PXE Awareness

National Association for Pseudoxanthoma Elasticum (NAPE, Inc.)

Volume 14, Issue 4, December 2008

Joy to the World

National Association for Pseudoxanthoma Elasticum (NAPE, Inc.)

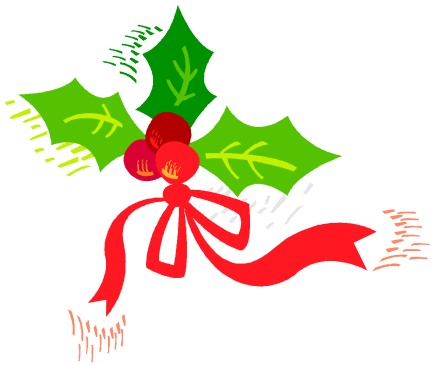
8760 Manchester Road, St. Louis, MO 63144-2724
Voice & Fax: 314-962-0100
Email: napestlouis@sbcglobal.net Web: www.napxe.org

NAPE, a non-profit 501(c)(3) support group whose mission is to provide education and support for PXE-affected persons, publishes *PXE Awareness*. Articles in this newsletter are provided for information only and are not a substitute for professional medical advice. You should not use information in this newsletter to diagnose or treat medical or health conditions. Please consult your healthcare provider before beginning or changing any course of treatment.

Board of Directors

Board of Medical Advisors

Table of Contents



President's Message

Dear NAPE Colleagues,

Holiday Greetings from our Board of Directors and office staff. We send our greetings to all those around the World who have PXE and who use our website for information about daily coping and research results. We have heard from patients across the U.S. and Canada as well as Europe, Africa, the Middle East, Australia, New Zealand, and Central and South America. We are pleased to respond to all who need information for themselves and their doctors. We feel privileged to help in this way.

In this season we are grateful for our doctors and researchers who have supported us with humanity, determination and creativity. NAPE members can note with pride their role in moving today's thriving PXE research effort. The search for ABCC6 was initiated in NAPE by Dr. Ken Neldner. Klaus Lindpaintner of Roche Pharmaceuticals arranged the funding of the genetics lab and appointed Dr. Berthold Struk to conduct the research as a post-doctoral study. In the fine tradition of science, Dr. Struk found and published the locus of the gene. Also in the fine tradition of science, other labs joined in the gene search. Four, including Dr. Struk's lab, identified ABCC6 in the same month, confirming Dr. Struk's seminal initial finding. Once ABCC6 was on the map, other research became possible. The recent metabolic findings reported in *PXE Awareness* provide the most current piece of the puzzle we are determined to complete. So NAPE members, toast your own role in this season of joy and hope.

In this issue we feature the research project in progress at the University of Missouri-Columbia. NAPE partially supports this effort along with much greater funding from the National Cancer Institute. The work led by Dr. Kattesh V. Katti seeks improved diagnostic and treatment methods for retina diseases and disorders. It stems from Dr. Katti's leadership of successful research to apply nanotechnology to the diagnosis and treatment of prostate and breast cancers. Dr. Ravi Shukla, colleague and co-researcher on the NAPE project, participated with Dr. Katti at the Salt Lake City meeting. Dr. Katti, Dr. Shukla and their colleagues at the University of Missouri-Columbia are advancing PXE science in ways we could not have imagined a short time ago. Their commitment to our

cause is very real, and we are thrilled and grateful for it. We thought you would enjoy knowing more about team members, their backgrounds and their work for us. Please read “Building A PXE Research Community” in this issue.

We had an excellent member meeting in Salt Lake City. NAPE members participated with ideas for our future. Officers were re-elected, except for the position of Vice President. Lenore Seeuwen accepted that position after serving on the Board for the past two years. We look forward to her leadership while also recognizing that of Linda Zeug who will continue on the Board but step down from her officer position. A committee of Lenore Seeuwen, Rosemary Atallian and Heidi Kevelin are busy planning our 2009 annual meeting. We expect to announce it in early 2009.

Seasons greetings to our NAPE family and friends!

