Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format.

1. **Grantee Institution:** The Pennsylvania State University

2. **Reporting Period (start and end date of grant award period):** 1/1/2010 - 12/31/2013

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** John Anthony, MPA

4. **Grant Contact Person's Telephone Number:** 814 935 1081

5. **Grant SAP Number:** 4100050904

6. **Project Number and Title of Research Project:** 59. Small Peptide Eye Drops for Diabetic Retinopathy

7. **Start and End Date of Research Project:** 3/12/2013 - 12/31/2013

8. **Name of Principal Investigator for the Research Project:** Joyce Tombran-Tink, PhD

9. **Research Project Expenses**.

   9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

   $ 102,565

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project	Cost
Anzor Gvritishvili, PhD	Research Associate	75	$24,492.00
Yanling Liu	Technician	60.74	5,502.41
Joyce Tombran-Tink, PhD	PI	10	10,161.00
Tiaosi Xing	Grad assistant		8,455.00

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project
None		

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
None		

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes___X_____ No_____

If yes, please indicate the source and amount of other funds:

$100,000 - QED

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes_____ No_____X____

If yes, please list the applications submitted (column A), the funding agency (National

Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
None	☐NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)		$	$

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes____X_____ No_____

If yes, please describe your plans:

Venture Capital
NIH - SBIR

12. Future of Research Project. What are the future plans for this research project?

Complete preclinical studies and begin clinical trials for diabetic retinopathy.

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes____X_____ No_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male				
Female		1		
Unknown				
Total		**1**		

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic		1		
Unknown				
Total		**1**		

	Undergraduate	Masters	Pre-doc	Post-doc
White				
Black				
Asian		1		
Other				
Unknown				
Total		**1**		

14. Recruitment of Out-of–State Researchers. Did you bring researchers into Pennsylvania to carry out this research project?

Yes_____ No___X_____

If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. Impact on Research Capacity and Quality. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes____X_____ No_____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

This CURE-funded project has helped to further strengthen and expand the base of research on diabetic retinopathy at Penn State Hershey and lay the groundwork for the development of potential new treatments.

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes___X_____ No_____

If yes, please describe the collaborations:

Interactions with QED consultants and Provid Pharmaceuticals

16(B) Did the research project result in commercial development of any research products?

Yes___X_____ No_____

If yes, please describe commercial development activities that resulted from the research project:

Patent application filed for licensing

16(C) Did the research lead to new involvement with the community?

Yes_____ No____X_____

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims for the period that the project was funded (i.e., from project start date through end date). Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project

work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

Specific Aim 1. *We will generate 10-15 structurally different analogs of serpxA1 and –*

(a) Identify metabolic sites within the 29-residue peptide
(b) Determine whether shorter analogs have better bioavailability and maintain the same bioactivity
(c) Assess the effect of site-specific mutations on peptide metabolism, bioavailability, and bioactivity

Specific Aim 2. *All synthesized compounds will be tested in a series of rigorous quantitative <u>in vitro</u> screens to select up to the 5 most effective and structurally diverse analogs of serpxA1.*

(a) To test efficacy of the compounds in preventing cell death, we will screen for their ability to (i) Increase cellular bioenergetic levels in an ATP assay; (ii) Reduce cell death in an LDH assay
(b) To test their action on inflammatory processes, we will examine their ability to decrease production of inflammatory cytokines using Luminex bead arrays
(c) To test their potential to reduce vascular leakage, analogs will be screened by qPCR for their ability to increase mRNA levels of ZO1 and occludin, two junction proteins essential to vascular integrity.
(d) To test peptide stability in the vitreous, compounds will be incubated with dissected vitreous humor and samples analyzed at various time points using Maldi TOF.

Specific Aim 3. *We will test efficacy of the 5 lead compounds from SA2 in longitudinal studies <u>in vivo</u> through the period when vascular leakage is opthalmascopically evident in rodent DR.*

Vascular leakage is first noticed in diabetic mouse retinas ~13 wks after onset of hyperglycemia (HG). Lead peptides will be tested at the effective dose of P78 for 15 wks at the onset of HG and the following measured -
(a) Reduction in vascular leakage will be assessed by fluorescein angiography using the Micron III retinal imaging real time acquisition system equipped with StreamPix 5.8.1.4. Fluorescein extravasation from vessels will be quantitated immediately after IP injections of AK-FLUOR (n=5)
(b) RGC death in the retina will be calculated by morphometric analysis and levels of inflammatory markers

Specific Aim 4. Because rodent and primate eye are quite different in size, the bioavailability of

2-3 of the most effective analogs from SA3 will be determined at two time points in African Green monkeys.

Summary of Research Completed

For Specific aim 1 we have completed the following:
1. Identified enzymatic cleavage sites within the P78 peptide using the PeptideCutter software and discussions with the peptide chemist team at GenScript, a leading biology CRO that focuses exclusively on early drug discovery, peptide modifications, and peptide synthesis service (New Jersey)

2. Designed and synthesized 10 analogs of P78 to improve stability and efficacy for testing in our in vitro assays for inflammation, angiogenesis, and cell death, the three leading pathological features of diabetic retinopathy. The peptide modifications include:

- Altering the charge of the peptides.
- Altering peptide stability
- Generating shorter fragments including one 29 mer and three 17 mer molecules.
- Changing two residues in a 17-mer fragment to Isoleucine.
- Changing two residues in a 17-mer fragment to Alanine.
- Changing four residues in a 17-mer fragment (2 Ile; 2 Ala).
- Addition of fatty acid modifications at the N-terminus and Pegylation at the C-terminus of a 29 mer and a 17-mer fragment, both of which contain the 4 residue changes (2 Ile; 2 Ala).

- Some of these modifications changed the charge of the analogs to a more positively charged molecule and the peptide Expasy software indicate that these changes improved the stability index, hydropathicity, and/or solubility of the peptides (Table 1).

- The results indicated that peptide 81-5 has the highest level of activity in all three in vitro tests – inflammation, angiogenesis, and cell viability with efficacy ranging between 15-41% greater than P78 and 18-65% improvement to the control stressed samples (Table 2).

- Two of the truncated (17mer) peptides have demonstrated biological activity that is better than that observed with P78. Peptide 81-2 shows good efficacy and bioavailability profiles of all the peptides tested so far. It is ~8-18% better than P78 and the control in the inflammatory and angiogenesis assays and 8-26% better than P78 and control in the viability assay. 81-5 is the strongest candidate from the in vitro studies but we have not yet completed the bioavailability study for this drug. If it has an equivalent or superior bioavailability profile compared to 81-2, it will be the lead compound in this series

3. Six truncated and/or modified peptides from the 44 mer (P78) were tested to date (spxA1, 81-1 to 81-5; Table 1) for their efficacy in reducing levels of TNF α, IFN γ, VEGF-A and increasing cell viability in *in vitro* assays. Efficacy was compared to both non-treated oxidative stressed controls and the ref standard P78.

In addition, bioavailability studies of the peptides in rodent eyes are completed for 4 of these shorter and/or modified peptides (spxA1,81-1 to 81-3) and the results compared to the ref standard P78 peptide

4. Four of the peptides were tested in bioavailability studies in rodents at 1, 2, 4, 8 hrs (n=4 each peptide/time pt). The animals were given a single eye drop containing 2 mg of each peptide. At each time point the animals were sacrificed, vitreous harvested, and bioavailability assessed by Maldi TOF.

5. We recently signed a CDA and a Service Agreement with Provid Pharmaceuticals, Inc., 9 Deer Park Drive, Monmouth Junction, NJ. Provid is a drug discovery service company with expertise in drug design of small molecules and peptide therapeutics of use in the discovery of new drugs or in the development of inhibitors or modulators of biological interactions. , Provid will carry out the following services for the QED project:

- Evaluate the initial peptide data and SAR from our lab
- Design a set of 25 candidate peptide derivatives for evaluation
- Based on the SAR evaluation a final set of 10-15 designs will be selected for synthesis by GenScript and testing in our lab.

Provid has entered into a Service Agreement with Penn State College of Medicine on March 21, 2013. I have had several discussions with Gary Olson, President and CEO and Dr Christopher Self the VP, Medicinal Chemistry of Provid Pharmaceuticals Inc., and have shared the current list of peptides analog sequence with them. The peptide chemist team at Provid plans to submit the first series of derivatives by April 9th so that we can continue with the SAR evaluation.

