominent intraretinal microvascular abnormalities in one or more quadrants and no signs of PDR.

PDR exists when one or more of the following is seen: neovascularization or vitreous/preretinal hemorrhage.

OPTIMAL PATIENT APPROACH

The optimal patient approach for the best care includes close interaction among health care providers (ie, primary care providers, endocrinologists and other clinicians), more frequent visits to the ophthalmolgist and the primary care provider, and the use of a clinical diabetes educator.

The level of PKC activity then correlates to the severity of diabetic retinopathy.

PKC

Protein kinase-C (PKC) activation appears to be a key player in many of the microvascular disease pathways.[2] PKC inhibition may help ameliorate hyperglycemia-induced cellular dysfunction. The PKC enzyme modulates molecules in the body and the beta isoform is primarily involved in the complications of diabetes and other vascular issues.[3] In the state of hyperglycemia, diaclyglycerol is synthesized, which in turn activates PKC. Therefore, the fundamental problem is that PKC is activated in the diabetic condition.

The level of PKC activity then correlates to the severity of diabetic retinopathy. PKC-beta activation plays a key role in mediating early retinal changes in diabetes. Even when PKC-beta was overexpressed in nondiabetic mice, retinal abnormalities were seen.[4]

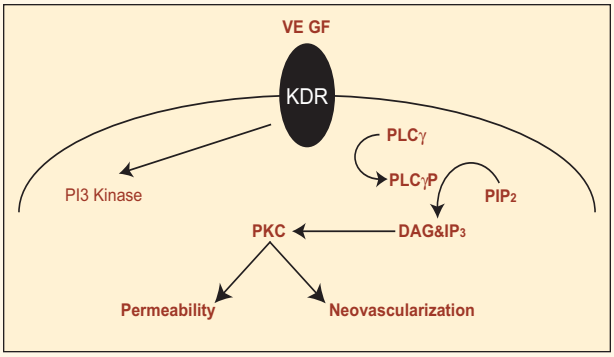
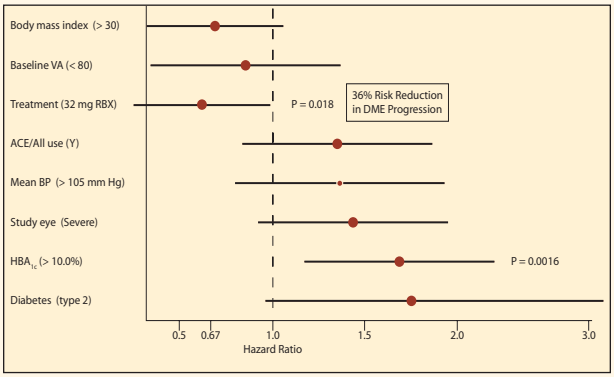
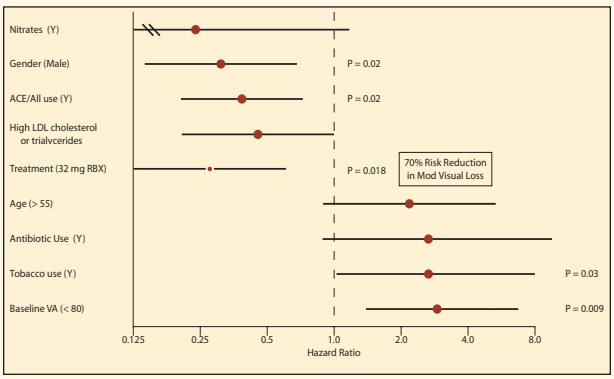


Figure 1. Mechanism of VEGF action.





Figures 2 and 3. Cox porportional hazard models for DME progression PKC DMES (top) and moderate visual loss.

VEGF

Growth factor mediation — particularly vascular endothelial growth factor (VEGF) — is part of retinopathy. PDR appears to be driven by ischemia in the retina. Growth factors in the eye lead to neovascularization.

VEGF is a 46 kDa homodimeric glycoprotein that was first identified in vascularized tumors. VEGF is produced by many ocular cell types and is induced by hypoxia. It binds to high-affinity receptors on retinal

endothelial cells and stimulates retinal endothelial cell growth. VEGF promotes vascular permeability and induces disassociation of tight junction components.