Warm regards,

Fran Benham

Salt Lake City Conference participants

Nanomedicine Approaches for the Design and Development of Therapeutic Agents in the Treatment of Pseudoxanthoma Elasticum (PXE) and Age Related Macular Degeneration (AMD)

By Ravi Shukla, Raghuraman Kannan, Dean Hainsworth, Kavita Katti and Kattesh V. Katti

Editor's Note: This article reviews research partially funded by NAPE last year and plans for continuing research during the coming year. The presentation at the Salt Lake City conference was introduced with basic information about nanotechnology and its application to medicine. The following article, based on that presentation, does not include the introduction. Instead, readers are encouraged to read "Nanomedicine: Should NAPE Be Interested?" by Kattesh Katti and Raghuraman Kannan (*PXE Awareness*, November 2006). That article explains how nanomedicine has been applied successfully to the diagnosis and treatment of prostate cancer and suggests its potential for treatment of AMD, PXE and related retinal disorders.

Hypothesis

Dr. Kattesh Katti and his colleagues have built a record of discovery in the diagnosis and treatment of cancers which has brought recognition and support from the National Institutes of Health. Dr. Katti and Dr. Raghuraman Kannan have discovered a library of biocompatible and tumor specific gold nanoparticles that have resulted in recognition by the National Cancer Institute. Among their discoveries is that early stages of various human cancers manifest formation and growth of new blood vessels – a process termed 'angiogenesis.' Eye disorders such as PXE and AMD manifest similar blood vessel growth. From this, they hypothesized that nanoparticle-based targeting techniques which can be used for the diagnosis and treatment of cancers should apply to the treatment of various disorders of the eye, including PXE and AMD.

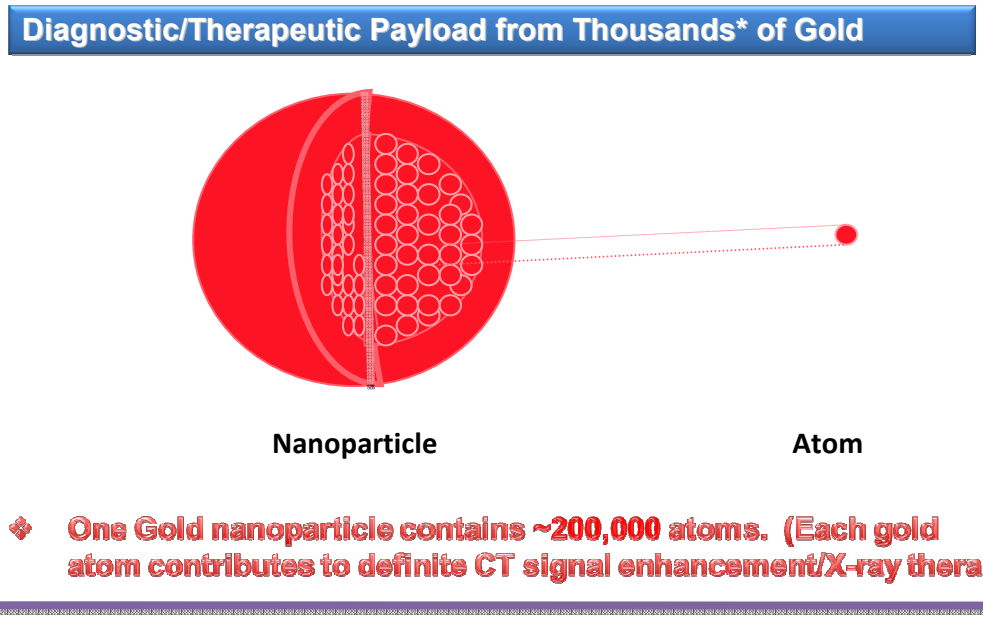
AMD/PXE

Age related macular degeneration (AMD), the leading cause of blindness affecting 90% of human populations across the globe, is characterized as a chronic degenerative disease that can lead to vision loss in either its dry or wet forms. In advanced wet AMD, new leaky blood vessels can cause rapid damage to the retina, typically resulting in central vision loss. The current treatment for wet AMD is repeated needle injections into the eye of Genentech's Lucentis or Avastin. These drugs target and inhibit

vascular endothelial growth factor (VEGF). VEGF is a sub-family of growth factors, specifically, of platelet-derived growth factor or cystine-knot growth factors. They are important signaling proteins involved in both vasculogenesis (the *de novo* formation of the embryonic circulatory system) and angiogenesis (the growth of leaky blood vessels from pre-existing vasculature).

