



International PC Consortium

Pachyonychia Congenita Project

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siRNA Clinical Trials Update Sancy Leachman, MD, PhD

As discussed at the recent IPCC meeting in Los Angeles, we are moving forward to perform a Phase Ib study in PC patients that carry an N171K K6a mutation. This drug, called TD101 (TransDerm, Inc.), is one of the in-vitro-tested siRNAs that demonstrates high specificity for the mutant K6a N171K allele (as shown previously by Roger Kaspar and Robyn Hickerson). During a pre-IND meeting on May 1, 2007, our strategy for advancing this drug into a Phase I clinical trial was reviewed with the FDA. Based upon these discussions we have moved forward to produce GMP quality drug from Agilent Technologies of Boulder, Colorado. Simultaneously, Sancy Leachman applied for IRB approval for the Phase I trial. On August 2, the University of Utah approved treatment of the first patient with TD101, contingent upon IND approval. Preparation of case reporting forms will start in September in consultation with Judy Meyer. Our goal is to submit the IND no later than mid-November.

In this application, we will request permission to treat the first patient prior to initiation of non-rodent model toxicity testing. We will also request approval of the IND, based on quality of the bulk drug substance, contingent upon the demonstration that the final "drug product" TD101 is of comparable quality.

If this is approved, the 14-week trial should be able to begin in early January.

The clinical trial will be a dose-escalation study in which dose is increased by both *volume* and concentration. The drug and vehicle will be given by intra-lesional injection into comparable, symmetric plantar calluses twice weekly on Mon. and Thurs. Dose escalations, first by volume and then by concentration, will occur every other dose (weekly). The starting dose will be 0.1

cc of a 1.0 mg/ml solution of TD101., and initial escalation will be accomplished by increasing the volume of the 1.0 mg/ml solution to 0.25, 0.5, 1.0, 1.5, and 2.0 cc per week over the following four weeks. If erythema, bruising, or other mild adverse reaction (considered a Grade 1 reaction) occurs, the site of local injection will be rotated, and the escalation will be continued. If erosion, blistering, ulceration or other more serious adverse event (considered a Grade 2 or 3 reaction) is experienced, the drug will be discontinued and one volume below the dose at which the adverse event was experienced will be considered the maximally tolerated volume. If the reaction was a grade 3 reaction, this will establish the maximally tolerated dose. In the absence of a grade 3 reaction and after resolution of any side effects that may have occurred, a concentration escalation will be continued at the rate of 0.5 mg/ml each week, resulting in concentrations of 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0 mg/ml concentrations over the next 8 weeks. Using the same clinical criteria described for the volume escalation, the maximally tolerated concentration will be established. If a Grade 2 adverse event is experienced, after resolution of the side effect, the 14 week course will be completed using the maximally tolerated dose (one level by both volume and concentration below that at which the side effect was experienced). If a grade 3 reaction is experienced at any time the trial will be stopped at that point. Thus, if the patient tolerates the complete escalation, it will be completed in 14 weeks and the maximal tolerated dose will be 2.0cc of a 5.0 mg/ml solution or total dose of 10 mg, but lower doses and concentrations may reach the dose-volume MTD and the duration of the trial may be extended by the length of time required for resolution of grade 2 reactions.

During the dose escalation, measures of potential efficacy will also be made. In addition to a clinical examination at the

time of each injection, photographs will be taken to document any clinical effects. A pain diary and quality of life questionnaire will also be completed before, during, and after the trial has been completed to permit a subjective evaluation. Callus shavings will also be obtained and examined for keratin expression using quantitative RT-PCR. Using this combined approach of subjective (patient and physician) and molecular measures, we hope to correlate any signs of efficacy with the dose escalation study. We will base pursuit of further investigation of TD101 on the results of this first trial.

In addition to the N171K mutation carriers in the U.S. there is also a family in Ireland with this mutation. We have recently partnered with Alan Irvine (Dermatologist) and Brendan Buckley (Clinical Pharmacologist), in Ireland, who will be working to approve and perform the Phase I dose-escalation trial in their PC patients as well. We are excited about this new collaboration because it offers us the opportunity to evaluate the toxicity and effect in a larger group of patients. In parallel with these clinical trial efforts, Roger Kasper's group continues to work on the delivery issue. Depending on the toxicity and side-effects observed, as well as the evaluations of efficacy, future plans for TD101 development may shift to a topical or micro-needle delivery method prior to completion of non-rodent pre-clinical toxicity studies. We look forward too keeping you posted on the progress.

Happenings at TransDerm

Research at TransDerm, led by Dr. Tycho Speaker, has resulted a new way of preparing strong hollow dissolvable arrays of microneedles as a device to facilitate skin delivery. This technology, termed STMNA (pronounced stamina for Soluble Tip MicroNeedle Array), has been shown in initial tests to efficiently

deliver (and express) fLuc expression plasmid to mouse ear, back and foot-pads. These experiments were performed in collaboration with colleagues at Stanford University including Drs. Emilio Gonzalez and Christopher Con-tag.

