



International PC Consortium

Pachyonychia Congenita Project

May 2006 Vol 3, No 2

“ENVISION SUCCESS: PC CLINICAL TRIALS” was the theme of the recent IPCC symposium. The IPCC is a ‘think tank’ in which decisions are reached through the synergy of information exchange. Following the meeting, each idea was evaluated, further investigation undertaken and an action plan formulated. Thanks to all participants!

IPCC Meeting - May 2-3 Philadelphia, PA

There is now a special website for IPCC members only at:

<http://www.pc-project.org/IPCC/>

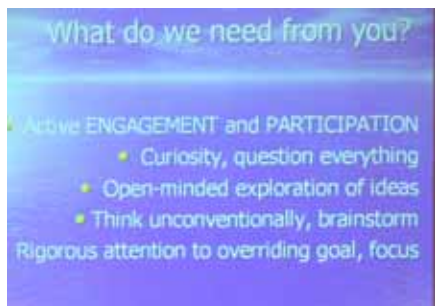
User name: IPCC

Password: member

All 2006 IPCC meeting presentations are available at this site.

Following is a brief review of the meeting presentations and a summary report of the **Action Plan** which has resulted.

Sancy Leachman outlined the success of MPS research over the last 10 years as a model for successfully developing and delivering therapy to PC patients. She challenged IPCC attendees to a productive and effective meeting focused on that goal.



Section 1: PC Research Progress

- **Frances Smith** - overview of the genotype/phenotype relationships in pachyonychia congenita which is more complicated than in some other keratin disorders. Frances has developed an siRNA inhibitor for the 3'UTR of K6a and is collaborating with Roger Kaspar and

Robyn Hickerson (TransDerm) to move this research forward to clinical trials.

- **Len Milstone** - has been successful in proof of principle experiments in using TFOs for gene correction and improvements using psoralen. The problem Len pointed out is that this approach may be 10-20 years from clinical application.
- **Irwin McLean** - out of an initial screening of only 200 small molecules, they had seven hits. Although this is a high risk project, this result appears promising. Irwin outlined new staff and facilities at the University of Dundee available to continue the drug discovery efforts.
- **Dennis Roop** - discussed the importance and use of ‘humanized’ mice. The schedule is set to inject cells in mice later this month (see update next page).
- **Robyn Hickerson** - reported success in development of siRNA specific for K6aN171K; screening in mice demonstrated specificity for the K6a mutant only with minimal effect on K6a wildtype.
- **Sancy Leachman** - developing biologic endpoints which will be used in PC clinical trials; showed results of *in vivo* confocal microscopy, tape stripping and skin shavings from 20-30 PC patients.
- **Mark Eliason** - in preparation for clinical trials, both a pain scale and a severity scale specific to PC are being developed. Initial mock-up versions are being circulated and

revised based on input from IPCC members and other specialists.

Section 2: Delivery

- **Devin Leake** (Dharmacon) - described potential mechanisms to enhance the delivery, increase the stability and minimize off-target effects of siRNA.
- **Bozena Michniak-Kohn** - presented various state-of-the-art delivery mechanisms including micro-needles, Micro Electro Mechanical Systems (MEMS), and the Bioject needless injection system.
- **Jonathan Rothbard** - discussed the possibility of using already available vectors and existing cellular communication structure (i.e. gap junction) for delivery. He advised to “follow, don’t fool, mother nature.”

Section 3: Overcoming Obstacles

- **Ervin Epstein** - summary of “Obstacles” conference (SFO Mar 2006); reviewed pros and cons of using siRNA, zinc finger nucleases and small molecule inhibitors to treat rare disorders.
- **Stephen Groft** (NIH/ORD), **Carl Baker** (NIH), **Peter L. Vaccari** (FDA/OOPD). Each representative outlined resources (funds and expertise) available through their agency which pertain to the goals of the IPCC and PC Project.
- **Roger Kaspar** - reviewed status in moving siRNA inhibitors towards FDA approval and clinical trials; posed questions for group discussion on decisions that have to be made immediately.