Research Plan

The similarity of the angiogenesis morphology in the growth of blood vessels in cancer and PXE/AMD led Dr. Katti's group to work on targeted nanoparticles. Their overall goal is to develop a Super Avastin to achieve superior visual acuity outcomes with fewer intraocular injections. They also are working to develop other new products for easier administration of medications in retinal disorders. This presentation focuses on details of the development of Super Avastin and related animal models to demonstrate retention of Super Avastin within the retina.



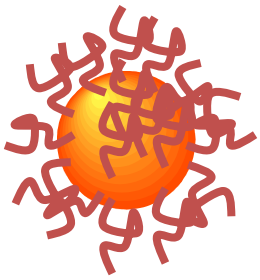
Gold nanoparticles have a great capacity for storing diagnostic and therapeutic pharmaceuticals on their surfaces. Because each nanoparticle can carry thousands of atoms on the surface, each atom can be attached with a specific pharmaceutical. For example, if each atom is attached with Avastin, a Super Avastin is created to deliver

hundreds/thousands of Avastin molecules in one injection. Traditional Avastin injections deliver only a few molecules of Avastin within the retina. Gold nanoparticle-bound Avastin can deliver hundreds of molecules of Avastin without crossing the toxic threshold.

Gold Nanoparticles
Can be conjugated with Avastin (Av/AuNP)

Avastin

Av/AuNP

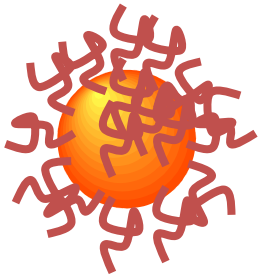


Super Avastin Nanoparticles

- Non-covalent bond
- 1 gold NP carries minimum of 20,000 Avastin molecules

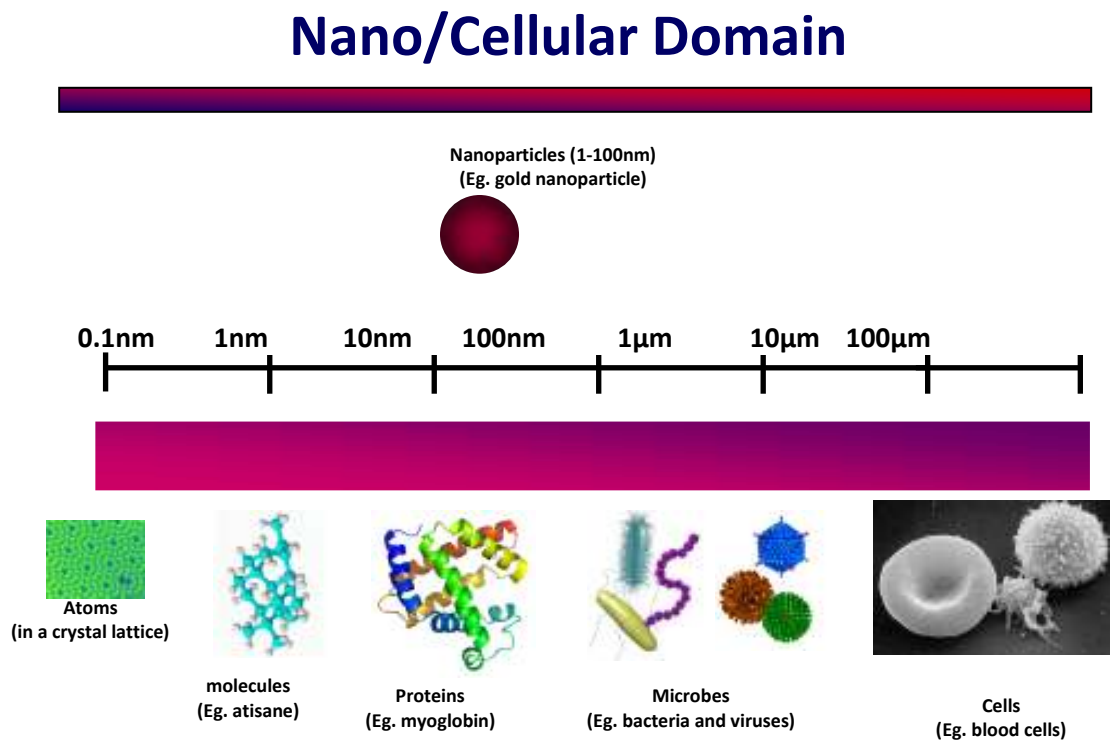
Avastin

Super Avastin



Nano Sizes

The size relations of gold nanoparticles to proteins, cells and various constituents of the living system is important since such gold nanoparticles can be designed in sizes which penetrate into blood vessels. Thus, VEGF-inhibiting Avastin, coated on gold nanoparticles, can directly penetrate blood vessels in patients with PXE/AMD to improve the therapeutic efficacy far better than traditional injections.



