



International PC Consortium

# Pachyonychia Congenita Project

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*We appreciate all who have expressed interest in knowing of our efforts and progress as well as many who have made our success possible. We hope that this Newsletter is useful in keeping you up-to-date on our activities. We welcome your active participation (see IPCC Working Groups attached). If you'd like more information on any topic, please send an email, and one of the IPCC leaders will give you more information. Mary Schwartz, Editor.*

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## **IPCC ANNUAL MEETING IN REVIEW May 9, 2007—Los Angeles**

More than 50 attended the IPCC annual meeting in Los Angeles held as an associate meeting of the SID. We were pleased at the many positive comments and the vitality of the discussion. Abstracts of relevant SID posters are attached and also available at [www.pachyonychia.org](http://www.pachyonychia.org).

**Robyn Hickerson** presented a siRNA stability study, including unmodified K6a.513a.12 siRNA, in preparation for the proposed Phase I clinical trial and topical cream formulation currently under development at TransDerm. The results showed that unmodified siRNAs are stable under a variety of storage conditions including multiple freeze/thaw cycles, extreme heat and prolonged storage (1 month) at room temperature. The siRNAs studied were also shown to be more stable than expected under physiological conditions, including incubation in human serum and exposure to RNases found on human hair and skin. (SID Poster #572.)

**Roger Kaspar** reported on recent work at TransDerm showing that the macrolide rapamycin causes selective down-regulation of keratin 6a expression and likely other inducible keratins. An analysis of the 5' untranslated region of the inducible keratins revealed the presence of multiple and extensive polypyrimidine tracks, which have been previously shown to be involved in gene regulation at the level of mRNA translation. Furthermore, it has been shown that rapamycin selectively down-regulates translation of mRNAs that contain these regulatory motifs. By western blot analysis of protein extracts prepared from rapamycin-treated HaCaT cells, the team at TransDerm, including

Robyn Hickerson and Manny Flores, showed that K6a expression was selectively repressed, with no decrease seen in K5, K14 or the non-keratin lamin A/C levels. Furthermore, the transcription start sites for K6a, K6b, K16, and K14 were determined by 5' RACE. An off-label rapamycin study in PC patients was undertaken by Dr. Sancy Leachman. The preliminary results suggest that rapamycin reverses some PC symptoms but the study was terminated due to gastrointestinal side effects. A rapamycin topical formulation for PC treatment is being actively pursued. (SID Poster #568.)

**Marianna Foldvari** described one of the biggest challenges in gene therapy which is the transfer of DNA, encoding a specific gene, into cells and the subsequent expression of a therapeutic protein in sufficient quantities in vivo. Dr. M. Foldvari and her team are investigating the use of gemini nanoparticles as novel delivery vehicles for topical application of many different genes. This nanoparticle technology is based on positively charged Gemini surface-active molecules. These cationic molecules clamp around the negatively charged plasmid DNA, causing the DNA to compact into very small 100-200 nm diameter particles in a characteristic structural pattern. The gemini nanoparticles were already used successfully in the Tsk1/+ scleroderma mouse model, where the therapeutic effect of the gene delivered after topical treatment was demonstrated.

**Fernando Larcher** reported on work he and his colleagues, including Marcela Del Rio (also present at the IPCC meeting), have performed on developing animal models of human skin disease by making skin equivalents from patient

biopsies and grafting these onto immunocompromised mice. Of great interest to IPCC/PC Project, mice containing skin equivalents from PC patients have been prepared. The grafted skin has some PC characteristics. Further experiments are underway to develop this as an animal model to test the effectiveness of (and the ability to deliver) mutant K6a-specific siRNAs in collaboration with TransDerm.

**Sancy Leachman** outlined details of the pre-IND meeting held May 1 with the FDA regarding TD-101 (a mutant-specific siRNA). A discussion regarding protocol for the clinical trial elicited many excellent comments. These notes have been typed and reviewed so that these thoughts can be utilized as we move forward. Information was also presented on development of the allele-specific RT-PCR measurement. (SID Poster #307.)