For Specific aim 2 we have completed the following:
Twenty P78 peptide derivatives were generated by either the PI or Provid Inc and synthesized by Genscript. The size of the analogs ranged from 35 to 12 amino acids in length. These peptides had internal amino acid substitutions of the sequence and/or helix stabilization and hydrophobic cap changes to improve peptide stability and efficacy. The peptides were all tested and compared to P78 and spxA1 for efficacy in cell viability (FIG 1) and release of TNFa, IFNg, and VEGFA (FIG 2) – three of the most potent inflammatory, vascular leakage, and angiogenesis markers in the retina. Several of the peptides showed activity differences ranging between ~5-20% when compared to P78. Several of the peptides were significantly less activity than P78.

Peptides of interest in this study include: 81-2, 81-5, 81-12, 81-20 .

We did not anticipate that the same peptides would elicit a better response in all the five assays (Fig 1 and Fig 2) so we were pleased to see this as it makes the selection process for taking the testing to the in vivo levels easier.

From the five studies, the peptides under consideration for in vivo testing then are: **81-2, 81-5, 81-12, 81-13, 81-20** .

Note: Peptides 81-10 and 81-19 have significantly better effects in reducing VEGFA levels but were less effective than P78 on all the other assays. These peptides may be considered specific anti-VEGF drugs.

SA2 (a) The effects of the peptides to increase cell viability by increasing levels of ATP were tested in two models of cell death: (i) serum starvation; (ii) oxidative stress caused by hydrogen peroxide toxicity (300 uM). The graphs in Fig 1 show the relationship of the peptides to the control and significance are given to P78. In the experiments (i) and (ii) presented in the graphs the following designs were used:

(i) Human ARPE19 cells were grown to ~85% confluency in 10% serum containing medium after which the cells were placed in serum-free medium for 48 hr in the presence or absence (control) of 25nM of each peptide. For ATP assay CellTiter-Glo reagents were used and luminescence determined after 12 min spectrophotometrically (n=4).

(ii) Human ARPE19 cells were grown to ~85% confluency in 10% serum containing medium. The cells were then treated with 300uM H_2O_2 for 48 hours in the presence or absence (control) of 25 nM of each peptide. Levels of ATP were measured and % luminences to controls and P78 quantitated (n=4). Changes to ATP levels are plotted against control values and p values are given to P78.

From the cell survival data (Fig 1) presented in the graphs, the following peptides were considered good candidates to move forward in the in vivo studies:

81-2, 81-5, 81-13, 81-20

Because the synthesis of all the peptides were completed about 3 weeks ago, we did not complete the LDH assays which is another assay for cell death. We believe that the information in the two assays in Fig 1 were meaningful in allowing us to select good cell survival candidates.

SA2(b). The effects of each peptide on the inflammatory and vascular permeability/angiogenesis cytokines. TNFa, IFNg, and VEGFA were next tested and compared to P78. The cumulative data from n=5 analyses are presented in the graphs in Fig 2.

In the experiments for SA2 (b) i-iii (Fig 3) human retinal RPE cells were grown to ~ 75% confluency in 10% serum containing medium. The cells were then exposed to serum starvation in the presence or absence (control) of 25nM of each peptide including the prototype P78 and the active fragment of P78, SpxA1. The supernatant from each treatment was tested for effects on the secretion of TNFa, IFNg, and VEGFA. The data represent the percent change to the control samples and p values are given for each peptide in relationship to the P78 treatment.

In summary, from both the cell survival assay (Fig 1) and cytokine studies (Fig 2), the peptides under consideration as candidates to move forward into vivo testing in a rodent model of diabetic retinopathy are: **81-2, 81-5**, **81-12, 81-13, 81-20** .

Table 1

Chemical Properties of P78 peptide analogs

Peptide	Residues/ modifications	MW g/mol	Ext Co $M^{-1} cm^{-1}$	P/ - pH	Net chrg at pH 7	Solubility	Instability index	Aliphatic index	Hydropathicity (GRAVY)
P-78	44 (N)	4667.3	2560	4.6	-2.8	Poor	46.81	130.91	0.423
SpxA1	29 (N)	3020.48	0	5.34	-0.9	Poor	36.49	141.38	0.597
188681-1	17 (N)	1855.04	0	5.34	-0.9	good	29.34	103.53	-0.353
188681-2	17 (M)	1855.04	0	5.34	-0.9	good	29.34	103.53	-0.271
188681-3	17 (M)	1738.97	0	10.89	1.1	good	6.68	115.29	0.353
188681-4	29 (M)/FA/PEG	3119.50	0	12.4	1.1	poor			
188681-5	17 (M)/FA/PEG	1954.24	0	10.45	0.1	good			
188681-6	15 (N)	1664.80	2980	4.20	-1.9	poor	66.11	110.67	0.087
188681-7	12 (N)	1183.46	0	6.01	0	poor	45.78	195	1.942
188681-8	13 (N)	1335.39	0	3.83	-2	good	25.80	75.38	-0.562
188681-9	13 (M)	1219.32	0	6.78	0	good	-3.83	90.77	0.362
188681-10	29 (M)	2904.41	0	10.89	1.1	poor	23.21	148.28	1.010

N=native; M = modified; Stability: <40 is stable
FA: fatty acid modification at NH2
PEG: mini-PEG2-COOH
Peptide property calculator software: Innovagen; ExPASy (ProtParam)

Table 2

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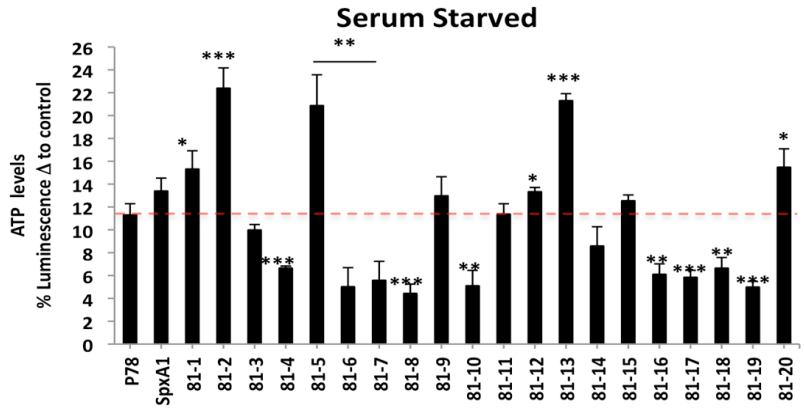
Analogs	residues	SEQ changes	* TNFα % To C	* TNFα % to P78	* IFNγ % to C	* IFNγ % to P78	* VEGF % to C	* VEGF % to P78	* ATP % to C	* ATP % to P78	^ after 4 hr
P-78	44	N	+2%	0%	-5%	0%	-5%	0%	+17%	0%	1200 ng/ml
SpxA1	29	N	-3%	-5%	-9%	-5%	-5%	0%	+5%	-11%	920 ng/ml
81-1	17	N	-3%	-5%	-4%	+1%	-8%	-3%	+9%	-11%	1800 ng/ml
81-2	17	M(2)	-13%	-15%	-12%	-8%	-18%	-14%	+26%	+8%	2000 ng/ml
81-3	17	M(4)	-7%	-8%	-9%	-4%	-12%	-7%	+11%	-6%	2280 ng/ml
81-4	29	M(4)	-7%	-8%	-15%	-11%	-11%	-6%	+7%	-9%	
81-5	17	M(4)	-18%	-19%	-20%	-15%	-20%	-16%	+65%	+41%	
81-6	15	N									
81-7	12	N									
81-8	13	N									
81-9	13	M									
81-10	29	M									

N=native; M = modified; *= In vitro assay - % INH. @ 25 nM ; ^ = % cmpd remaining in vitreous 4 hr after eye drops were given
ATP: Viability assay - H_2O_2 stress
$n \geq 4$