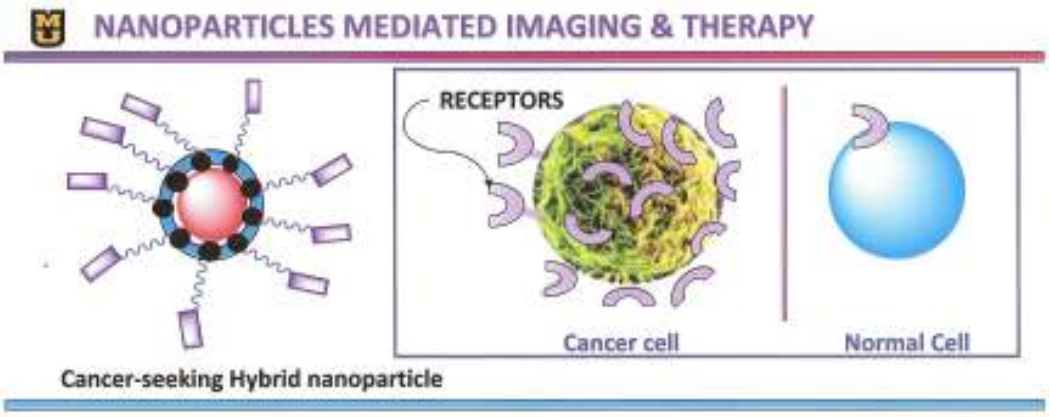
Non-Toxic Gold Nanoparticles

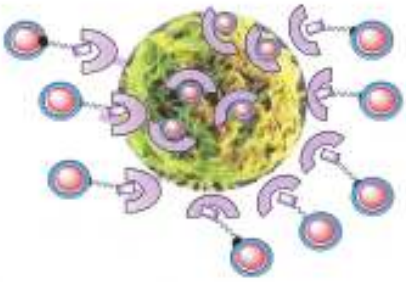
The discovery of the process for forming biocompatible gold nanoparticles using Gum Arabic as a stabilizing agent has opened the door for its use in medical applications. Gum Arabic is widely accepted in the food and pharmaceutical industries. Several tests recently performed at the National Institutes of Health funded Nanocharacterization Laboratory in Frederick, MD, have confirmed the non-toxic features of Gum Arabic stabilized gold nanoparticles. This means they can be used to develop non-toxic applications for treating cancer and PXE/AMD. The discovery, published in 2007, has been cited widely in science and medical media.

It was learned also that the imaging capabilities of biocompatible Gum Arabic protein-coated gold nanoparticles are quite remarkable. Such images provide realistic opportunities for the use of biocompatible gold nanoparticles as diagnostic agents for the detection of early stage cancer, AMD, PXE and related diseases which manifest growth of blood vessels and leaky vasculature.

Targeting Disease Sites

A sense of direction can be attached to gold nanoparticles by tagging them with certain types of chemical molecules called peptides. Specific peptides, such as bombesin peptide, have affinity to cancer cell receptors found in prostate and breast tumors. Attaching gold nanoparticles to tumor targeting peptides allows them to home in on tumor sites when injected in cancer patients. Once gold nanoparticles enter tumor sites, they can be used for both diagnosis and therapy. Similarly released Avastin bound gold nanoparticles should persist within the retina for a longer time than that achieved by traditional injection. Longer retention of Avastin in the retina would mean less frequent need for injections.





Blood Vessel Penetration

Because gold nanoparticles have a high affinity for blood vessels present in angiogenesis in retinal regions of PXE/AMD patients, administration of Avastin-conjugated gold nanoparticles allows penetration into blood vessels. This provides a highly effective delivery mechanism for Avastin at angiogenesis sites. It is most interesting to recognize that gold nanoparticles even without Avastin might be used in reducing/eliminating leaky blood vessels in PXE/AMD.