**Irwin McLean** reported progress in ongoing drug screening. Initial results show that several drugs may affect genes relevant to PC including one of the more common drugs used in controlling high cholesterol levels. Another finding of interest relates to recently published studies on the variability in the human genome with numerous gene duplications and deletions. A study is being done by the McLean lab to identify persons with a missing K6a, K6b, K16 or K17 gene. The study will start by evaluating DNA from no less than 10,000 control subjects. The results will be of importance in pursuing other PC treatments. (SID Poster #564.)

**Edel O'Toole** spoke about the effect of PC1 mutations on desmosomal protein expression in keratinocytes. K16 mutations also cause in vitro loss of cell-cell

adhesion which may be one of the reasons why blistering occurs in PC.

**Frances Smith** presented results on mutations identified in new PC patients together with a summary of all PC mutations. An update on the K6a 3'UTR siRNA studies was given. All four K6a 3'UTR siRNAs significantly reduce endogenous K6a in the two keratinocyte cell lines tested with little/no change to other keratins. From these experiments one K6a 3'UTR siRNA was chosen for testing in a mouse footpad *in vivo* assay. These experiments were carried out in collaboration with Roger Kaspar & Robyn Hickerson (TransDerm) and Christopher Contag & Robert Reeves (Stanford University.) The K6a 3'UTR siRNA was shown to be effective in reducing exogenous K6a using this system. Preliminary results were shown for the new K16 and K17 3'UTR siRNAs that have been tested in cell culture assays. (SID Poster #563.)

I apologize I do not have proper summaries for the presentation by **Birgit Lane**: *PC Cell Lines* and **Liangdan Sun** (student of Xue-Jun Zhang): *A PC Case Study*.

**IPCC 2008**. At the outset of the 2007 IPCC meeting **Xue-Jun Zhang** announced the next IPCC meeting which will be held in Hefei, China on May 17-19, 2008. A beautiful Invitation Program was presented to each IPCC participant. For the 2008 meeting, PC Project will provide a travel stipend of \$1,800 to 16 scientists and physicians to assist with travel costs for the 2008 PC Symposium in Hefei. To qualify for this stipend, we ask that you

- Be an active member of the IPCC and a member of one of the working groups
- Apply by 1 January 2008 for the stipend by sending a 100 word statement to PC Project about your interest in the meeting
- Commit to attend the meeting if the stipend is granted

**TOPICAL DELIVERY SYSTEMS**. The IPCC meeting was shortened to a half-day session this year, so that we could

adjourn to attend the special delivery session co-hosted by IPCC member Leonard Milstone which featured speakers Paul Wender (Stanford University), Samir Mitragotri (UCSB), Richard Heller (University of South Florida) and Mark Prausnitz (Georgia Institute of Technology). PC Project hosted the delivery session speakers at the IPCC dinner where active discussions continued.

**IPCC WORKING GROUPS**. At the IPCC meeting, 'working groups' (not committees) were organized. The attached table shows initial participants. Please notify PC Project of the group(s) you'd like to join. Initial emails and assignments pertinent to each group have been sent.

The **IPCC FOCUS MEETING, *Planning the PC Future***, will be held June 15, 2007 in Salt Lake City, Utah immediately following the 2007 PC Patient Support Meeting. A report will be sent to IPCC members.

#### **POSSIBLE USE OF BOTULINUM TOXIN FOR TREATMENT OF PC**

PC Project is delighted that a group of physicians and scientists in Sweden, led by IPCC member Carl Swartling, are actively pursuing a controlled clinical trial using Dysport® for PC treatment. The significant factors that we hope will be emulated by other PC members include:

- Identifying an existing treatment and exploring possibilities for PC
- Contacting relevant funding sources including drug companies
- Coordinating and collaborating with PC Project for patient identification and recruitment
- Collaborating with other IPCC members in genetic testing, development of measurement tools and other aspects of the study

Whatever the outcome of the study, we highly commend Dr. Swartling (and other IPCC members who are making similar efforts) for his pro-active work for PC treatment. We invite other physicians to contact PC Project for similar collaborations. This is an good example

of PC Project & IPCC collaborations.

