PXE Awareness

National Association for Pseudoxanthoma Elasticum (NAPE, Inc.)

Volume 15, Issue 1, April 2009

Return Of A Special Friend

Matthew Lange

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

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President's Message

Dear NAPE Colleagues,

Colorful blooms, warm breezes lift our spirits. With the welcomed seasonal change, NAPE is making changes too. A remarkable one was to learn from Matthew Lange of his return to school to earn a PhD in nutrition. This could not have come at a better moment. His letter explains how he will use newly earned research skills to study the potential of Vitamin K supplements to treat PXE. Please read his letter, and if possible, offer to participate in his research. What would be more satisfying than to have a PXE patient develop a treatment that overcomes the impact of our mutant gene. Welcome home, Matthew!

Our joy in Matthew's return is dampened by the loss of the services of Linda Austin in the NAPE office. Linda has added to her very full life as wife, mother and active community member, the role of primary caregiver for her elderly mother struggling with Alzheimer's. We will miss Linda's excellent services and cheerful presence. We are especially grateful that in her time of difficulty, Linda is continuing to help us in our transition.

On a happier note, we are pleased to share another recognition for Dr. Kattesh Katti, who has been named a "Chancellor Professor" by the University of Missouri-Columbia. This is the highest honor bestowed on faculty at the University and is one we know to be well deserved. Dr. Katti and his team have provided us an update on their efforts to improve the treatment for AMD and PXE retina neovascularization. Please read their article which describes their findings and research plans.

Rosemary Atallian, Lenore Seeuwen and Heidi Kevelin are working on details of this year's fall meeting. They will be shared in the next issue of *PXE Awareness*.

As we enter into a new spring season, we who live with PXE have much reason for hope. Let's use it to fuel our efforts to live full, joyful lives.

With warm good wishes from the NAPE office,

Fran Benham

Matthew Lange and Vitamin K Research

Hello Fellow NAPxErs,

Some of you may remember me. I served on the NAPE Board of Directors a few years back and served as the legislative advocate in the late 1990's. In that capacity, I attended various legislative meetings, and lobbied with groups like the National Organization for Rare Disorders and the Coalition of Patients with Heritable Skin Disorders on Capitol Hill in order to increase both awareness and funding for necessary research. Two things you may not realize are that: 1) in my regular job, I was a database architect and built all kinds of databases - both for health-related research, as well as for large Fortune 500 companies and 2) I really love food and nutrition and majored in this when I went to college. More recently, in an attempt to unite my two passions, I decided to return to school to get my PhD in Food Science Biochemistry, with a focus on using bioinformatics techniques to determine diets and foods that would be most appropriate for people based on their genes and other parameters.

Now, I have always followed the medical research related to PXE - so you can imagine how thrilled I was when I read some of the most recent theories about the PXE gene being related to vitamin K. As you may have guessed by now - I have decided to make this the focus of my doctoral dissertation. Fortunately, I am in great company here at UC Davis, where I am doing my research. This campus is at the forefront of research in personalized nutrition. It is an NIH Center of Excellence in Nutrigenomics (the study of nutrient/gene interactions). I can't tell you too many specifics about the study at this point, but I can tell you that we are going to start with a small clinical trial. We hope to gather about a half dozen people in these categories: PXE diagnosed, family members of PXE diagnosed, and people who are not carriers of the ABCC6 gene mutation. It's going to work something like this: We will ask participants for a spit swab; we will use that to sequence your ABCC6 gene DNA. Then we will ask you to avoid vitamin K rich foods for a while (length to be determined). After this length of time, we'll ask you to go to a local lab where they can collect and send to us a blood/plasma/urine sample. Then we will likely give some participants a Vitamin K supplement, and some a placebo. After participants have been on the supplement for a while, we'll do another sample collection from your lab. Then there will be some time off of the supplement, and another sample collection.

When we receive the samples back here at UC Davis, we will analyze the blood/plasma/urine samples to see if there was any change in some of the different by-products (known as metabolites) that are known to be specific to PXE. When we do the analysis, we will look to see if there was any correlation between the vitamin K supplementation and a change in metabolites. Of course, I have left out many details, and anyone who decides they might like to participate will receive complete disclosure about all aspects of their participation. We will be starting with a relatively small trial at this point, and if there is some evidence of effect, then we will do another similar study with more participants. Hopefully, we will have something to report at the next NAPE meeting. If you are interested in possibly participating please drop me a line at mclange@ucdavis.edu with "PXE Study" in the subject line. Otherwise, you can send me mail at:

Matthew Lange
Department of Food Science and Technology
1 Shields Avenue , UC Davis
Davis, CA 95616

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Advances in Nanomedicine for Treating PXE and AMD: Functionalization of Avastin on Biocompatible Gold Nanoparticles