Retinal VEGF levels are elevated in diabetes and VEGF can cause diabetic retinopathy-like changes in the eye.[5-7] Data shows that VEGF plays a key role in mediating retinal neovascularization and vascular permeability under ischemic retinal conditions, such as diabetic retinopathy.

> Another therapeutic option would be to block PKC-beta activation along the pathway.

If an agent was able to block VEGF, we might be able to prevent diabetic complications in the eye. VEGF inhibition in mice and primate models was shown to be successful.[8,9]

OPPORTUNITES FOR INTERVENTION: ANTI-VEGF THERAPIES

Current anti-VEGF approaches that are being studied include pegaptanib sodium, ranibizumab, VEGF trap and bevacizumab.

- Pegaptanib sodium (Macugen; OSI/Eyetech, and Pfizer, New York, NY) acts as an intravitreal aptamer VEGF inhibitor. Its potential indication is DME treatment and it is in phase 3 trials. It has been FDA approved for the treatment of neovascular or wet age-related macular degeneration (AMD).
- Ranibizumab (Lucentis; Genentech, San Francisco) acts as an intravitreal humanized anti-VEGF antibody fragment. Its potential indication is DME and studies are planned. Genentech will soon seek FDA approval for the indication of the treatment of neovascular or wet AMD.
- The VEGF Trap (Regeneron) is an intravitreal VEGF receptor construct that is being studied for the potential treatment of DME.
- Bevacizumab (Avastin; Genentech, San Francisco) is an intravitreal anti-VEGF antibody that is being studied in DME treatment. This use is off-label. Currently, it is approved as first-line treatment for patients with colorectal cancer that has spread to other parts of the body. Mechanism of VEGF action is shown in Figure 1.

PKC-BETA INHIBITION

Another therapeutic target option would be to block PKC-beta activation along the pathway. A PKC-beta inhibitor should be highly PKC selective, highly beta isoform selective and orally bioavailable. Eli Lilly and Company's ruboxistaurin, currently in development, is all of these things. In clinical studies it has been shown to reduce retinal neovascularization, reduce diabetes-induced permeability and normalize retinal blood flow.

A ruboxisaurin phase 2/3 trial studied 252 total

TABLE 1. ANTI-SIGNALING APPROACHES

Agent	Action	Indication	Status
PKC-beta inhibitor OTDRS (ruboxistaurin)	Oral, PKC-beta inhibitor	RBF MCT	Phase 1b completed
PKC-beta inhibitor DRS1 (ruboxistaurin)	Oral, once-daily PKC-beta inhibitor	PDR NPDR	Phase 2/3 completed
PKC-beta inhibitor DMES (ruboxistaurin)	Oral, once-daily PKC-beta inhibitor	DME	Phase 2/3 completed
PKC-beta inhibitor DRS2 (ruboxistaurin)	Oral, once-daily, PKC-beta inhibitor	Visual loss	Phase 3 completed
PKC-beta inhibitor DMES2 (ruboxistaurin)	Oral, once-daily, PKC-beta inhibitor	DME	Phase 3