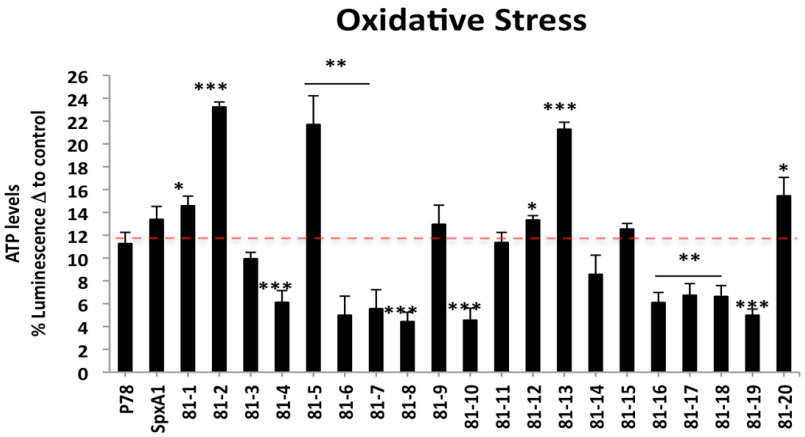
Fig 1

% Δ to control
Significance to P78

(i)



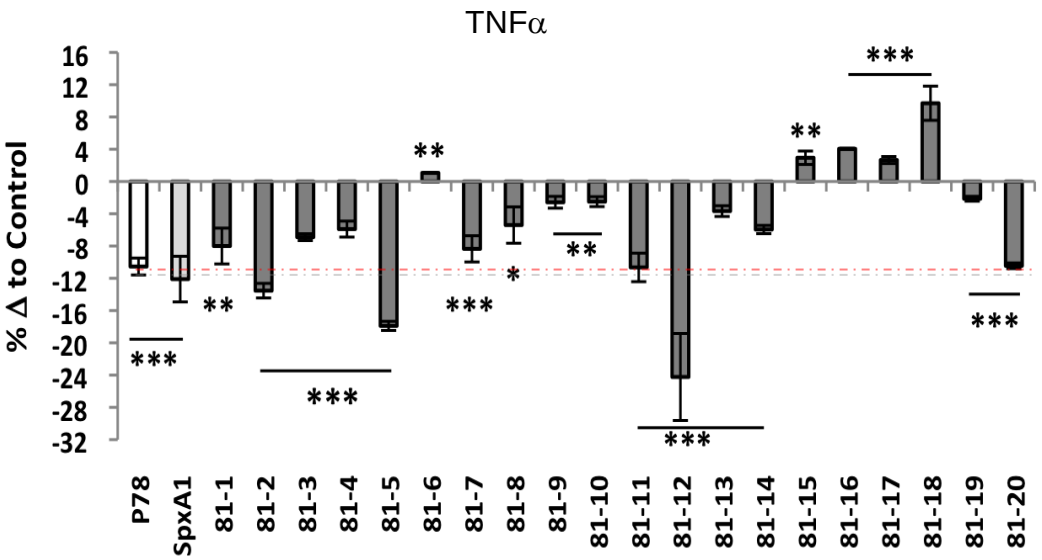
(ii



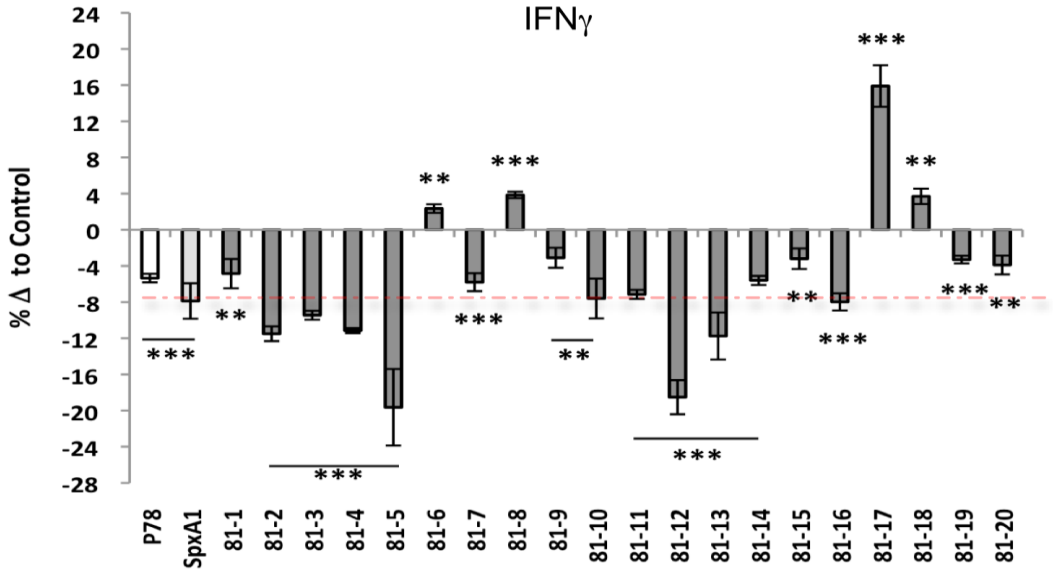
***p ≤ 0.05; **p≤0.01; ***p≤0.001**

Fig 2

(i)

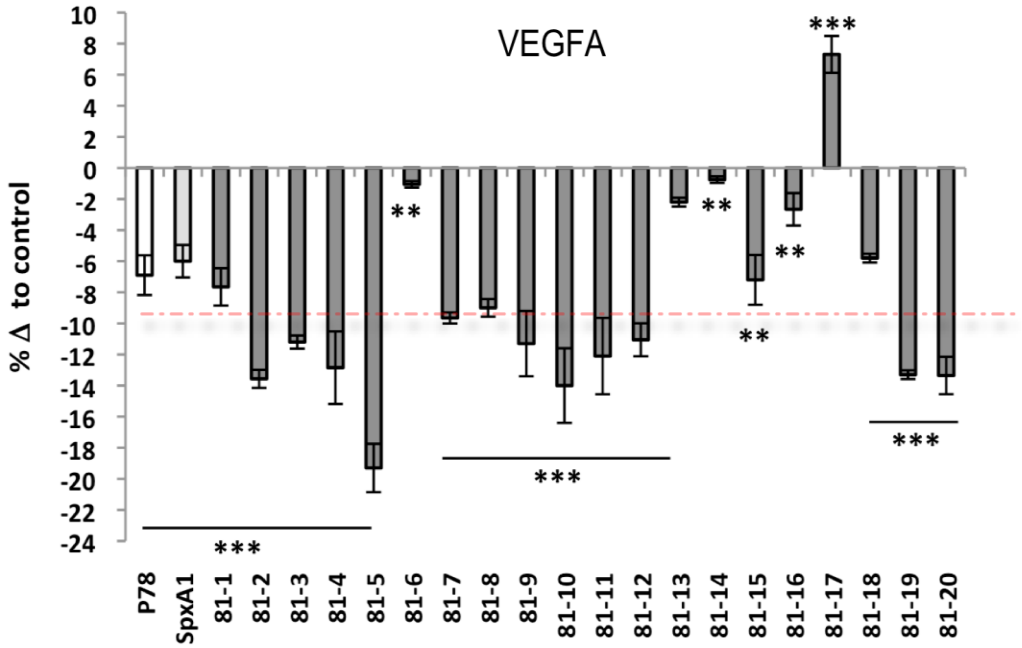


(ii)



*p ≤ 0.05; **p≤0.01; ***p≤0.001

(iii)



SA3. We will test efficacy of the 5 lead compounds in longitudinal studies through the period when vascular leakage is evident in DR.

From the in vitro P78 peptide analog screening studies conducted in Aims 1 & 2 (Annual Progress Reports 1 and 2), we selected five structurally diverse analogs with best biological activity to test their efficacy in the diabetic mouse eye. In vivo effects of these peptides were tested in the Ins2Akita mouse model of DR, a well-characterized DR model that has a mutation in the insulin gene. Heterozygote Ins2Akita mice (Jackson Lab) become hyperglycemic (HG) at ~4.5wks of age. RGC death and inflammation occur within the first 4-6 wks of DR and vascular pathology is first noticed ~13 wks after the onset of diabetes. Our strategy in this aim was to identify peptide candidates with pleotrophic effects on cell death, inflammation, and vascular leakage in the diabetic retina.

Male hyperglycemic mice with blood glucose levels >300 mg/dL were used and treatments carried out essentially as we have described for P78 ((Liu Y et al., Mol Med 2012). Diabetic mice were treated 2x/wk for 15wks at the onset of HG using a single dose of 5μg/5μl artificial tears for each drug, a dose at which P78 is effective. Both eyes received the same treatment to avoid drug cross contamination between the eyes of an individual animal. At the end of the longitudinal studies, eyes were enucleated from anesthesized animals, vitreous collected, and retina dissected and embedded in paraffin for morphometric analysis of RGC survival and vascular leakage.