Animal Studies

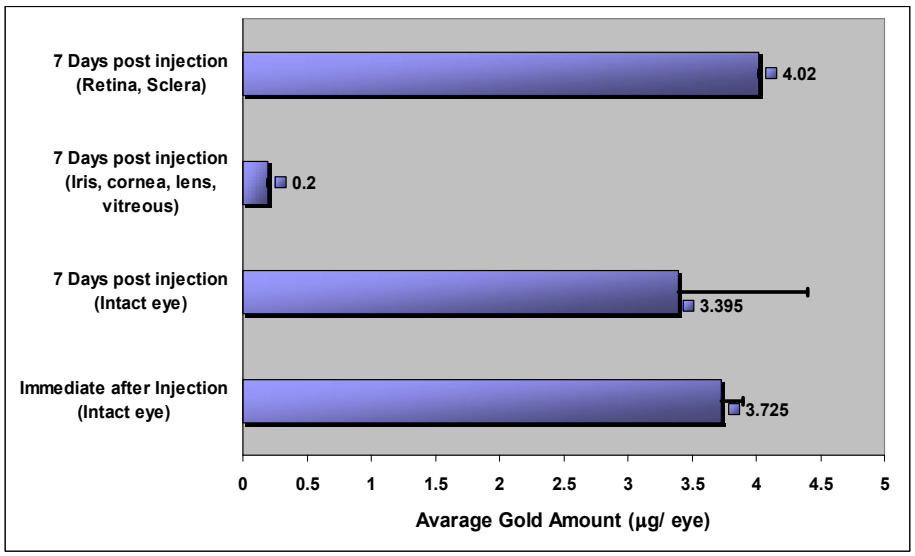
Brown Norway rats were chosen for initial study due to their eye pigmentation characteristics. Rats were injected with 5 µL hybrid gold nanoparticles intravitrially and the eyes were visualized with a fundus camera. The gold nanoparticle solution was rapidly dispersed throughout the retina and the blood vessels could not be visualized. When fluorescence angiography was performed seven days post-nanoparticle injection, the presence of gold nanoparticles in the retina was noted. Gold nanoparticle aggregates were found in blood vessels over the retina as well as in laser-induced choroidal neovascular lesions.

In order to quantify the amount of nanoparticles associated with the retina during the course of our studies, rats were sacrificed on day one as well as seven days post-nanoparticle injection. Eyes were enucleated and dissected in two major steps wherein retina and sclera were separated from the rest of the eye including cornea, lens, iris and vitreous. Gold distribution in the tissues was studied by Neutron Activation Analysis. Results confirmed that 95% of the injected gold nanoparticles went to the retina. To further confirm and visualize the presence of gold nanoparticles in the retina, the retinas were detached from sclera seven days post-nanoparticle injection and both sides of the retinas were processed for scanning electron microscopy. Back scatter images of both sides of the retina confirmed the presence and association of gold nanoparticles. Study results clearly demonstrate the selective retinal localization of gold nanoparticles in the eye.

Avastin vs Super Avastin Retention in the Eye

The traditional biological half-life of Avastin in eyes is around 8-10 hours. However, Super Avastin which was produced through the conjugation of Avastin on gold nanoparticles, exhibited the presence of Avastin in the retina and sub-retinal space even after seven days post-nanoparticle injection. Immunohistological findings, one hour post-injection, suggest that gold nanoparticle aggregates are visible beneath the lens in supra-retinal space. Indeed, the clearance of nanoparticles from the supra-retinal regions increased with time providing strong evidence for the interaction of nano aggregates with the retina and associated blood vessels. In sharp contrast to traditional Avastin, seven days post-Super Avastin injection high fluorescence was observed confirming increased biological half-life of Avastin following conjugation to gold nanoparticles.

Fate of Super Avastin NPs after Intravitreal injection in Rats (Neutron Activation Analysis)



Summary

In summary, gold nanoparticles conjugated with Avastin are biocompatible. Super Avastin nanoparticles selectively localized and penetrated through the retina and could, therefore, be clinically used to deliver Avastin, related drugs or antibodies into eyes. Our studies

also demonstrate that gold nanoparticles under specific concentration ranges do not lead to ocular toxicity, thus presenting a realistic prospect for the creation of a new generation of gold nanoparticulate-based diagnostic and therapeutic agents for the treatment of PXE, AMD and related ophthalmic diseases and disorders. In the coming year our team will continue to investigate and refine these conclusions.