**SUMMARY**: On March 16, 2007, Carl Swartling hosted an all day program at Uppsala University Hospital to discuss a proposed controlled clinical trial using Dysport® for PC. Attendees (in addition to Dr. Swartling) included Professor Anders Vahlquist, Jan Weis, and Mattias Karlqvist of Uppsala University, Mary Schwartz and Sancy Leachman from PC Project, and four PC patients. Also attending the meeting was Andreas Wallengren, a representative from Ipsen (maker of Dysport®, a botulinum toxin A product).

#### **REPORT ON UPPSALA, SWEDEN MEETING BY SANCY LEACHMAN, MD, PHD AND CARL SWARTLING, MD, PHD**

The day began with re-treatment of three patients of the plantar keratoderma with botulinum toxin. A fourth patient who had been treated successfully with this technique also came to the meeting but did not receive injections. The botulinum toxin treatments are modeled after the treatments that Dr. Swartling provides for hyperhidrosis. He generally injects about 2 units of toxin every 15 mm on the plantar surface of the feet, and for PC patients may focus more around prominent callosities. A template is used to assure that the distance between injections remains uniform. He has found that he needs to perform deeper injections for PC patients than are required for hyperhidrosis to obtain an effect. The Uppsala University Hospital has now performed over 1000 botulinum toxin procedures for hyperhidrosis with very few complications.

PC patients have significantly more pain associated with the procedure than the hyperhidrosis patients and therefore require either a general or regional anesthesia. Both forms of anesthesia were utilized on the day of the meeting. The regional anesthesia utilized was an IV method (Bier's block) in which blood pressure cuffs are placed around the leg just above the ankles at 100 mm Hg above the systolic pressure and 0.8 ml prilocaine 0.5%/Kg) is injected intravenously through a vein in the foot.

When the procedure is completed, the cuffs are released one at a time to avoid a systemic effect of the prilocaine. This procedure resulted in good pain control for the PC patient we observed and had the benefit of more rapid resolution of the anesthesia relative to both general or regional block methods. We discussed the procedure and the perceived risks and benefits of the procedure with each patient. Each of these patients felt that the benefit they received from having the injections outweighed the risk and inconvenience of having the procedure. The patients also noted it is usually within 2-6 weeks after the first treatment before they began to feel an improvement in the pain, and that the pain relief lasts about 2-3 months. Patients are very eager to receive additional treatment following recurrence of the pain. Patients and Dr. Swartling agree that the keratoderma improves after a couple of injection cycles, but a comparison of before and after images show that even before injection, the keratoderma was mild relative to many of the PC patients in the IPCRR.

The informal lectures in the afternoon began with an introduction to the GENESKIN project by Professor Vahlquist. This lecture allowed everyone to appreciate the interest and resources devoted to the investigation and treatment of genodermatoses such as PC in Uppsala. Mary Schwartz presented an overview of PC Project and its mission. Carl Swartling and Mattias Karlqvist presented cases of PC that have been treated empirically with botulinum toxin and one case in which only one foot was initially treated. In this single foot trial, data suggested that the treated foot could withstand greater pressure than the untreated foot and that less blistering was present in the treated foot. Jan Weis presented a MRI-based micro-imaging technique that was used on the pilot trial patient and the method suggested less blistering on the Dysport-treated foot. Sancy Leachman presented early data on an RT-PCR assay that might be useful to quantitate keratins in skin from PC patients before and after treatment with potential therapeutics.