Section 4: Related research

- **Brian Nickoloff** - showed that TNF inhibitors (e.g. Humira) decreased K16/K17 in psoriasis. This effect may be a direct action on keratinocytes and may represent a way to reduce K16 expression in PC. Brian noted “for every complex problem, there is a simple solution. And, it is always wrong.” (1949, H.L. Menken).
- **Reinhard Windoffer** - used real time *in vitro* imaging to show that intermediate filaments are dynamic and continually cycle from granular to filament to granular form by a regulated process.
- **Irene Leigh** - classified many keratodermas and compared and contrasted PC with a number of those disorders.
- **Elizabeth Rugg** - presented protein folding and stability as a strategy for treating keratin disorders
- **Xue-jun Zhang** - presented a case study of a novel PC keratin mutation.

Awards & Recognition

- **Extraordinary Leadership Award.** The 2006 IPCC meeting was dedicated to Sancy Leachman, founding member and chair of the IPCC since 2004.
- **PC Career Development Award** from PC Project (\$150,000/year 2005-2007) renewed for Frances J.D. Smith.
- **Outstanding Achievement Award** to Robyn Hickerson for development of mutant specific siRNA.
- **Award of Excellence** presented to Devin Leake for Dharmacon, Inc. for providing siRNA material for PC research.
- **Special Appreciation** to GeneDx, Inc. for providing CLIA-quality testing for PC.
- **Outstanding Publication** to W.H. Irwin McLean and Frances

J.D. Smith (with a number of collaborators including many IPCC members) for a paper in *Nature Genetics* 2006 38(4):441-6 which reported a strong correlation between individuals with filaggrin mutations and atopic dermatitis. In addition to the IPCC recognition, Irwin’s abstract received one of the highest recognitions at the SID meeting and his presentation was made to the full SID audience. Irwin and Frances (again with many collaborators) also recently made a major breakthrough in identifying *filaggrin* as a genetic cause of ichthyosis vulgaris. *Nature Genetics* 2006 38(3):337-42.

IPCC at SID

IPCC members included on PC-related posters and presentations at SID include Mark Eliason, Robyn Hickerson, Roger Kaspar, Sancy Leachman, Irwin McLean, Len Milstone and Frances Smith.

IPCC Action Plan

Following the 2006 IPCC symposium, all input from the meeting was considered and additional investigation was undertaken to gather further information and involve additional collaborators. The questions posed and the plan of action for IPCC/PC Project follows:

1. CONTINUE EFFORTS TO BRING siRNA TO CLINICAL TRIAL. At this time siRNA has the most promise for early delivery to patients. The goal is to submit an IND by October 2006 and request the meeting with the FDA at that time. (We are advised the FDA has 60 days in which to hold the meeting after the IND is submitted.)

a) Do we use K6a mutation specific or K6a 3’UTR targeting siRNA? We will do tox testing on rodents using the most effective siRNA inhibitor for both (1) K6aN171K mutation specific and

(2) K6a 3’UTR. An order has been placed with Dharmacon, Inc. for 3 grams of each siRNA sequence and is scheduled for delivery in August for the toxicity studies.

b) Use modified or unmodified siRNA? We will use unmodified.

c) How/where to conduct toxicity testing? A second more extensive tox study with GMP material may need to be performed (probably in minipigs) following FDA recommendations. We are seeking collaboration with a quality lab outside the US for initial tox testing.

d) How to best test off target & long term effects of siRNA? It was agreed that the most likely off-target effects would be seen in other keratins and possibly skin intermediate filaments. The approach to determine off-target effects has not been finalized, but we will not use a global profiling method.

e) How to prepare for clinical trials? We have retained a consultant to assist in the IND preparation and in initial clinical trial protocol. The orphan product designation application has been submitted for the K6aN171K mutation specific inhibitor. We continue to refine the severity scale by analyzing the inter-rater variability and modifying the photographic content. We continue to refine the molecular endpoints for PC trials and will use shavings from the PC patients’ feet to follow keratin expression (rather than tape stripping or confocal microscopy.)

f) How to overcome delivery obstacles? We will initially use intradermal injections and later work towards more patient friendly delivery methods. We are currently testing saline solution injections in PC patients to establish volume and delivery technique.