Ravi Shukla, Satish Nune, Kavita K. Katti, Dean Hainsworth, Raghuraman Kannan and Kattesh V. Katti
Departments of Radiology, Ophthalmology, and Physics, University of Missouri – Columbia, Columbia, MO 65212, USA

Neovascularization, or the growth of new blood vessels, is a well known response to injury and disease, exemplified by the healing of skin wounds or new vessels that aid in response to ischemic cardiac injury. In the human eye, however, neovascularization is an ominous sign of end-stage disease, often resulting in poor vision. Although not limited to these diseases, ocular neovascularization is typified by the blinding disorders macular degeneration, proliferative diabetic retinopathy and pseudoxanthoma elasticum (PXE). Age-related macular degeneration

(AMD) is an aging process of the macular retina that results in distortion and haziness of central vision, making reading and driving difficult. Unfortunately AMD is often complicated by the growth of new blood vessels beneath the retina that lead to scarring of the macula. These new blood vessels are called choroidal neovascular membranes (CNVM) and result in loss of central vision, precluding reading and driving in affected patients. CNVM associated with AMD is the leading cause of legal blindness in patients 65 years and older in the US and worldwide.[1, 2] Similar to AMD, subretinal neovascularization and retinal hemorrhages also lead to the loss of central vision in PXE.

The primary stimulus for the development of CNVM in patients with AMD and PXE is the production of vascular endothelial growth factor (VEGF), which is directly involved in new blood vessel growth.[3] A promising treatment for CNVM associated with AMD is the use of monoclonal antibodies that bind to VEGF. Bevacizumab (Avastin), one of two formulations of these antibodies, first became available for treatment of AMD patients about three years ago. It has become the standard for vision care worldwide.[4-5] Although Avastin has dramatically improved the visual prognosis for patients with AMD associated CNVM, it is administered through frequent intraocular injections. Such clinical procedures can be painful and inconvenient, and markedly increase risks associated with injections inside the eye including infection, bleeding, retinal detachment and cataract.

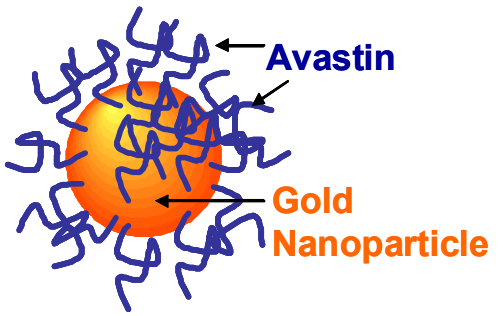

Figure 1: *Molecular structure of Super Avastin*

Currently, there are no sustained release forms of Avastin available, nor are there delivery systems that specifically target neovascularization. Nanomedicine is an emerging area of medicine that utilizes nanoparticles for the detection and treatment of various diseases and disorders.[6-15] Nanoparticles are tiny fragments of metals (or non metals) that are 100,000 times smaller than the width of human hair. Nanoparticles within the size range of about 1-50 nanometers have a size that correlates to cells, viruses, proteins and antibodies. The size resemblance of nanoparticles to living cells and cell components is of great interest in medical research because cells are primary components of all life (human and animal). Gold nanoparticles have a number of important potential medical applications. They are highly reactive, but biocompatible, making them especially well-suited for *in vivo* imaging and therapy. Gold nanoparticles can also be coated with specific biomolecules including monoclonal antibodies, proteins and peptides. Receptor specific protein-coated nanoparticles are used mainly for

targeting different markers that are over expressed on cancer cells or in various diseases or disorders. In order to develop a sustained released form of Avastin we have developed a 'Super Avastin' gold nano construct by conjugation of Avastin to gold nanoparticles and assessed their applicability for intraocular applications. The multitudes of atoms available on the surface of nanoparticles allow direct conjugations with diagnostic or therapeutic probes. Such multiplexing capabilities of gold nanoparticles and their favorable biocompatible characteristics presented new possibilities for the creation of 'Super Avastin' construct wherein hundreds of Avastin molecules would be conjugated on one gold nanoparticle (See Figure 1). 'Super Avastin' construct has the potential to serve as a nano-vehicle for sustained release of Avastin, thus providing effective binding and inhibition of VEGF for effective treatment of eye disorders in patients with PXE and AMD. Gold nanoparticles are biocompatible and do not show signs of toxicity in rat eyes at various concentrations of gold nanoparticles. When injected in rat eyes, over 90% of the injected dose of 'Super Avastin' homed into the retina. The ability of Avastin coated gold nanoparticles to selectively localize in the retina has important clinical implications. 'Super Avastin' with further testing may find applications for use in treating patients with AMD and PXE. Our studies to date have demonstrated 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 (Please see *PXE Awareness*, December 2008). Further, we have compared the half life of Avastin in rat eyes following intraocular injection with Super Avastin. Brown Norway rats were chosen for the studies due to their characteristic pigmentation in the eyes. Rats were injected intraocularly either with free Avastin or Super Avastin. Rats from each injection group were sacrificed at one hour, one day and seven days post injection. The eyes were enucleated and four eyes from each time point were stored in modified Davidson fixative for 24 h. This solution