TransDerm was pleased to host Dr. Marta Garcia from CIEMAT in Madrid, Spain, during the month of July. Dr. Garcia has been able, with assistance from her colleagues Marcela del Rio and Fernando Larcher, to prepare a PC animal model in which human skin equivalents are prepared from patient biopsies, which are grafted onto the backs of immunocompromised mice. As reported at the recent IPCC annual meeting, these full-thickness human skin graphs retain many of the disease characteristics of PC skin. The results of this research are being prepared for publication.

Status of PC Research at the University of Dundee **W.H. Irwin McLean**

A number of chemical compounds that affect keratin 6 expression have been identified from small molecule library screening. These are being investigated for their effects on various keratins within cultured cells. One class of compound that has already been developed for clinical use in an indication unrelated to PC has been investigated in more detail and from this, one drug already on the market appears to decrease activity of the K6A promoter. Further work on protein expression in cells is on-going.

One type of assay has been developed for rapidly screening large DNA collections for the copy number variation reported to cause deletion of K6A, B and C genes in 3% of the population. However, it is felt that this assay may not be as fast, reliable or economical for the intended screening project. Thus, a different type of assay based on real-time PCR is in development. Once a reliable assay for high-throughput use has been established, a population of 10,000 or more individuals linked to detailed

medical history will be screened with the aim of finding an individual homozygous for loss of K6A and/or nearby keratin genes. If such an individual lacks a PC or other skin disease phenotype, this will validate a gene-specific siRNA approach to treatment of PC.

New PC Mutations Identified **Frances J.D. Smith**

Genetic testing of new PCers registered with the IPCRR project and those that contact the Dundee lab directly continues.

Until recently all reported PC mutations have been missense or insertion/deletion mutations but we have recently identified several splice site mutations. A manuscript reporting a number of mutations has recently been accepted for publication by the Journal of Dermatological Science. In this paper we have renumbered the recurrent mutation, K6a N171del as K6a N172del to bring our numbering in line with the most recent version of the mutation nomenclature (in a stretch of repetitive sequence it is the most 3' amino acid deleted that is numbered).

Identification of siRNA for K6a, K16 and K17

Frances Smith reports that siRNAs designed against the 3'UTR of K6b, K16 and K17 have been tested in a cell culture system and shown to be effective against the target genes. Cell culture studies are ongoing to look at the effect on other keratins when the level of each of these target genes is individually reduced in primary keratinocyte cultures.

Progress on real time PCR assay for mutant & wildtype K6a **Robyn Hickerson**

Drs. Lana Pho and Sancy Leachman have developed an allele-specific RT-qPCR assay that is capable of distinguishing the single nucleotide difference between wildtype K6a and the K6a N171K mutant at the mRNA level. This assay was developed as a clinical endpoint to establish efficacy of K6a

N171K mutant-specific siRNA (TD101) treatment in human skin by assessing wildtype and N171K mutant allele expression in the shavings from PC patients before and after treatment with TD101. In addition to the work done at the University of Utah, Dr. Robyn Hickerson at TransDerm has nearly completed the assay validation according to the ARUP guidelines for clinical assay development (ARUP is a CLIA-certified national clinical reference laboratory) and verified the specificity of the assay. The assay has been shown to be specific to K6a, as other keratins such as K6b and K5 are not detected. RNA has been isolated from non-PC patient shavings, the shavings of three different PC patients with the N171K mutation and from six different PC patients with a mutation other than K6a N171K. The RT-qPCR results were consistent with the known genotype.

Dr. Emilio Gonzalez has successfully used the validated allele-specific assay to show N171K mutant specific inhibition by TD101 in immortalized primary keratinocytes harboring the N171K mutation from a PC patient (cells were immortalized in the laboratory of Dr. Leonard Milstone and the initial keratinocyte culture was obtained from a biopsy taken by Dr. Sancy Leachman). This assay will be utilized in the Phase I clinical trial to monitor the K6a wildtype and N171K mutant mRNA levels upon treatment with TD101.

Off-label study of rapamycin in PC patients **Roger Kaspar**

As reported in the previous newsletter and at the IPCC annual meeting in Los Angeles, rapamycin selectively inhibits K6a expression in human keratinocytes. These in vitro data supported off-label treatment of a small number (n=3) of pachyonychia congenita patients, performed under the direction of Dr. Sancy Leachman at the University of Utah (Salt Lake City). One PC patient with a K6a(N171K) mutation and two patients with the K16(N125D) mutation were invited to participate in a three to five

month off-label use of oral rapamycin (Rapamune®, Wyeth Pharmaceuticals, Inc.) Rapamycin was given in the standard fashion following typical recommendations for use as an adjunct therapy in renal transplantation. The starting dose was initially low and trough levels were obtained at steady state after 2 weeks. The dosage was increased every two weeks until trough levels reached a therapeutic range of 9-12 ng/mL (known for immunosuppression). Patients were re-evaluated for effects and side effects every two weeks. Appropriate laboratories were performed to ensure safety.