ACKNOWLEDGEMENTS

This work has been supported by funds from the National Association for Pseudoxanthoma Elasticum and the National Cancer Institute through a Cancer Nanotechnology Platform grant.

Building A PXE Research Community

By Fran Benham

NAPE is a relatively small, though highly committed, patient advocacy organization. We are dependent on the good will of physicians and researchers, many of whom prefer to focus on major disorders, such as cancer. Often they have no choice as PXE patients are widely dispersed in the population and comparatively few in number. Funding for research for such rare disorders is not readily available. Our disorder is as devastating in individual lives as are diseases that are more common. We fully appreciate our situation and are most grateful that our efforts have attracted physicians like Kenneth Neldner and Berthold Struk and researchers like Kattesh V. Katti. Several years ago Dr. Katti, an internationally respected scientist with significant discoveries in the new science of nanomedicine, knew little about PXE. His research group was making ground-breaking advances in the diagnosis and treatment of prostate cancer. They had recognized the potential to advance their findings into the problems of age related

macular degeneration. When approached about PXE, Dr. Katti quickly noted similarities in PXE and AMD. He realized the need for attention to rare disorders and committed to try to help us. Today Dr. Katti and his colleagues are working to improve diagnostic and treatment methods in PXE. As well, they are using their wide-ranging expertise to develop other treatments to make our lives easier. Those of us in NAPE who are privileged to know Dr. Katti and his colleagues know that they are deeply committed to using their scientific knowledge and skill to relieve human suffering. This article provides brief introductions to these remarkable and caring scientists.

Dr. Kattesh Katti has been recognized by many organizations in many countries. His work, reported in over 130 peer reviewed articles, over a dozen book chapters and through fifteen patents, is widely cited in the scientific media, a significant sign of leadership, productivity and great respect. Most recently he was named one of the 25 2008 most influential scientists in his field. The award citation refers to recipients as "movers and shakers" who shape and advance their research agendas. Those are apt words to describe Dr. Katti who after many years of research success is still enthusiastic about new discoveries and learning opportunities as well as teaching and guiding the next generation of scientists. He is a remarkable ambassador for introducing nanotechnology to scientists and lay audiences. He truly is the catalyst for his team's interest in and research on PXE.

Ravi Shukla's post doctoral research involves the development of biocompatible gold nanoparticles for use in the design and development of diagnostic and therapeutic agents for detection and treatment of prostate, breast and lung cancers. For the NAPE project, he is working on Avastin conjugation chemistry with gold nanoparticles for the production of Super Avastin. His research includes complete in vitro analysis of Super Avastin and evaluation of in vivo properties in order to understand its releasing capabilities for the

controlled delivery of Avastin at neovascular sites within retinal regions. Ravi is uniquely prepared for this research after earning the PhD from the National Centre for Cell Science, India, where his dissertation was on the assessment of the biocompatibility of nanoparticles for biomedical applications. Dr. Shukla expects to work under the guidance of Dr. Katti for five years before returning home to India. He is performing great service for PXE patients. He will take home expert knowledge of PXE and its impact on individuals. We are most grateful to this fine scientist for his enthusiasm and determination to understand PXE and to reduce its impact. He has become a special friend of all who suffer from PXE. His studies are conducted with Dean Hainesworth, MD, from the Opthalmology Department of the University of Missouri-Columbia.

Dean Hainesworth, MD, recently became a member of Dr. Katti's research team. Initial results of the PXE/AMD project were so positive our cautious scientists felt they needed the objective evaluation of a skilled ophthalmology researcher. Dr. Hainsworth replicated the research, confirmed the findings and became interested in its potential. He arranged for Dr. Katti to speak to a national meeting of ophthalmology researchers about the possible application of nanotechnology to their work. Dr. Hainesworth has become interested in PXE/AMD and is now a valued participant in the NAPE project. He hopes to participate soon in our annual conference. Dr. Hainsworth earned his MD at the University of Kentucky. His research interests are the retina and vitreous. He works closely with Dr. Shukla in the evaluation of the animal studies underway. We of NAPE are grateful for Dr. Hainsworth's willingness to join with Dr. Katti's team to assure that the retinal results are informed by a retina specialist.