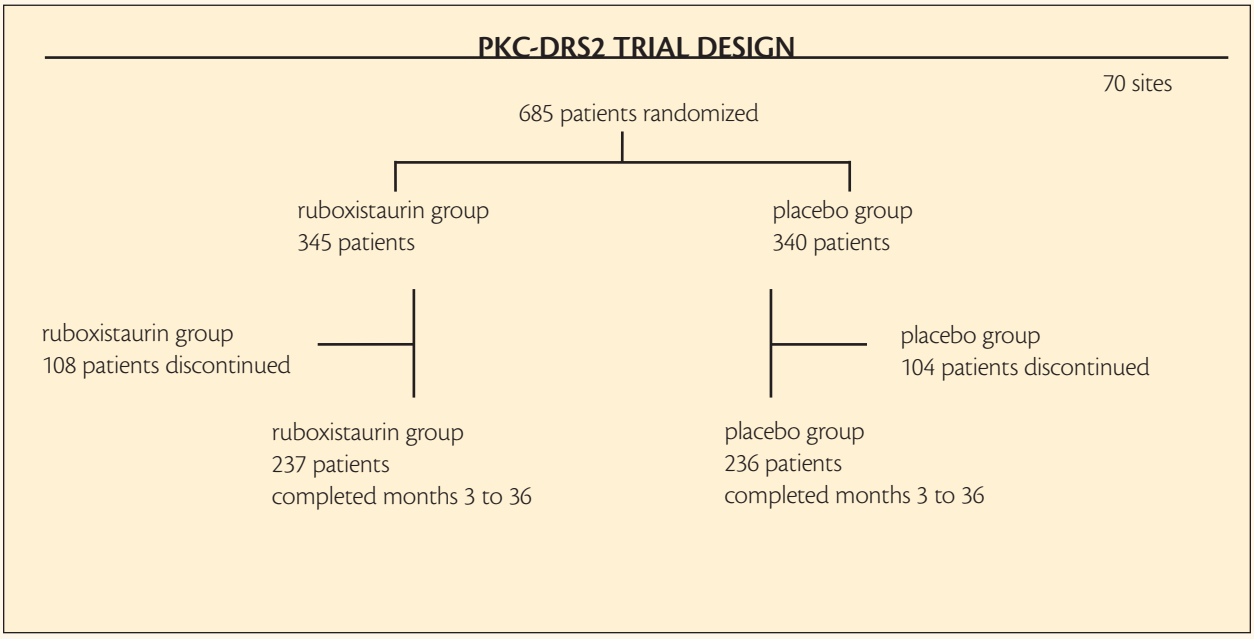


Figure 4. PKC-DRS2 phase 3 trial design.

patients in the diabetic retinopathy arm and 426 total patients in the DME arm. In the diabetic retinopathy arm, the overall goal of the trial was to see if the agent slowed the progression of (NPDR) or prevented laser treatment. Diabetic retinopathy severity was 47b to <53e (moderate to severe NPDR). Patients had no prior panretinal photocoagulation but could have prior focal treatment and could have macular edema at baseline (Table 1).

The results of the PKC-DRS2 study were presented at the 2006 ARVO and ADA meetings.

In the DME study, the outcome was the slowing or reversal of the progression of macular edema or to prevent laser treatment. The diabetic retinopathy severity was 20 to ≤47 a (mild to moderate NPDR). Patients did not have prior panretinal photocoagulation or focal treatment and did have macular edema at baseline. The Cox proportional hazard model is as shown in Figure 2.

The primary study endpoint in the PKC-DRS was the progression of diabetic retinopathy, meaning a 3+ step change in the Early Treatment of Diabetic Retinopathy Study (ETDRS) severity scale (patients with two eyes <level 61) or 2+ step change in the ETDRS severity scale (patients with only one eye <level 61) or panretinal photocoagulation. The Cox proportional hazard model for moderate visual loss is shown in Figure 3.

The PKC-beta inhibition study showed that ruboxistaurin decreased the development of macular edema threatening the center of the macula and the occurrence of visual loss. Ruboxistaurin did not prevent the progression of diabetic retinopathy nor the combined outcome of DME progression or the application of laser photocoagulation. Two large, high-power, single-dose trials evaluating the impact of ruboxistaurin on these endpoints are underway (Figure 4).

You can read about the results of PKC-DRS2, which were presented at the ARVO and ADA meetings, on page 25 of this issue. —Eds.

INTRAVITREAL STEROIDS

The potential of intravitreal steroid treatment is that it can reduce retinal edema, but there is a time limited effect. The visual acuity response of this method of treatment does not always commensurate with reduction in retinal edema. Intravitreal steroids come with a high risk of cataract, intraocular pressure elevation and endophthalmitis, and often it is an off-label use.

Intravitreal steroids are being investigated as part

of the Diabetic Retinopathy Clinical Research Network (DRCR.net). The DRCR.net is dedicated to multicenter clinical research of diabetic retinopathy, macular edema and associated disorders.

Intravitreal steroids are being investigated as part of the Diabetic Clinical Research Network. The DRCR.net is dedicated to multicenter clinical research of these associated disorders.

Triamcinolone acetonide is being studied in a phase 3, multicenter, randomized clinical trial. The treatment is delivered as a preservative-free, pH balanced solution, in a single-dose prefilled syringe. Patients are randomized to one of three treatment groups: standard of care (conventional treatment including modified ETDRS photocoagulation), intravitreal injection of 1 mg triamcinolone acetonide or intravitreal injection of 4 mg triamcinolone acetonide.