However, before the longitudinal efficacy studies were carried out in vivo, we examined the bioavailability of the five analogs in comparison to the parent compound P78 and its active 29-mer derivative, SpxA1. The studies are outlined below.

(a) Peptide Bioavailability in the retina

Bioavailability of each peptide was determined in vitreous samples obtained at various time points after eye drops were given. Vitreous samples were harvested from enucleated eyes and immediately analyzed by mass spectrometry. Spectral intensities were compared to spectra of known concentrations of each analog to calculate amounts of peptides reaching the vitreous. The eye drops administered to normal mouse eyes contained 5μg of the test compound. Bioavailability was studied at three time points: 1, 4, 6 hr using four animals/time point (n=8 eyes). The vitreous was rapidly dissected from each group after the drops were given and immediately analyzed by MALDI TOF. Several of the analogs showed significantly better access to the retina compared to P78 and SpxA1 but all showed peak levels at approximately 1 hr after the drops were administered. Analog 81-5 showed the best stability profile in the vitreous after 6 hr compared to the parent compound and its truncated 29-mer SpxA1. The quantitative bioavailability data obtained by Mass Spectrometry are given below in Fig 1 (pg 20). The amount of peptides reaching the retina 1 hr after eye drop administration was also visualized by confocal microscopy. In this experiment, eye drops containing the peptides were given to the diabetic Ins2Akita mice for 1 hr. The animals were then euthanized, eyes dissected, fixed in 4% paraformaldehyde overnight, and whole globes were sectioned in OCT. Sections were then immunolabeled with an antibody again the P78 peptide, which also recognizes the full length PEDF and the analogs. As controls, C57BL/6 (wt) and diabetic Ins2Akita mice treated with vehicle (Diabetic Control) were immunolabeled to compare with the peptide treated groups. The data in Fig 2 (pg 20) shows weak endogenous PEDF staining in the wt retina and an upregulation of endogeneous PEDF in the diabetic control retinas suggesting that in diabetes, PEDF is upregulated, possibly as an endogeneous therapeutic approach by the eye. The intense labeling after the peptide eye drops were given largely represents the peptide analogs present in the retina and indicates that a significant amount of the peptides is delivered to the retina by topical routes. Staining is visible throughout the retina, but is more strongly seen in the Choroid, RPE-Photoreceptor layers of the retina. The intense labeling seen in the choroidal vasculature suggests that these vessels may be a route of delivery of these peptides (Fig 2-pg 20).

Thus, both the mass spectrometry and immunolabeling studies provide strong evidence that these small therapeutic peptides can be delivered to the back of the eye when administered topically and is represents a non invasive approach to treating retinal diseases.

(b) Reduction of inflammation

Vitreous samples harvested at the termination of the 15 weeks efficacy study were analyzed to detect levels of proinflammatory cytokines using the Bioplex multiplex platform. This system utilizes polystyrene luminex bead arrays and the xMAP technology. Target inflammatory markers examined were TNFα, IFNγ, and IL-6, and the major proangiogenesis cytokine, VEGFA. In Fig 3 we show the expected rise of the

proinflammatory cytokines in the diabetic retina compared to wt samples and a marked reduction in all three by the P78 peptide analogs. These peptides showed similar anti-inflammatory profiles in vitro experiments as well so their effects in vivo were not entirely surprising. While the peptides showed similar effects in reducing TNFα and IFNγ, 81-5 and 81-3 showed slightly but significantly better effects in reducing IL-6 (Fig 3).

(c) VEGF Levels

Levels of VEGF were also quantitated in the vitreous of the 15 wks peptide treated and control groups using the Luminex bead technology. From this experiment, we also noted a significant increase in VEGF levels as we have shown before (Liu et al., Mol Med 2012) in the vehicle treated diabetic controls compared to the age and weight matched wild type C57BL/6. All peptide treatments resulted in reduced levels of VEGF in the vitreous relative to the diabetic controls with analogs 81-5 and 81-12 slightly but significantly more effective (Fig 4).

(d) Reduction in vascular leakage

Vascular hemorrhaging was quantitated by measuring extent of albumin extravasation in the retina by leaky retinal vessels. In our proprosal, we planned to measure vascular leakage weekly at 13-15 wks after the onset of diabetes by obtaining fluorescein angiograms using the Micron III retinal imaging real time acquisition system. However, while this approach worked well for us in early diabetic stages without vascular complications and in control animals, several of the advanced-stage diabetic animals were too sick to undergo this procedure and died during the process. We were however, prepared for alternate strategies using an ELISA approach (Fig 5) to quantitate extent of albumin leakage from blood vessels into the retina parenchyma and confocal microscopy using an antibody to mouse albumin to detect changes in albumin levels in the controls and treatment groups (Fig 6). In Figure 5, we show that there was a significant increase in vascular leakage in the diabetic control retinas compared to the wild type animals at 15 weeks of diabetes. The selected panel of analogs was also effective in reducing albumin content in the retina with Spx, 81-2 and 81-5 having a small but significant advantage over the other analogs.

This analysis was confirmed by microscopic evidence on retinal sections immunolabeled for albumin. From this study, it was evident that the diabetic retinas contained higher levels of albumin throughout the retinal parenchyma and in blood vessels (arrows) as show in Fig 6 (20x). Higher magnification of these retinal images (40x) shows increased albumin levels in the photoreceptor inner and outer segment areas, vascular leakage into the retinal parenchyma in the outer plexiform layers (OPL; A, arrow) and the retinal ganglion cell layer (RGC; B,C,E,F, arrows), and a large blood vessel in the RGC layer (D). The data suggest that not only is there leakage from the microvessels in the inner retina but that the RPE-Choroidal vasculature adjacent to the photoreceptor layer maybe compromised in diabetic retinopathy.

In Figure 8, the effects of the peptides on vascular leakage as measured by albumin extravasation into the retina are shown. All peptide-treated retinas immunolabeled with the albumin antibody showed less fluorescence intensity throughout the retina including the photoreceptor IS/OS compared to the diabetic controls. Albumin levels were comparable to the wild type retinas although inner retinal microvessels appeared larger than the wt.

The microscopy data thus confirms the ELISA quantitative measurements (Fig 5) which argued for increased vascular leakage in the diabetic retina and a reduction after peptide treatments.

(e) RGC survival.

RGC loss occurs early in diabetic retinopathy in both humans and rodents. In this study retinal sections from peptide treated and control groups were stained with DAPI to manually count the nuclei of surviving RGCs. Six non-serial sections from each eye in the peripheral and central retinas were used for morphometric analysis. Stained nuclei in the RGC layer were counted in all retinal eccentricities using high- resolution confocal optical slices of DAPI stained retinas. Cell counts were taken from 6x250 mm zones along the length of the retina from centrally located fields adjacent to the optic nerve to the periphery. 6 fields/retina were analyzed and data presented as the avg # cells/400 μM. (n=6). The results of this experiment are presented in Fig 9-10.

The morphometrics data in Figure 9 show a decrease in the number of surviving retinal ganglion cells (RGCs) in untreated diabetic control retinas (DC) as we have published. The extent of the neuroprotective actions of the peptide treatments on these cells was also significant for each treatment and comparable RGC counts in the wild type retina. The efficacy profiles of this group of peptides for RGC survival are comparable and suggest that this set of P78 analogs are clearly potent in preventing diabetes-induced degeneration of RGC cells. Whether the neuroprotective effect of the analogs is a direct action on RGCs themselves or is indirectly mediated through the reduction of proinflammatory cytokines is worthy of further investigation. Although the peptide analogs are structurally diverse, the modifications were conservative. Although they are much shorter fragments that P78, each contain the same core group of peptides. These peptides were selected because their in vitro activity was superior to P78. Without a doubt, the in vitro and in vivo efficacy profiles indicate that these peptides contain the active core sequence of P78 and constitute a panel of therapeutic analogs for DR.

The micrographs in Figure 10, are representative images of DAPI and TUNEL stained retinas of peptide treated diabetic mice and control groups. The RGC layer is indicated as the single cell layer in the inner retina. DAPI staining was used to count the nuclei in the RGC layer and TUNEL assay (green fluorescence) was used to detect ongoing cell death after treatment. Several of the peptides showed little ongoing cell death in the RGC layer and increased numbers of surviving RGCs compared to the vehicle treated diabetic animals. Of interest is the abnormal morphology and disorganization of the nuclei comprising the inner nuclear layer (INL) in the untreated diabetic group. The INL also

appears to be affected by the peptide treatments and is shown to be more like the wt after treatment.

