Achieving Successful Delivery of Nucleic Acids to Skin: 6th Annual Meeting of the International Pachyonychia Congenita Consortium

Roger L. Kaspar[1], W.H. Irwin McLean[2] and Mary E. Schwartz[3]

Journal of Investigative Dermatology (2009) **129,** 2085–2087. doi:10.1038/jid.2009.220

The 2009 Annual Meeting of the International Pachyonychia Congenita Consortium (IPCC)* centered on the need to develop patient-friendly technologies to effectively and efficiently deliver nucleic acids to skin. The IPCC is a group of physicians and scientists who have agreed to work together to develop therapeutics for the rare skin disorder pachyonychia congenita (PC) (a list of IPCC members can be found at http://www.pachyonychia.org). Each year's IPCC meeting is devoted to the most pressing issues related to developing PC therapeutics and to identifying future directions toward achieving realistic goals. The consortium fully recognizes and expects that research success in this realm will be of immediate benefit not only to patients with PC but also to those suffering from other skin disorders.

The 2009 meeting addressed the difficulty of delivering nucleic acids to skin. The impetus for this topic was the recent phase Ib pachyonychia congenita clinical trial using a mutation-specific small interfering RNA (siRNA), TD101. The side-by-side, dose-escalation toxicity trial of intralesional injections of this mutation-specific siRNA exhibited no toxicity at the higher doses, and a clear clinical response was observed in the siRNA-treated site, but not in the site injected with vehicle alone (Leachman *et al*., submitted). Unfortunately, pain from the injections was intolerable, and a decision was made that better delivery options were needed before further trials were pursued.

The meeting was divided into four areas: (i) a historical overview highlighting the need for nucleic acid skin delivery, (ii) delivery technologies used in recent clinical trials, (iii) delivery technologies currently under development, and (iv) future directions in the development of nucleic acid skin delivery technologies that will be acceptable and effective.

The need to deliver nucleic acid therapies to skin

Nicholas Dean explained the urgent need for better ways to deliver nucleic acids. He pointed out that steady progress has been made in the development of oligonucleotide-based drugs over the past 20 years and that the pharmacokinetics, toxicology, and pharmacology of many classes of single- and double-stranded oligonucleotides are understood in detail. Furthermore, many of the challenges to development and commercialization of these drugs are being overcome, with more than 30 oligonucleotide drugs now progressing in non-skin clinical trials. However, important technological hurdles must be overcome before the therapeutic potential of oligonucleotides in skin can be realized.

Joseph Carroll (formerly of Sirna Pharmaceuticals) shared his experience in developing topical formulations while directing the skin siRNA program at Sirna. The short time needed to identify and manufacture potent and specific siRNAs is attractive to the pharmaceutical industry, and the cost of production is now decreasing. This project was discontinued after Merck acquired Sirna, but several formulations were optimized at Sirna to allow efficient delivery of labeled siRNA through the stratum corneum, although they were not functionally active. A point of discussion throughout the meeting was that delivery of siRNA through the stratum corneum is necessary but not sufficient for delivery to epidermal cells and that additional steps must be taken to facilitate nucleic acid uptake by keratinocytes (and endosomal release) to allow access to the RNA-induced silencing complex (i.e., no "magic bullet currently exists for both barrier disruption *and* intracellular delivery"). Dr Carroll noted the complexities in formulating siRNA, including the need for compatibility of excipients with siRNA, development of penetration enhancers, avoiding inflammation (which causes epidermal thickening, reducing siRNA penetration),

[1]*TransDerm, Santa Cruz, California, USA;* [2]*Division of Molecular Medicine, University of Dundee, Dundee, UK and* [3]*Pachyonychia Congenita Project, Salt Lake City, Utah, USA*

Correspondence: Prof Irwin McLean, Division of Molecular Medicine, Medical Sciences Institute, Dow Street, Dundee DD1 5EH, UK. E-mail: w.h.i.mclean@dundee.ac.uk

limiting off-target effects, and testing of formulations on preclinical models (with subsequent re-optimization in humans).

Success in clinical trials

Several presenters described successful clinical trials using nucleic acids delivered to the skin. Sancy Leachman reported the first clinical trial of an siRNA (TD101) delivered to skin (it is also the first siRNA to target a mutant mRNA). Although signs of efficacy were clearly apparent, with the treated skin reverting to "normal" (non-tender) skin at the site of siRNA injection, pain associated with intralesional injections required regional nerve blocks and oral pain medication to make the administration bearable. No adverse effects occurred, and the upper dose limit was not reached. The clear conclusion from the study was that a more patient-friendly method of delivery was needed. Richard Heller discussed the successful use of electroporation to facilitate delivery following intradermal injection of a plasmid encoding IL-12 to reduce or eliminate tumors in melanoma patients.