The injection volume is always 0.05 mL and follow-up is 3 years. The first patient was enrolled in July 2004 and more than 417 patients have been enrolled to date. The goal is 690 patients total. I presented this information at the 2005 American Academy of Opthalmology Annual meeting in Chicago.[10]

CONCLUSION

Diabetes is a systemic disease. Diabetic retinopathy is the leading cause of blindness in working age adults. Diabetic neuropathy is the leading cause of nontraumatic amputations and diabetic nephropathy is the leading cause of end-stage renal disease. Also, with regard to the macrovascular picture, patients with diabetes have a two- to fourfold increase in cardiovascular mortality and stroke.[11] ■

1. Early Treatment Diabetic Retinpathy Study Research Group. Fundus photographic risk factors for profession of diabetic retinopathy. ETDRS report No 12. *Ophthalmology.* 1991;98:823-833.
2. Sheetz MJ, King GL. Molecular understanding of hyperglycemia's adverse effects for diabetic complications. *JAMA.* 2002;288:2579-2588.
3. Ishii, Koya D, King GL. Protein kinase C activation and its role in the development of vascular complications in diabetes mellitus. *J Mol Med.* 1998;76:21-31.
4. Wakasaki H, Koya D, Schoen FJ, et al. Targeted overexpression of protein kinase C beta2 isoform in myocardium causes cardiomyopathy. *Proc Natl Acad Sci U S A.* 1997;94:9320-9325.
5. Aiello, Avery RL, Arrigg PG, et al. Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy an other retinal disorders. *N Engl J Med.* 1994;331:1480-1487.
6. Adams AP, Miller JW, Bernal MT, et al. Increased vascular endothelial growth factor levels in the vitreous of eyes with proliferative diabetic retinopathy. *Am J Ophthalmol..* 1994;118:445-450.
7. Qaum T, Xu O, Joussen AM, et al. VEGF-initiated blood retinal barrier breakdown in early diabetes. *Invest Opthalmol Vis Sci*. 2001;42:2408-2413.
8. Tolentino MJ, Miller JW, Gragoudases, et al. Intravitreous injections of VEGF produce retinal ischemia and microangiopathy in adult primates. *Ophthalmology.* 1996;103:1820-1828.
9. Tolentino MJ, McLeod DS, Taomoto M, et al. Pathologic features of vascular endothelial growth factor-induced retinopathy in tghe nonhuman primate. *Am J Ophthalmol.* 2002;133:373-385.
10. Aiello LP. Molecular biology of diabetic retinopathy: Opportunites for therapeutic intervention. CME Symposium presented during the the 2005 American Academy of Ophthalmology meeting. October 15, 2005. Chicago.
For a downloadable pdf of this article, including Tables and Figures, click here.
11. Aiello L, Haller JA, Zinman B, Bressler NM. The Eyes Have It: Preserving vision in patients with diabetes. Presented at the American Diabetes Association 66th Scientific Sessions. June 12, 2006. Washington, DC.

This activity may also be completed online at www.cmetoday.net.

CME QUESTIONS

Circle the most appropriate answer in the "ANSWER SECTION" on the following page.

1. What is the principal underlying cause of the complications of diabetes?
a. hyperglycemia
b. growth factors
c. ischemia
d. PKC-beta

2. How often should a diabetic patient with no evidence of retinopathy vist an ophthalmologist?
a. every other year
b. every 5 years
c. yearly
d. monthly

3. Which of the following is NOT a sign of severe NPDR:
a. >20 intraretinal hemorrhages in each of the four quadrants
b. definite venous beading in two or more quadrants
c. no signs of PDR
d. microaneurysms only

4. The use of a clinical diabetes educator in the optimal patient approach to care is not advised.
a. true
b. false

5. What PKC isoform is primarily involved in the complications of diabetes and other vascular issues?
a. alpha
b. beta
c. gamma
d. delta

6. Which of the following statements is NOT true about VEGF?
a. It is a glycoprotein
b. It was first identified in vascularized tumors
c. It binds to high-affinity receptors on retinal endothelial cells and stimulates retinal endothelia cell growth
d. Retinal VEGF levels are decreased in diabetes