From these studies we selected 2 active, structurally diverse peptides 81-5 and 81-13 to test their availability in the primate eye when given topically. Peptide 81-13 was chosen over 81-12 because it was much smaller in size.

SA4. Test bioavailability of 2-3 lead compound(s) in primate eyes - There are substantial differences between the eyes of rodents, primates, and humans. The most obvious is size of the aqueous and vitreous fluid compartments. Fluid flow forward from the ciliary body in primates/humans is countercurrent to drug movement to the back of the eye and may influence drug concentrations in the vitreous. In this study, we tested whether two of our lead compound(s) had access to the retina when given topically to primate eyes as they do in rodents. This experiment was carried out at the RxGen facility, St Kitts Biomedical Research Foundation. This facility has an experienced staff in drug delivery in vervets. Peptide doses used were scaled up by 8-20 fold as an estimate to account for differences in eye volume between rodents and primates.

Adult males and females were equally distributed into 3 treatment groups (Table 3) and randomized by weight criteria. Animals were fasted overnight then sedated with ketamine (8mg/kg, I.M) and xylazine (1.6 mg/kg, I.M) prior to all procedures. Two monkeys received a topical dose of 40 mg (40 ,ml) of 81-5 OU (both eyes) due to limited supply of this peptide and underwent vitreocentesis at ~1 hr (Group 1) after dosing. Four monkeys received a topical dose of 100 mg (50 ml) of 81-13 OU and underwent vitreocentesis at ~1 hr (Group 2; 2 animals) or ~2 hr (group 3; 2 animals) after dosing (Table 3). Animals remained under continuous sedation prior to vitreous humor collection. Eyes were manually blinked (4 blinks/min) for 2 minutes after dosing to mimic ocular delivery in a non-sedated animal. Prior to vitreous humor collection, topical local anesthesia was administered (0.5% proparacaine) and eyes disinfected with 5% Betadine. A 25-gauge, 0.5 inch needle was placed 2 mm posterior to the limbus in the inferior temporal quadrant, targeting the central vitreous. A volume of 100 mL of vitreous humor was aspirated gently from the right (OD) and left (OS) eyes and transferred to labeled pre-tared cryovials. A larger volume of vitreous humor (200 ml) was withdrawn into the syringe from animal X429 because the operator had to pull harder to overcome the viscosity of vitreous. The cryovials were flash frozen in liquid nitrogen and shipped to Penn State College of medicine. Vitreous humor collection was followed by topical administration of a triple antibiotic ointment (neomycin/polymyxin B sulfates/bacitracin zinc). Animals were returned to the colony after vitreocentesis. Since both eyes were used for the same peptide, the n value was 4 for each peptide/dose/time.

Upon receipt at Penn State College of Medicine, the samples were immediately analyzed by mass spectrometry and concentrations calculated using a standard curve that plotted intensity vs known concentration. Although the levels of the peptides reaching the vitreous compartment in the primate eye reached therapeutic levels (bioactivity = 20-50 ng/ml), peptide concentrations were lowered by ~2 fold than that seen in the rodent eyes possibly because of the drug:volume ratio and the countercurrent fluid flow forward in

the anterior chamber of the eye. However, the study suggested that increasing dosage may result in increased concentrations of the drugs in the vitreous compartment as observed with peptide 81-13 which showed comparable bioavailability profiles with 81-5 in rodents. The peptide concentration in the vitreous was not significantly different between the 1 and 2 hr treatment and was even a bit lower at 2 hr, suggesting that peak concentrations were similar to the rodent at 1 hr after topical administration.

In summary, the in vivo data in diabetic mice indicate that the selected set of P78 analogs that showed the best activity in vitro were also active in vivo in reducing hallmark pathologies of diabetic retinopathy, namely inflammation, vascular leakage, and cell degeneration. While the minor differences in efficacy in vivo was unexpected, these structurally diverse molecules represent a therapeutic panel of active compounds for diabetic retinopathy and have advantages for the development of more effective next generation compounds. The primate ocular bioavailability study is very encouraging and holds promise for delivery of these small therapeutic peptides to the human eye to treat ocular diseases.

Hurdles and Alternate approaches

One of the hurdles encountered in the study was that we did not predict animal loss during the live retinal imaging we planned to detect vascular leakage during the late stage pathology. This method worked safely during early stage diabetes but the animals were unable to withstand the anesthesia required for us to obtain the fluorescein angiograms and died before or during the retinal imaging. Because of this, we deviated from the original study and instead assessed vascular leakage quantitatively using an ELISA measurement of albumin content in the retina and confirmed leakage by confocal microscopy.

The second problem encountered was that we had a limited supply of analog 81-5 after the 15 weeks eye drop treatments. Although we synthesized another batch of this analog we were unable to ship it in time for the primate studies because of the ice storms during December. We still were able to use two different doses and two time points of vitreous sampling between the two peptides tested. This gave us relatively useful information about dosage and peak levels of the peptides in the primate vitreous. The relatively lower dose of 81-5 was still within the targeted dose range and the interpretation of study results was minimally effected.

In the primate study, collection of vitreous humor was initially attempted using a 27 G needle to minimize trauma associated with vitreocentesis. Due to the viscosity of vitreous humor in some eyes a 25 G needle was required to withdraw the target volume. In one animal, twice the volume was collected because of the pull force used to overcome vitreous viscosity.

Commercial Potential

A patent application for the peptide technology and its use for retinal diseases and diabetic complications was filed in September 2013.

We are currently in negotiations with several interested parties in licensing the technology or supporting the research program so that we can move forward with studies required by the FDA to move these peptide analogs towards a clinical path.

We are fortunate to have the Penn State office of Technology that works closely with our research team and have given invaluable counsel on filing the patent application and negotiating licensing agreement. However, there is ongoing need to leverage additional funds to develop the technology further by completing dosage and toxicology studies, validate results in a second model of DR, and study mechanism of action in vivo.