Patients also completed a daily pain diary (rating their level of pain on a scale of 1-10 several times per day) and a bi-weekly well-validated life quality evaluation (DLQI) at the time of their clinic visits. Patient reported pain (morning assessment) and DLQI quality of life scores were evaluated over time and with respect to dose of drug received and trough level over that time period. None of the patients experienced any laboratory abnormalities or serious side effects. However, all patients experienced one of the previously reported gastrointestinal side effects that ultimately resulted in discontinuation of use of the drug. Significantly, in spite of these side effects arising from systemic administration of rapamycin, all three patients reported an increased quality of life that paralleled the rapamycin trough level. As might be expected, the subjective pain scale was more variable than the DLQI scores, but as a general trend, the lower pain scores corresponded to higher trough levels. The simple pain scale used in this study was not as good as the DLQI in adequately capturing the overall subjective experience of pain in these patients. Patients reported that they felt their pain was improved, but the pain scores did not reflect their general impression. These patients reported that this was due in part to the increased level of activity that spontaneously occurs when pain decreases. An improved pain scale is currently under development, which incorporates activity level into the subjective reporting system.

In addition to subjective improvement

of plantar pain and improved quality of life, patients also demonstrated important clinical changes in the plantar calluses. Before-and-after photographs demonstrate decreased keratoderma following treatment, particularly in the K6a PC patient. Of note, this patient has long noted that the level to which she is able to remove callus is determined by the level at which the blade reaches capillaries and associated pain fibers. At the 12 week time point, the depth at which these neurovascular structures were found appeared to be regressed relative to baseline.

Overall, subjective and objective clinical data suggest that oral rapamycin was able to reduce the pain and keratoderma in these PC patients. The lack of long-term tolerance of the oral form of the drug raises the question of whether a topical form, similar to that recently reported for psoriasis, might be a viable alternative formulation of the drug. It is unclear what the exact mechanism or mechanisms of action of rapamycin are in alleviating the pain associated with PC, but the expression data suggests that direct regulation of keratin translation may be involved. We cannot exclude the possibility that the immunosuppressive function of rapamycin may also be playing a role in the response, presumably through indirect immunologic effects on keratin expression. The results of this study are being prepared for submission for publication.

Future investigation of topical formulations of rapamycin may permit treatment of PC patients without the associated side effects observed with the oral form. TransDerm and the Paul Wender laboratory (Stanford University) are currently collaborating to prepare and test rapamycin prodrugs that have skin-penetrating activities. The wealth of experience achieved by Dr. Paul Wender and colleagues at Stanford University (and Cellgate) in successfully converting cyclosporine, an agent that does not penetrate skin, into cyclosporine transporter conjugates that readily penetrate multiple layers of human skin using oligoarginine or lipid transporters with linkers that release free

drug is being incorporated into the rapamycin-conjugate design. The goal of this collaboration is to develop rapamycin analogs that can be applied locally at high concentrations to problematic areas of PC patients (such as the soles of the feet), bypassing the undesirable side effects associated with oral rapamycin administration.

World Dermatology Congress (October) in Buenos Aires

Robyn Hickerson and Roger Kaspar will be giving oral presentations at the upcoming World Dermatology Congress (October) in Buenos Aires and Mary Schwartz will represent PC Project. Please let us know if you will be attending as we are planning a dinner meeting for IPCC participants. We hope to see many of you there.

1st World Congress on Genodermatology (November) in Maastricht

IPCC member Maurice van Steensel is on the organizing committee for this event. We note that several IPCC members will be giving presentations at this meeting. Please let us know if you will be attending as we are planning a dinner meeting for IPCC participants. We hope to see many of you there.

Publications Added to PC Bibliography

Hickerson, RP, Smith, FJD, Reeves, RE, Contag, CH, Leake, D, Leachman, SA, Milstone, LM, McLean, WHI, and RL Kaspar, Single Nucleotide-Specific SiRNA Targeting in a Dominant Negative Skin Model. *J. Invest. Dermatol.*, in press

Smith, FJD, Hickerson, RP, Sayers, JM, Reeves, RE, Contag, CH, Leake, D, Kaspar, RL, and McLean, WHI, Development of Therapeutic SiRNAs for Pachyonychia Congenita. *J. Invest. Dermatol.*, in press

Wang, Q., Ilves, H., Chu, P., Contag, CH, Leake, D, Johnston, BH, and RL Kaspar, Delivery and Inhibition of Reporter Genes by Small Interfering RNAs in a Mouse Skin Model. *J. Invest. Der-*

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