2. CONTINUE DEVELOPMENT OF THE PC MOUSE. Mouse embryonic stem (ES) cells, in which one wild-

type K6a gene has been replaced with a humanized mutant K6a gene, have been injected into blastocysts, which were subsequently transferred into recipient females. The humanized mutant K6a gene contains a region of human K6a encoding exon 1 and the mutation N171K, but not the 3' UTR. After germline transmission is confirmed, we will mate these mice to mice containing the inducible Cre recombinase to create bigenic mice that will allow us to induce expression of the mutant K6a protein in a restricted area of the skin. Dennis Roop and Jiang Chen continue this work. There may also be a need for a fully humanized mouse for testing.

3. EXPLORE USE OF 'GRAFT CHAMBER' TECHNOLOGY to create xenografts of primary or immortalized keratinocytes containing PC keratin mutations in nude mice. Dennis Roop and Roger Kaspar collaboration.

4. FURTHER siRNA EFFICACY TESTING IN MICE. This will be coordinated by Roger Kapar with Dennis Roop, with Stanford lab and with others and will involve immunostaining using tomato and plum reporter expression cDNAs as well as the eGFP.

5. DEVELOP IMMORTALIZED CELL LINES THAT EXPRESS K6A AND K16 MUTATIONS. Biopsies are scheduled for a K6a patient with Sancy Leachman and Len Milstone collaboration. Also, Irwin McLean and Irene Leigh are working on the K16 line. The 293 cells may be utilized as needed for testing siRNA effect on keratins.

6. CONTINUE SMALL MOLECULE DRUG SCREENING. Although often stated that this is territory only for 'big pharma,' we feel this approach has merit especially with the increased expertise now available at University of Dundee. Irwin McLean will continue this project as long as

results are promising.

7. CONTINUE GRANT APPLICATIONS. The Center of Research Translation (CORT) grant application for PC was scored and ranked in the top 1/3. We do not expect this will result in funding. However, we are moving forward with on-going collaborations and additional grants are being considered including (1) "Clinical Trials Planning Grant" and (2) "R21 Exploratory Grant" from NIH/NIAMS and (3) "Orphan Product Development Grant" from OOPD/FDA. (4) a grant soon to be released for clinical trial outcomes instrument development.

8. DEVELOP PC 'SYNTHETIC HUMAN SKIN.' The biopsies scheduled for a K6a patient will also be used for this research with collaboration between Sancy Leachman and Roger Kaspar-US and Fernando Larcher and Marcela Del Rio-Spain.)

PC Clinical Trials

Carl Swartling recently published a case series of three PC patients that he has treated for over two years with botulinum toxin type A in the *British Journal of Dermatology* 2006 154(4):763-5.

The patients were treated with intradermal injections of botulinum toxin in their feet in an effort to treat the plantar pain and hyperhidrosis. All three patients reported significantly decreased plantar pain and decreased hyperhidrosis. The duration of relief varied from 6 weeks to 6 months. The patients tolerated repeated injections over a two-year period with no reported side effects or tachyphylaxis. Dr. Swartling has contacted PC Project for cooperation in extending the botox clinical trial to additional patients.

Sancy Leachman and Brian Nickoloff are collaborating to perform staining on tissue blocks from PC

patients with several mutation types. To investigate the clinical effects of Humira on PC, Sancy Leachman has begun a two-month off label trial with Humira on a PC patient with a K16 mutation. This trial is sponsored by PC Project. Based on the results of these efforts, further actions will be scheduled.

Sancy Leachman recently completed a four week trial of 0.1% Tazorac applied daily to the plantar surface of a PC patient. There was no change in the keratin expression after treatment, suggesting lack of effective penetration.

IPCC Member News

Pierre Coulombe has two recent publications on keratin function:

- Tong X, Coulombe PA Keratin 17 modulates hair follicle cycling in a TNF{alpha}-dependent fashion. *Genes Development* 2006 20:1353-64.
- Kim S, Wong P, Coulombe PA A keratin cytoskeletal protein regulates protein synthesis and epithelial cell growth. *Nature* 2006 May 18; 441: 362-65.

Gerald Krueger has been named as an honored recipient of the Edna Benning Presidential Endowed Chair in the University of Utah School of Medicine. This honor "recognizes extraordinary effort and achievement on the part of the chair holder".

THANKS for 2006 IPCC symposium funding support from PC Project, NIH/NIAMS and NIH/ORD.

IPCRR Statistics

- 322 PC patients identified
- 310 registered with PC Project
- 109 completed the Questionnaire

The Registry now includes genetic test results for 47 families (108 individuals).

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