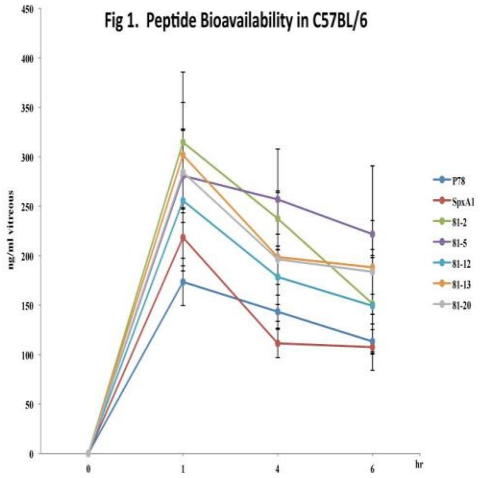


Fig 2 Peptide Delivery to the retina – 1 hr eye drop treatment

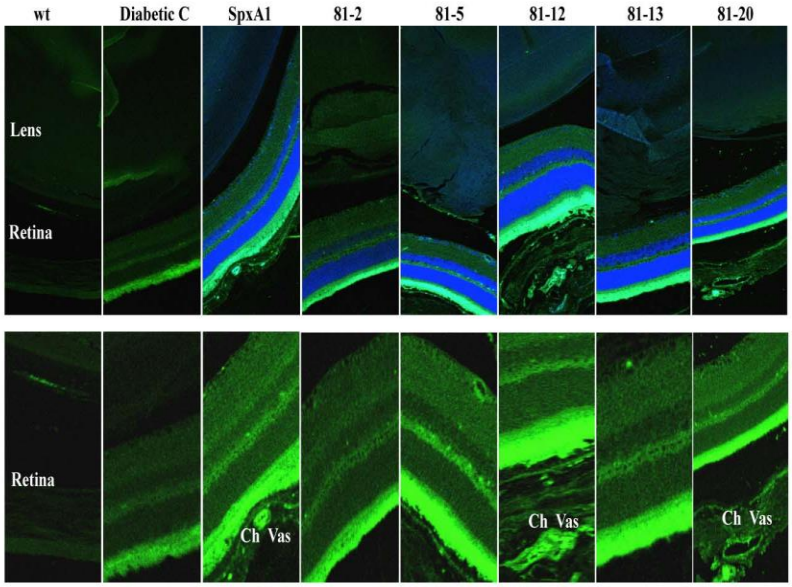


Fig 3. Inflammatory cytokines

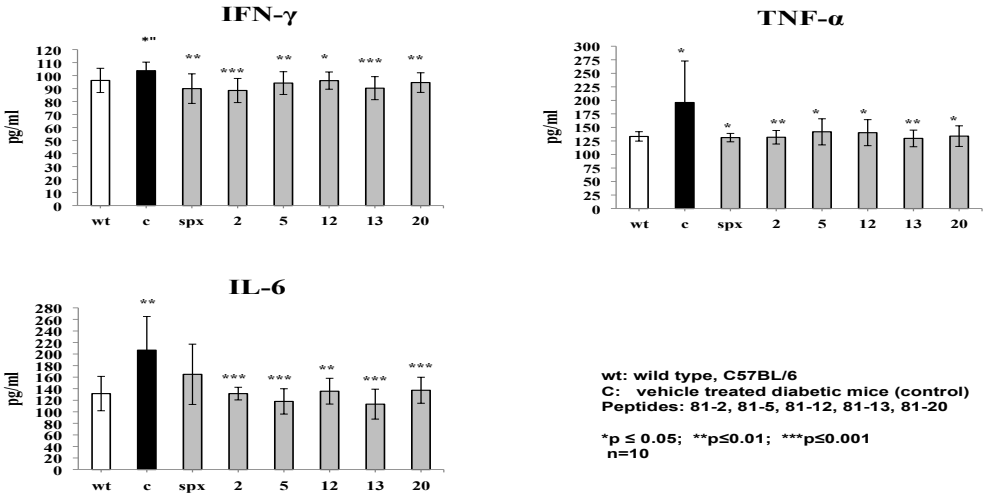
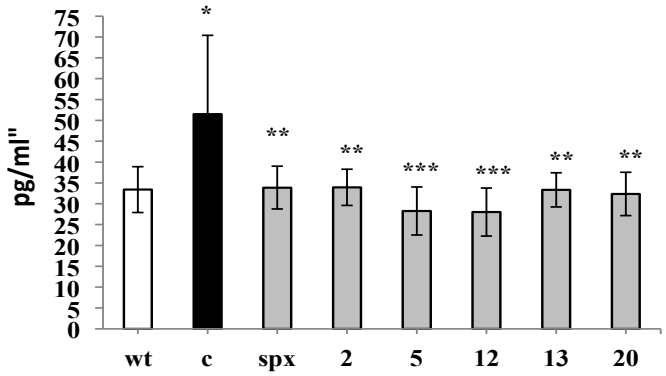


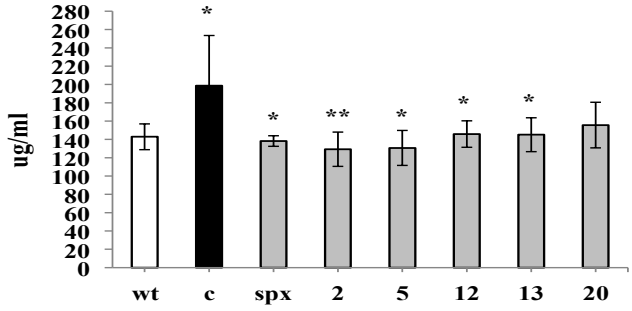
Fig 4. VEGF Levels



wt: wild type, C57BL/6
C: vehicle treated diabetic mice (control
Peptides: 81-2, 81-5, 81-12, 81-13, 81-20

***p ≤ 0.05; **p≤0.01; ***p≤0.001**
n=10

Fig 5. Albumin Leakage in the retina



wt: wild type, C57BL/6
C: vehicle treated diabetic mice (control
Peptides: 81-2, 81-5, 81-12, 81-13, 81-20

***p ≤ 0.05; **p≤0.01**
n = 10

Fig 6. Vascular leakage: Albumin content

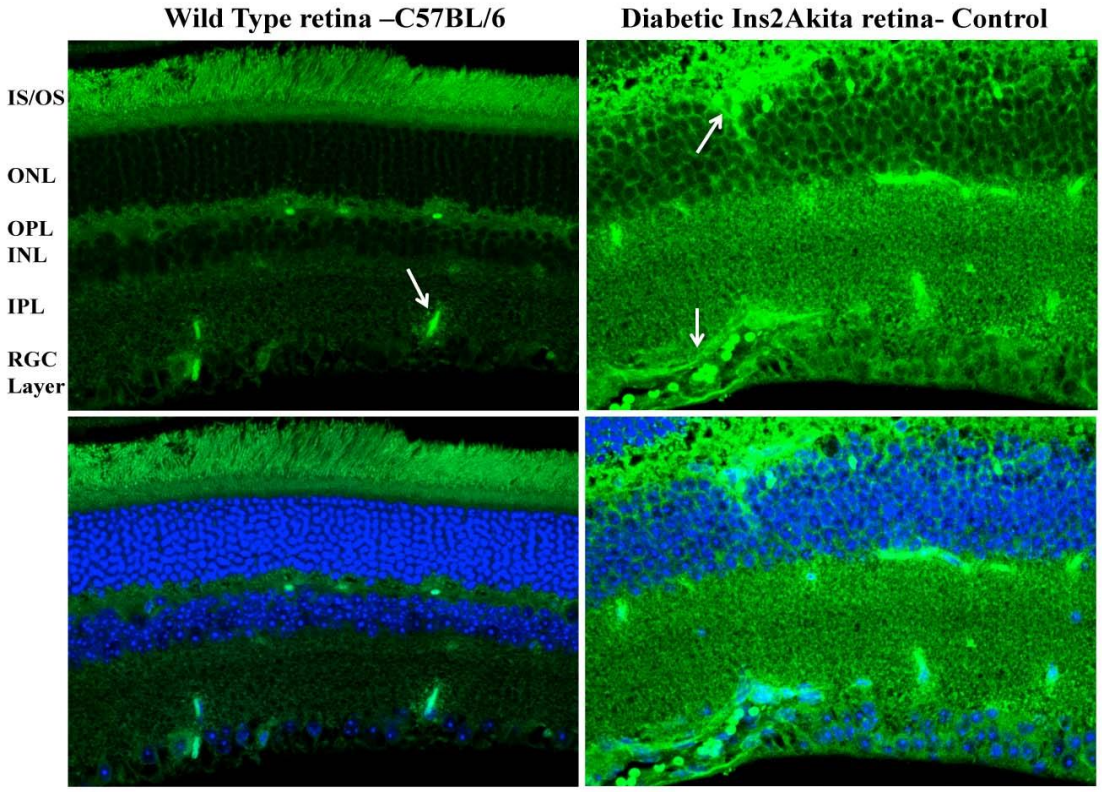


Fig 7. Vascular Leakage: Albumin extravasation in the retina

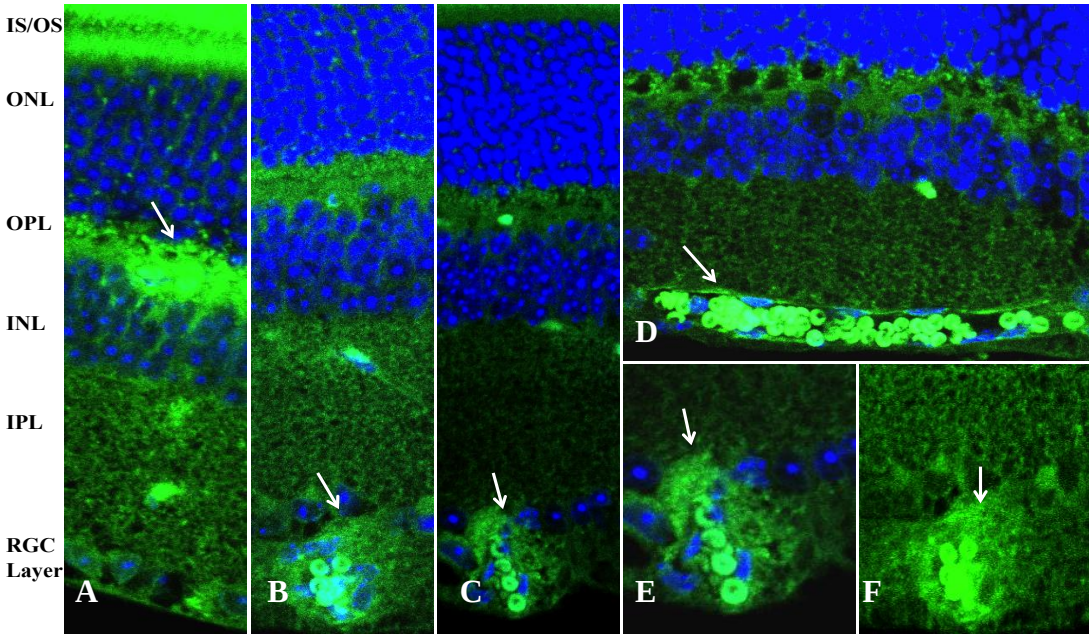


Fig 8. Vascular Leakage after peptide treatment – 15 weeks : Albumin immunolabeling