Thomas Schulze reported encouraging clinical results in a psoriasis study using decoy oligonucleotides (delivered by unguentum emulsificans) designed to bind and neutralize transcription factors. Dan Yarosh described his experience with topically applied liposomes to deliver proteins (although they are not nucleic acids, these proteins share many of the same delivery issues—both classes of macromolecule are high in molecular weight and are highly electrostatically charged), including a DNA repair enzyme (bacteriophage T4 endonuclease V). He presented promising clinical trial results in patients with xeroderma pigmentosum, actinic keratoses, and basal cell carcinomas.

Emerging skin delivery technologies

Marianna Foldvari and Roger Kaspar presented work on topical formulations. Although the formulations presented appear to penetrate the stratum corneum and deliver plasmid or siRNA to the epidermis (and dermis), there was some question as to whether functional nucleic acids were taken up by keratinocytes, because limited activity was observed. Kaspar reported results generated by Emilio Gonzalez at Stanford University, who demonstrated that the uptake of plasmid DNA and siRNAs by keratinocytes following intradermal injections is probably the result of pressure ("pressure-fection"). Following intradermal injection of a fixed amount of plasmid DNA, enhanced expression of plasmid DNA correlated with increasing vehicle volume. Paul Campbell described how ultrasound (sonophoresis) can generate targeted convection of microbubbles (microjetting) that enhance the delivery of nucleic acids. Marc Brown gave an overview of several topical formulations and physical transfer technologies with the potential to facilitate epidermal delivery. Samir Mitragotri described the use of needle-free liquid jets to generate high force and pressure to facilitate nucleic acid delivery.

The technique of microneedle arrays was well represented, with three presentations (by Tycho Speaker, Yeu-Cun Kim, and James Birchall) describing how projections of various types can penetrate the stratum corneum to either deliver nucleic acids directly or generate breaches in the stratum corneum into which topical formulations can be applied. Finally, Daniel Gibson explained that iontophoresis would help deliver fluorescently tagged oligonucleotides into the cornea and the skin of pigs.

Formulating a path to success

Dennis Roop gave a provocative presentation on the future of skin therapeutics, including the potential therapeutic use of reprogrammed induced pluripotent stem (iPS) cells from patients ("individualized medicine"). He also described elegant proof-of-principle mouse siRNA experiments in which isolated keratinocyte stem cells were transduced with lentivirus expressing allele-specific siRNA, cultured, and reconstituted into skin equivalents that were subsequently grafted onto mice, which exhibited improved phenotype when compared with controls. He noted that, after listening to reports of the many delivery approaches at the meeting, he thought it likely that combinations of delivery technologies would be needed and that comparisons of delivery data generated within the community are essential.

During the culminating panel discussion (led by Christopher Contag, Peter Hull, and Irwin McLean), which focused on the best path forward for development of nucleic acid skin therapeutics with an emphasis on delivery technologies, a consensus emerged that the field would benefit from standardized animal models, skin-equivalent cell culture models, and organ culture models that use human skin. These validated skin models, along with common pools of reagents, would allow direct testing and evaluation of the various delivery strategies (i.e., allow comparison of "apples to apples"). The possibility of applying for American Reinvestment and Recovery Act stimulus funds—National Institutes of Health (NIH) Grand Opportunities (GOs)—was proposed as a way to fund such projects. Following the meeting, a GO proposal was prepared and submitted to the NIH by US-based IPCC members, with the aims of developing skin models (human and mouse) and sharing common reagents (expression plasmids, siRNA, and triplex oligonucleotides) with researchers interested in testing and comparing delivery systems in well-characterized model systems. A complementary program grant application was made to the Medical Research Council by UK-based IPCC members to develop a range of animal models for validating and optimizing delivery of siRNA and other gene-silencing therapeutics into the skin and cornea.

Overall, there was enthusiasm throughout the meeting regarding the future of siRNA and other nucleic acid therapies, with the realistic recognition that further development will require improved delivery technologies. A number of working groups were established to foster international communication and collaboration and to continue the spirit of collegiality of the 2009 meeting.