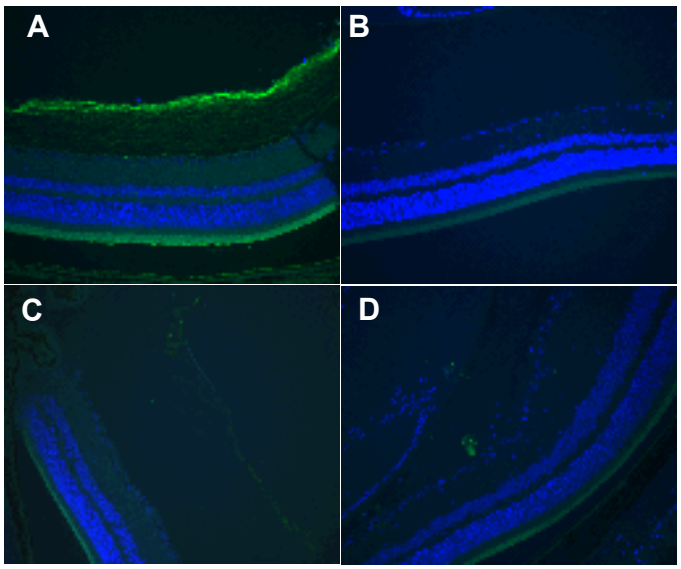

Figure 2: *Immunohistochemical analysis of (A,B) Avastin (green) and (C,D) Av-AuNP 1h(A,C) and 7d(B,D) post injection. Slides were counter stained with DAPI (blue)*

preserves tissue and eye architecture and is commonly used for histological eye studies. The eyes were processed for Immunohistochemistry (IHC). Immunohistochemistry is a well established technique widely used in the diagnosis of abnormal cells such as those found in cancerous tumors. It takes its name from the roots "immuno," referring to antibodies used in the procedure, and "histo," meaning tissue. IHC is also widely used in basic research to understand the distribution and localization of biomarkers and differentially expressed proteins in different parts of a biological tissue. An antibody which specifically binds to a biomarker is used to probe the locations of biomarkers in the tissue. Visualizing an antibody-antigen interaction can be accomplished in a number of ways. In the most common instance, an antibody is conjugated to a fluorophore and the fluorescence produced by it can be observed under a fluorescence microscope (see Figure 2). In our studies, IHC refers to localization of Avastin in thin tissue sections from the eye by exploiting the principle of antibody binding specifically to Avastin.

For IHC, the eye tissues were processed and 5 micron sections were cut. The slides were placed in a 60^{o}C oven for 30 min. Slides were deparaffinized by placing them in Xylene for 10 min with two changes of Xylene, two changes of absolute alcohol and two changes of 95% alcohol and then rinsed in distilled water. In histological examination of eyes, no retinal, ciliary body, iris or corneal abnormalities were noted at either one hour or one week after Super Avastin injection into the vitreous. However, dark reddish Super Avastin clumps were visible in the vitreous 1h post injection. In order to perform immunohistochemistry, the sectioned, deparaffinaized slides were kept in wash buffer (Dako, Carpinteria, CA cat# S3006) for 5 min followed by 20 min incubation with FITC conjugated affinity purified goat anti human IgG, F(ab') (Jackson Immuno Research Labs, Inc, PA, Cat# 109-095-097) in dark at 25 ^{o}C. Since Avastin is a humanized monoclonal antibody, antibody specific for human IgG allowed the presence of Avastin to be determined in rat eyes. When free Avastin was injected intraocularly, fluorescent staining for human IgG demonstrated Avastin to be diffusely present in the rat vitreous at one hour. However, at one week no Avastin remained, consistent with the short half life observed with this antibody (Please see Figure 2a, b). Similar anti human IgG staining in eyes injected with Super Avastin demonstrated focal areas of fluorescent staining both at one hour and at one week, indicating continued presence of sustained released intraocular Avastin (Please see Figure 2c, d). The results from these experiments unequivocally confirmed the long term slow release of Avastin from Super Avastin in rat eyes.

Further experiments are underway to establish the efficacy of Super Avastin for ocular applications. At laboratory scales, we routinely synthesize 5 mL Super Avastin in a batch. In order to perform detailed experiments, we are developing kit formulations to produce large amounts of Super Avastin. We are currently standardizing synthesis protocols for a 50 mL batch. We would further perform *in vivo* experiments in rats to assess the longer half life and capabilities for sustained release of Avastin for ultimate applications of 'Super Avastin' in human patients.

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