Raghuraman Kannan, PhD, is an Assistant Professor of Radiology and Co-Director, Nanoparticle Production Core Facility at the University of Missouri-Columbia. Dr. Kannan has developed a novel pathway for generating biocompatible and radioactive gold nanoparticles in aqueous medium providing a major breakthrough in utilizing nanogold radiotherapeutic agents for curing cancer. His research focus areas

are: (i) Nanoparticle production and conjugation to tumor-avid peptides, (ii) Biocompatible and angiogenesis targeting Avastin-conjugated gold nanoparticles for use in the treatment of AMD and PXE diseases; (iii) Hybrid nanoparticles for tumor optical imaging and (iv) Radioactive nanoparticles for cancer therapy. Raghu received his PhD from the Indian Institute of Science, India, and pursued post-doctoral studies at the University of Missouri-Columbia with Dr. Katti (2000-2005) prior to accepting a tenure track appointment in the Department of Radiology at the University of Missouri in 2006. Raghu's quiet confidence and determination bring hope to PXE patients.

Kavita K. Katti is a Senior Research Chemist in the Department of Radiology at the University of Missouri Medical School in Columbia. She earned the BS in chemistry and biology at Karnatak University in Dharwad, India. She has been involved in the development of target specific and biocompatible gold and silver nanoparticles. She also is working on the development of Avastin and Lucentis conjugated gold nanoparticles for potential applications as therapeutic agents in the treatment of AMD and PXE. Kavita has published over 25 articles in peer reviewed journals and is co-inventor on ten patents. She is married to Kattesh Katti, and they have twins (Nahush, son, and Sumidha, daughter). In addition to her commitment to science and family, Kavita has many interests, including vegetarian cooking. She plans to contribute simple, healthy, Indian vegetarian recipes for our NAPE website to help the PXE community.

NAPE is deeply grateful to these scientists who have added to already demanding research schedules our disorder. Their achievements to date encourage us to work harder to support their efforts.

Salt Lake City Conference

Lenore Seeuwen
and Dr. Struk

Dr. Ravi Shukla
with Claudia McCallister

Jennifer and Todd
Redd

Claudia McCallister
Sally Dawoud
Heidi Kevelin

Stef Seeuwen

Dr. Katti with
Sally Dawoud

National Association for Pseudoxanthoma Elasticum

8760 Manchester Rd., St. Louis, MO 63144-2724

Donations - Membership

No membership fee is required, although donations are needed to pay operational expenses, including telephone, fax, email, website and newsletter services.

Donations can be made in Honor or Memory of a loved one, for the Research Fund and/or for the Low-Vision Fund. All donations are tax deductible in the USA.

❒**Operations** ❒**Honor** ❒**Memory** ❒**Low-Vision** ❒**Research**

Name of Loved One: _____
Address for Acknowledgement:

PLEASE COMPLETE THE SECTION BELOW IF YOU HAVE PXE, THINK YOU HAVE PXE, OR ARE FILLING THIS OUT FOR SOMEONE ELSE

Name: _____ **Phone:** _____

Email: _____ **Fax:** _____

Address: _____

City: _____ **State:** _____ **Zip:** _____ **Country:** _____

Male ❒ **Female** ❒ **Birthdate:** _____ **Age:** _____

I am diagnosed with PXE ❒**Yes** ❒ **No** **Newsletter:** ❒ **Print** ❒ **CD**

Are you legally blind? ❒ **Yes** ❒ **No** ❒ **Email notification**

Do others in your family have PXE? ❒ **Yes** ❒ **No** **If so, who? (Mother, Father, Sibling, etc. & Name)** _____

_____

Please list any medical problem(s) you are experiencing: e.g., eye involvement, skin lesions, heart problems, gastric bleeding, etc., and comments/questions (use another page if required):

_____

_____

_____

Are you willing to be contacted by another who wishes to talk with someone else who has PXE? ❒ **Yes** ❒ **No**

Have You Changed Your Address?

Please help by letting us know. Please be sure to print your new zip code number, including the extra four digits, if possible. When we use the full zip code, our costs of mailing in the United States are lower. Please help.

New Address
Name: _____

Street: _____

City, State, Zip _____

Old Address
Name, if different: _____

Street: _____

City, State, Zip _____

PLEASE PRINT NEATLY

National Association for Pseudoxanthoma Elasticum
NAPE, Inc.
8760 Manchester Road
St. Louis, MO 63144-2724

ADDRESS SERVICE REQUESTED