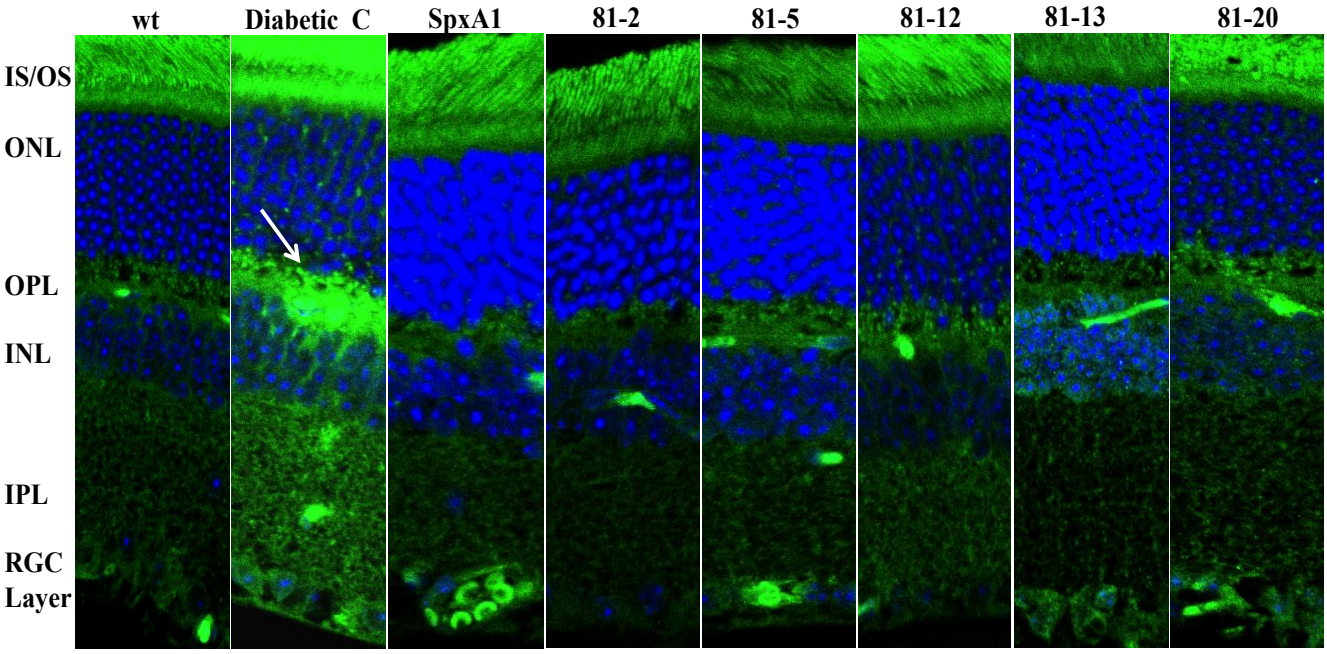


Fig 9. RGC survival

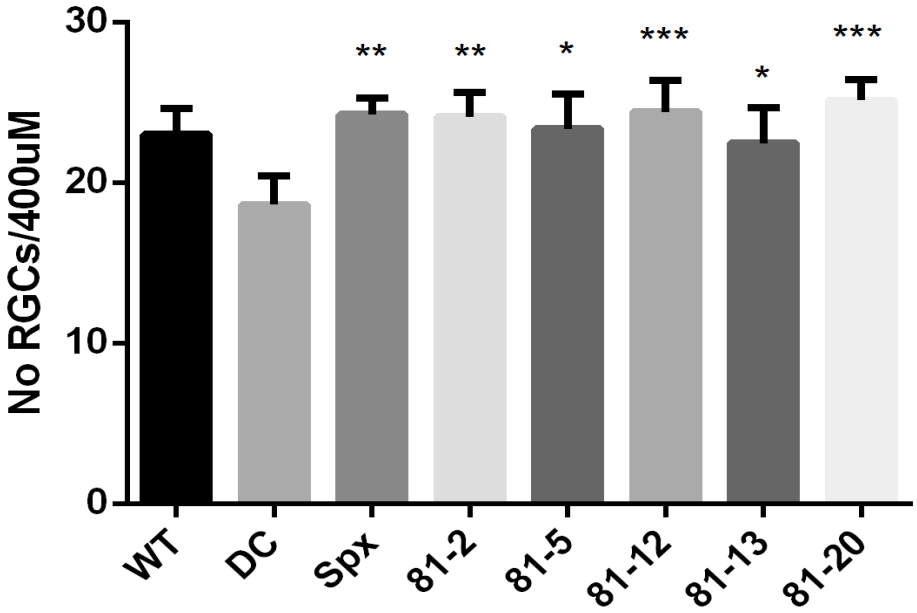


Fig 10. Retinal Ganglion Cell Survival

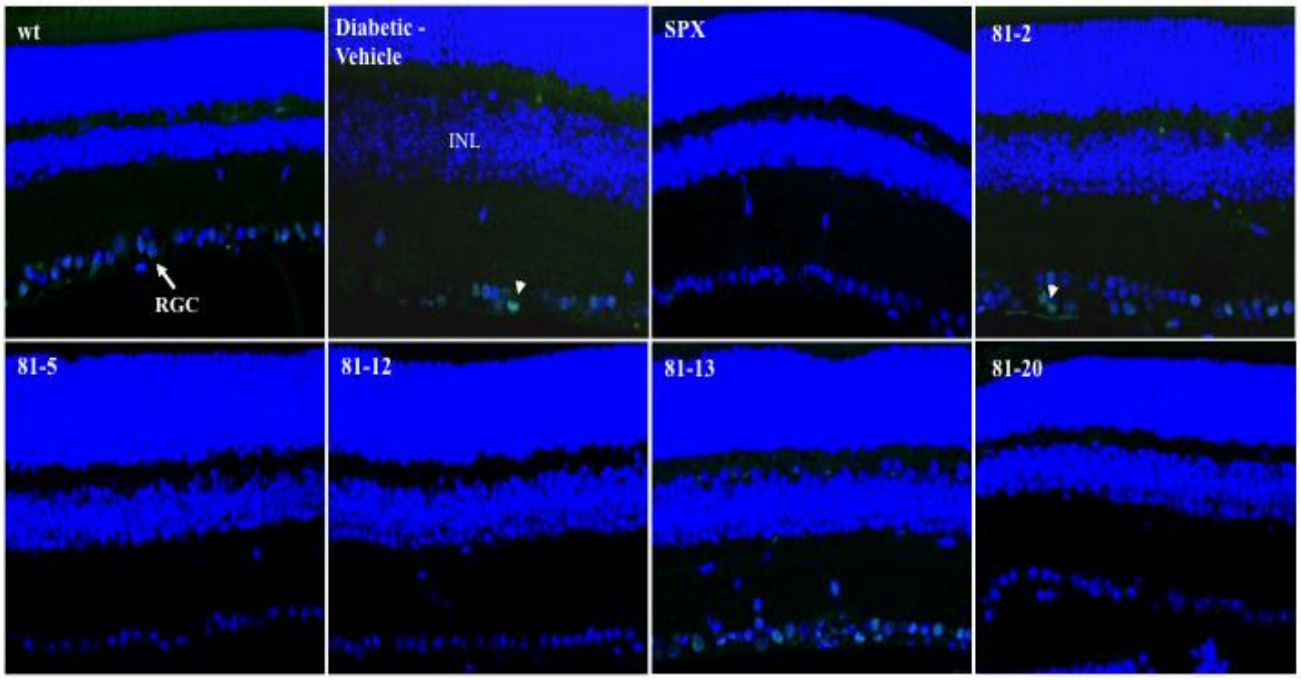


Table 3. Peptide bioavailability in primate (Vervets) eyes

Group	Animal ID	Sex	Body weight (kg)	Eye	Test article	Dose/ Topical	Vitreous humor collected after dosing	Volume collected	Mass Spec Avg Conc ng/ml
1	Z998	Male	6.54	OU	81-5	40µg/40µL/eye	OD:67 min OS:69 min	OD:100 µL OS:100 µL	
1	K099	Female	3.78	OU	81-5	40µg/40µL/eye	OD:65 min OS:66 min	OD:100 µL OS:100 µL	Group 1 (n=4) 158.5±15.5
2	V715	Female	4.68	OU	81-13	100µg/50µL/eye	OD:59 min OS:60 min	OD:100 µL OS:100 µL	
2	K146	Male	5.92	OU	81-13	100µg/50µL/eye	OD:58 min OS:64 min	OD:100 µL OS:100 µL	Group 2 (n=4) 237.5±32.5
3	X429	Female	4.98	OU	81-13	100µg/50µL/eye	OD:120 min OS:118 min	OD:100 µL OS:200 µL	
3	K169	Male	8.28	OU	81-13	100µg/50µL/eye	OD:125 min OS:128 mi	OD:100 µL OS:100 µL	Group 3 (n=4) 212.5±15.0