7. Which of the following is NOT a VEGF therapy that was discussed in the activity?
a. Macugen
b. Lucentis
c. triamcinolone acetonide
d. Avastin
e. VEGF Trap

8. Which of the following is NOT true with regard to ruboxistaurin?
a. it is highly PKC selective
b. it is highly beta isoform selective and orally bioavailable
c. it has been shown to reduce retinal neovascularization
d. it has not been studied in patients with existing macular edema

9. Intravitreal steroids are being investigated in the DRCR.net.
a. true
b. false

10. What is the leading cause of blindness in working age adults?
a. diabetic retinopathy
b. diabetic neuropathy
c. diabetic nephropathy
d. CVD and stroke

REGISTRATION/EVALUATION FORM: MOLECULAR BIOLOGY OF DIABETIC RETINOPATHY: HOW TO BEST PRESERVE VISION IN THESE PATIENTS

To obtain AMA PRA Category 1 Credit™, you must:

- Read the learning objectives and the CME article and complete the self-assessment test;
- Photocopy and complete this registration/evaluation form and record your test answers in the Answer Section below;
- Send the Registration/Evaluation form to **The Dulaney Foundation, Post Office Box 44408, Phoenix, AZ 85064, or fax to 602-508-4893;** and
- Retain a copy of your test answers. Your answer sheet will be graded, and, if you achieve a passing score of 70% or better, you will receive a CME credit letter awarding 1 *AMA PRA Category 1 Credit™* within 4 weeks. If you do not achieve a passing score, you will be notified and offered the opportunity to complete the activity again.

ANSWER SECTION

Circle the best answer for each question on page xx.

1. A B C D	2. A B C D	3. A B C D	4. A B	5. A B C D
6. A B C D	7. A B C D E	8. A B C D	9. A B	10. A B C D

REGISTRATION FORM

First name _____ Last name _____ Degree (MD, PhD) _____

Specialty _____

Institution or practice name _____

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City _____ State _____ Zip Code _____ Country _____

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I attest that I have completed this activity as designed, and I am claiming ____ (up to 1 credit) AMA/PRA Category 1 Credit™

Signature _____ **Date** _____

Credit for this activity is available until July 31, 2007.

The planning and execution of useful and educationally sound continuing education activities are guided in large part by input from participants. Please assist us in evaluating the effectiveness of this activity and make recommendations for future educational offerings by completing this evaluation form. Your response will help ensure that future programs are informative and meet the educational needs of all participants. Please note: CME credit letters and long-term credit retention information will only be issued upon receipt of this completed evaluation. Thank you for your cooperation.

OBJECTIVES

(Please circle the number that is most accurate; 5 represents strongly agree, and 1 represents strongly disagree.)

After successful completion of this program, you should be able to:

• describe the diagnosis, classification and manifestations of diabetic retinopathy and DME and its underlying molecular biology	5	4	3	2	1
• evaluate currently available therapeutic strategies for intervention	5	4	3	2	1
• discuss the DRCR.net initiatives and the role of the diabetes treatment team in interacting with each other and the patient toward providing optimum care.	5	4	3	2	1

OVERALL EVALUATION

(Please circle the number that is most accurate; 5 represents strongly agree, and 1 represents strongly disagree.)

• The information presented increased my awareness/understanding of the subject.	5	4	3	2	1
• The information presented will influence how I practice.	5	4	3	2	1
• The information presented will help me improve patient care.	5	4	3	2	1
• The faculty demonstrated current knowledge of the subject.	5	4	3	2	1
• The program was educationally sound and scientifically balanced.	5	4	3	2	1
• The program avoided commercial bias or influence.	5	4	3	2	1
• Overall, the program met my expectations.	5	4	3	2	1
• I would recommend this program to my colleagues.	5	4	3	2	1

- If you anticipate changing one or more aspects of your practice as a result of your participation in this activity, please provide a brief description of how you plan to do so: _____
- Please provide any additional comments pertaining to this activity (positive and negative) and suggestions for improvements: _____

_____

- Please list any topics you would like to see addressed in future educational activities: _____

_____