PRESENTERS AND PRESENTATIONS

James Birchall, MRPharmS, PhD
Welsh School of Pharmacy, Cardiff University, Cardiff, UK
"Gene Delivery to Excised Human Skin via Microchannels"

Marc Brown, PhD, CChem, MRSC
MedPharm, Ltd., Surrey, UK
"(Trans)dermal Gene Delivery: To Be or Not To Be?"

Paul Campbell, PhD, FInstP, FRAS
Carnegie Physics Laboratory, University of Dundee, Dundee, UK
"Sonoporation and Sonophoresis: Transdermal Delivery Mediated by Ultrasound"

Joseph Carroll, PhD
University of Colorado at Boulder, Boulder, CO, USA
"Topical Delivery of siRNAs: Challenges and Lessons Learned"

Nicholas M Dean, PhD
Excaliard Pharmaceuticals, Carlsbad, CA, USA
"Nucleic Acid Delivery to Treat Skin Disorders—An Urgent Need"

Marianna Foldvari, PhD
University of Waterloo School of Pharmacy, Waterloo, Ontario, Canada
"Topical Non-Invasive Delivery of Nucleic Acids"

Daniel J. Gibson, PhD
University of Florida, Gainesville, FL, USA
"Electromotive Delivery of Oligonucleotides into Rabbit Corneas"

Richard Heller, PhD
Frank Reidy Research Center for Bioelectrics, Old Dominion University, Norfolk, VA, USA
"Plasmid DNA Delivery with Electroporation: Results from Pre-clinical and Clinical Studies"

Roger L. Kaspar, PhD
TransDerm, Santa Cruz, CA, USA
"Developing Topical Formulations for siRNA Skin Delivery"

Yeu-Chun Kim, PhD
School of Chemical and Biomolecular Engineering, Georgia Institute of Technology, Atlanta, GA, USA
"DNA Delivery Using Coated Microneedle Patch"

Sancy A. Leachman, MD, PhD
Department of Dermatology and the Hunstman Cancer Institute, University of Utah, Salt Lake City, UT, USA
"TD101 Clinical Trial: Intradermal Injection of siRNA"

Samir Mitragotri, PhD
Department of Chemical Engineering, University of California, Santa Barbara, Santa Barbara, CA, USA
"Needle-Free Liquid Jet Injectors"

Dennis R. Roop, PhD
Department of Dermatology, University of Colorado at Denver, Aurora, CO, USA
"The Future View"

Thomas Schulze, DrRerNat
Avontec, GmbH, Martinsried, Germany
"AVT-01 Decoy Oligodeoxynucleotides Show a Clear Drug Potential in Psoriasis Clinical Phase IIa Studies Associated with a Very Specific Th-17 Pathway Inhibitor Signature"

Tycho Speaker, PhD
TransDerm, Santa Cruz, CA, USA
"Pros and Cons of Using Microneedle Delivery Systems"

Daniel B. Yarosh, PhD
The Estee Lauder Companies, Melville, NY, USA
"Topical Delivery of Proteins by Liposomes"

PANEL DISCUSSION
Developing an Action Plan for Delivery

Christopher Contag, PhD
Stanford University School of Medicine, Stanford, CA, USA

Peter R. Hull, MD, PhD
Division of Dermatology, Royal University Hospital, Saskatoon, Saskatchewan, Canada

W.H. Irwin McLean, PhD, DSc, FRSE, FMedSci
Epithelial Genetics Group, Division of Molecular Medicine, Colleges of Life Sciences and Medicine, Dentistry, and Nursing, Dundee, Scotland, UK

CONFLICT OF INTEREST
Roger Kaspar and Irwin McLean have filed patents relating to siRNA therapy for pachyonychia congenita.

ACKNOWLEDGMENTS
We thank Frances Smith of the University of Dundee and Robyn Hickerson of Transderm for providing their additional notes on the meeting. Support for this symposium was provided by the National Institute of Arthritis and Musculoskeletal and Skin Diseases and the National Institutes of Health Office of Rare Diseases (1R13AR057174-01), the Pachyonychia Congenita Project, TransDerm, Avontec, Agilent Technologies' Nucleic Acid Solutions Division, and Orfagen.

*The 6th Annual Meeting of the International Pachyonychia Congenita Consortium was held at the Palais de Congres Convention Center in Montreal, Quebec, Canada, 5–6 May 2009.

Additional information about past IPCC symposia and ongoing PC Project activities can be found at http://www.pachyonychia.org.

7th Annual Meeting of the International Pachyonychia Congenita Consortium

May 4–5, 2010,
Hilton Atlanta Hotel,
Atlanta, Georgia