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
__X___No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___X__No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
___X_ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
____Yes
____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, and an abbreviated title of the publication. For example, if you submit two publications for Smith (PI for Project 01), one

publication for Zhang (PI for Project 03), and one publication for Bates (PI for Project 04), the filenames would be:

Project 01 – Smith – Three cases of isolated
Project 01 – Smith – Investigation of NEB1 deletions
Project 03 – Zhang – Molecular profiling of aromatase
Project 04 – Bates – Neonatal intensive care

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1. None				☐Submitted ☐Accepted ☐Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes_____X____ No_____

If yes, please describe your plans:

Because we were in the process of filing a patent application for this work, the results were withheld and not disclosed in either a paper or abstract format. However, we have a manuscript in preparation and plan to submit the paper this year.

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project.

Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

The study showed that several PEDF peptide eye drops can change the progression of diabetic retinopathy in a mouse model of diabetes. The peptides are small analogs (13-17 mer) of the active region of the PEDF and when given topically can reduce both the early onset of inflammation and neuronal degeneration and the late onset of vascular leakage in the diabetic retina

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

In this study we have developed a panel of small PEDF peptide mimetics that represent a set of unique and patentable compounds for diabetic retinopathy. The peptides have several advantages over current treatments for ocular diseases.

1. The drugs are small and can be administered topically. They have access to the retina in therapeutic doses.
2. They can be applied with ease to the eye, as needed, and does not require a visit to the physician's office
3. The drugs address three major pathologies observed in diabetic retinopathy: Inflammation, vascular complications (angiogenesis, leakage), and neuronal degeneration. There are no current biological treatments for diabetic retinopathy. Those currently available for AMD are anti-VEGF therapies that hold promise for also treating diabetic retinopathy but only address a single pathology in the diabetic retina.

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes__X_____ No_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention: Derivatives of PEDF

b. Name of Inventor(s): Joyce Tombran-Tink, PhD., Colin J Barnstable, DPhil

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

The use of small PEDF derivatives for the treatment of diabetic retinopathy and other diabetic complications. The derivatives are small stretches of amino acids derived from the parent protein and contain modifications that render them unique.

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes X___ No___

If yes, indicate date patent was filed: Provisional patent filed: September 13, 2013

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No___X
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No___X

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No X

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____X____ No_____

If yes, please describe your plans:

We are in the process of finalizing a licensing agreement with a small Biotech Company to develop the products for commercialization.

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages.

BIOGRAPHICAL SKETCH

NAME Tombran-Tink, Joyce	POSITION TITLE Professor
eRA COMMONS USER NAME (credential, e.g., agency login) TOMBRANTINKJ	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Eastern Nazarene College	B.S.	1982	Biology
University of Southern California (USC)	Ph.D.	1990	Cell and Neurobiology
National Research Council	Res. Assoc.	1990-1992	Molecular Biology
National Eye Institute, NIH	Staff Fellow	1992-1997	Retina, Cell & Mol Biol

A. Personal Statement

Dr Tombran-Tink studies mechanisms of cell death and survival of the neural retina. During her doctoral studies at USC, she identified and isolated pigment epithelium-derived factor (PEDF), which is shown to be a key player in neuroprotection and angiogenesis in the retina. As an NRC Associate and Fellow at NIH, she continued to explore the role of PEDF in maintaining the health of the retina and has laid the foundation for over 800 peer reviewed publications by more than 2000 international Scientists, who have shown either a trophic/survival or antiangiogenic effect of PEDF in the nervous system. Her current research focuses on elucidating mechanisms of retinal cell death and developing small molecule interventions and non-invasive delivery strategies for retinal pathologies. She is an inventor on numerous patents for technologies related to neurodegenerative conditions. Her work has been funded by the intramural program at NIH and by grants from several organizations and foundations including the Juvenile Diabetes Research Foundation, Novo Nordisk, Ben Franklin, the American Diabetes Association, and Lions Eye Conservation Research. These have allowed her to establish strong international collaborations, successfully complete studies, publish findings in peer-reviewed journals, and support and train students and post doctoral fellows, many of whom now hold leadership positions internationally in industry, medicine, and academia. As a result, she has developed expertise, experience, and leadership strengths necessary to provide collaborative support in guiding the experimental designs and data analysis of the numerous programs she engages in at Penn State University and in international projects. Dr Tombran-Tink is an ARVO Fellow, served on numerous ARVO committees, including the ARVO Advocacy and ARVO International members committees and is a member of the Weaver Leadership committee and the ARVO Foundation Board of Governors. Dr Tombran-Tink is a Consultant to the Collaborative Research and Development Foundation where she contributes to Global Nonproliferation objectives that promote application of science and technology to economic growth by conducting international training programs that foster invention, innovation, entrepreneurship, and commercialization of technologies.

Positions and Employment

1990-1992 National Research Council, Res Assoc Fellow, National Academy of Sciences
1992-1997 Fellow, National Eye Institute, NIH
1994-2001 Lecturer, Johns Hopkins University

1996-	Consultant: Collaborative Research and Development Foundation
1997-2001	Res Assoc Professor, Neuroscience, CNMC & George Washington University
2000-2006	Associate Professor, University of Missouri Kansas City
2004-2007	Visiting Professor, Department of Ophthalmology, Yale University
2007 -	Professor, Neural & Behavioral Sciences, Penn State University School of Medicine
2011-	Visiting Professor, Xi'an Fourth Military Medical School
2013-	Visiting Professor, Henan Eye Hospital & Henan Eye Institute

Other Experience

2004-	Editorial Board, Journal of Molecular Neurobiology
2004-	Ophthalmology Series Co-Editor, Springer & Humana Press – 7 book published
2006-	Editorial Board, Journal of Molecular Neuroscience
2007-	Editorial Board, Journal of Ocular Biology, Diseases, and Informatics
2007-	Co-Editor-in-Chief, Journal of Ocular Biology, Diseases, and Informatics
2009-	Editorial Board, Open Systems Biology Journal (OSBJ)
2000	Study Section: NASA, DoD, CRDF, NIH, UM Research Board, AIBS Spars, Henry Smith Charity, Univ College London
2008-	WEAVR/ARVO Leadership committee
2009-	AFER/ARVO Host-A-Researcher Program Committee
2012	Board of Governors - ARVO Foundation
2013:	Editorial Board: Asia Based International Open Access Journal of Science (Science Postprint)

Honors

1988	Fight for Sight Citation award/ARVO
1990-1992	NRC Research Fellow
2011	Fellowship ARVO (FARVO)
2011	Dowling Society
2012	Dan Walter Memorial Award, Lion's International
2013	Melvin Jones Fellow, Lions International Foundation

C. Selected Peer-reviewed Publications (Selected from ~70 peer-reviewed publications)

1. Steele FR, Chader G.J, Johnson LV, **Tombran-Tink J**. PEDF: neurotrophic activity and identification as a member of the serine protease inhibitor gene family. *PNAS.* 1993, 90:1526-1530.PMID:8434014
2. **Tombran-Tink J**, Shivaram SM, Chader GJ, Johnson LV, Bok D. Expression, secretion and age-related down regulation of PEDF, a serpin with neurotrophic activity. *J Neurosci.* 1995, 15:4992-5003.PMID:7623128
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