After the informal lectures, there was a

discussion about how to design and finance a clinical trial with Dysport® botulinum toxin in PC patients. It was agreed that a split-body, placebo controlled trial would be necessary. Newly identified, untreated participants would be ideal and PC Project will be involved in making the IPCRR participants in close proximity to Sweden aware of this potential research opportunity once IRB approval has been obtained. It was also agreed that quantitative endpoints should be used to confirm or refute the utility of this treatment method. Specific endpoints discussed included: Use of the pressure gauge, use of RT-PCR quantification of keratin, use of an improved diary with pain scale, and use of a validated life quality scale - the DLQI. We also discussed the possibility of using a modified micro-MRI protocol that would be less complex and easier to apply to a larger number of lesions on more patients. The conclusion of the meeting was that we would collaborate to perform a well-designed randomized, placebo-controlled trial on interested and appropriate PC patients as soon as funding and IRB approvals are in place.

## PUBLICATIONS

**Kim S, Wong P, Coulombe PA.**

A keratin cytoskeletal protein regulates protein synthesis and epithelial cell growth. *Nature* 2006 May 18; 441: 362-65.

**Sharma VM, Stein SL.** A Novel Mutation in K6b in Pachyonychia Congenita Type 2. *J Invest Dermatol.* 2007 Apr 12; [Epub ahead of print]

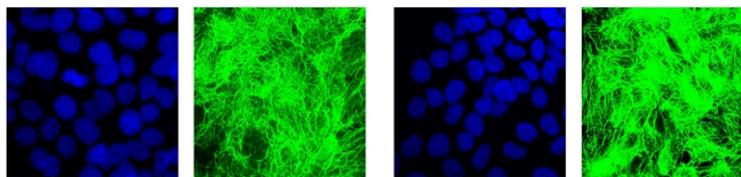
**Tong X, Coulombe PA.** Keratin 17 modulates hair follicle cycling in a TNF {alpha}-dependent fashion. *Genes Dev.* 2006 May 15; 20:1353-64.

**Woll S, Windoffer R, Leube RE.** p38 MAPK-dependent shaping of the keratin cytoskeleton in cultured cells. *J Cell Biol.* 2007 May 29; [Epub ahead of print]

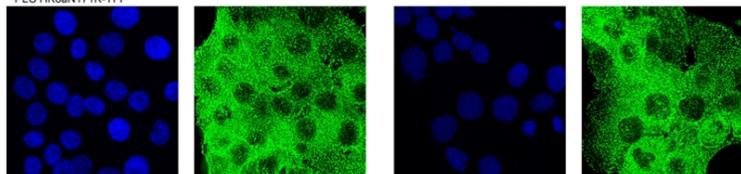
**Wang Q, Ilves H, Chu P, Contag CH, Leake D, Johnston BH, Kaspar RL.** Delivery and Inhibition of Reporter Genes by Small Interfering RNAs in a Mouse Skin Model. *J Invest Dermatol.* 2007 May 24; [Epub ahead of print]

**IPCC SHARED REAGENTS.** Dr. Rudolf Leube writes “we now have available cell lines with very even expression of wild type and mutant keratin 6a. A panel of representative regions of the cell lines is shown below. These cell lines may be useful to test RNAi or any other treatment that potentially interferes with mutant keratin granule formation.” A list of all shared reagents available to IPCC members can be found at [www.pachyonychia.org](http://www.pachyonychia.org).

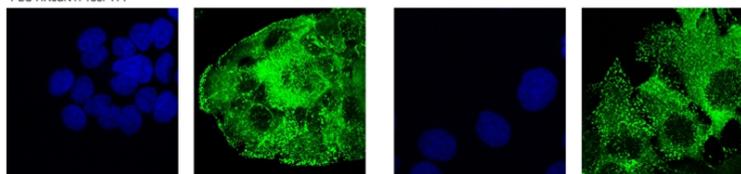
PLC HK6a-YFP



PLC HK6aN171K-YFP



PLC HK6aN171del-YFP



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