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STP Speaker Abstracts

S-1

Skin Deep: The Thick, the Thin, and the Therapeutic Target
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Skin is our largest organ, with constant interaction with our environment. It is a barrier, a portal, and home to an extensive microbiome. It is also a sensory organ, and an active participant in immune sensing, trafficking and regulation. Skin has an extensive ability to repair itself; although not always perfectly. The adnexa are involved in thermoregulation, protection, and sexual attractiveness. Since skin and hair are what we see, aesthetic concerns with skin are often closely tied to feelings of self-confidence, attractiveness, and well-being. Due to this multiplicity of functions, there are numerous potential local targets within the skin for therapeutic manipulation. Additionally, transdermal administration is an alternative for systemic delivery, and can be attractive for the patient due to ease of access and application. This talk will be an overview of these aspects of dermal anatomy and function.

S-2

Dermal Drug Development and Delivery to the Skin
Jon Lenn, MS, PhD, GlaxoSmithKline, Research Triangle Park, NC

Human skin has evolved to protect the body from physical, mechanical, and chemical insults while preventing endogenous water loss. This is predominantly achieved by a thin (10-30 µm) cornified outermost layer, the stratum corneum, that also severely limits drug delivery into and through the skin. The development of a dermal product involves a good understanding of the properties of the active pharmaceutical ingredient (API) and the vehicle used for local delivery of the molecule into the skin. One of the advantages in the treatment of skin diseases is direct access to the target tissue, which allows for the critical assessment of the pharmacokinetics (PK) and pharmacodynamics (PD) of the drug product during formulation development and optimization. There are several in vitro and in vivo models that can be used in combination to evaluate the performance of the topical drug product.

S-3

Look into the Skin
Jonathan Stauber, PhD, Imabiotec, Loos, France

Analyzing where the drug and metabolites are localized in the skin and what the effects of these molecules are on cells are crucial to investigating drug efficacy and toxicity. A new advanced label-free approach based on the combination of Quantitative Mass Spectrometry Imaging (QMSI) and classical histology techniques is presented. This innovative technique provides an assessment of the quantitative
distribution of molecules directly on tissue sections (fresh punch biopsy, reconstructed 3D skin, etc.), and can support various types of preclinical or clinical dermatology projects. Examples of drug penetration, quantification, and co-localization with biomarkers for the evaluation of drugs and appropriate formulations will be presented.

S-4

An Overview of the Pathogenesis and Lesions of Immune-mediated Skin Injury

Dimitry M. Danilenko, DVM, PhD, DACVP, Genentech, Inc., South San Francisco, CA

One of the skin’s primary functions is to serve as a physical and physiologic protective barrier against injury from the external environment and from loss of water and solutes from the body. When the skin is exposed to irritants, such as xenobiotics, infectious agents, or ultraviolet radiation, that may damage or disrupt this barrier, it mounts an inflammatory and proliferative response in order to prevent further damage and to restore a morphologically and physiologically functioning barrier.

This barrier disruption manifests in the form of both morphologic and physiologic alterations that will vary depending on the degree of barrier damage, and to some extent on the specific irritant, although most pathophysiologic responses are generalized and independent of the specific initiating factor(s). A core premise underlying the skin’s response to injurious stimuli is that the epidermis, and particularly epidermal keratinocytes and dendritic cells, including Langerhans cells, are central to the initiation of the skin’s response to injury.

The hypothesis that the epidermal keratinocyte is a primary initiator of the skin’s response to noxious stimuli has gained widespread acceptance. Epidermal keratinocytes can recognize pathogen-associated molecular patterns (PAMPs) of microbial origin, and danger-associated molecular patterns (DAMPs), such as xenobiotics and other irritants through Toll-like receptors (TLRs), including TLR-1, TLR-2, TLR-4, TLR-5 and TLR-6 on their surface and TLR-3 and TLR-9 in their endosome, that trigger an inflammatory cascade leading to the generation of anti-microbial peptides such as \( \beta \)-defensins, cathelicidins and S100 family proteins, pro-inflammatory chemokines such as IL-8, CXCL9, CXCL10, CXCL11, CCL27 and CCL20, and pro-inflammatory cytokines such as IL-1\( \beta \), TNF, IL-6 and IL-18.

These chemokines and cytokines recruit and activate leukocytes and convert the initial innate immune response to an adaptive immune response. In addition, keratinocytes express nucleotide-binding domain, leucine-rich repeat-containing (NLR) proteins that recognize cytoplasmic PAMPs, DAMPs and UV radiation. When engaged, NLRs trigger a pro-inflammatory signaling pathway through a large multiprotein complex termed an inflammasome formed by an NLR, an adaptor protein termed ASC, and pro-caspase 1. Inflammasome assembly activates caspase-1, which in turn cleaves pro-IL-1\( \beta \) to active IL-1\( \beta \) (Figure 1).

Figure 1. Keratinocytes as sensors of danger: Keratinocytes are central skin sentinels and can recognize foreign and dangerous agents, for example pathogen-associated molecular patterns (PAMPs) of microbial origin and danger-
associated molecular pattern (DAMPs), such as irritants and toxins, through Toll-like receptors (TLRs) and the inflammasome. Reprinted from Nestle F.O., et al. Skin immune sentinels in health and disease Nat Immunol. 2009 9: 679-91 with permission from Macmillan Publishers Limited.

The immune response triggered by keratinocyte activation is believed to be central to the skin’s response to a wide range of stimuli, and leads to the stereotypic morphologic response(s). Even the immune-mediated/autoimmune disease psoriasis, is now believed to be at least partially caused by inappropriate or poorly regulated activation of epidermal keratinocytes, which in turn leads to inflammation and the hallmark morphologic changes associated with this condition. The ability of cytokines such as IL-22 and oncostatin M to induce morphologic and differentiation features in keratinocytes that mimic those found in psoriatic epidermis in 3-dimensional reconstituted human epidermal models skin in the absence of blood vessels and leukocytes reinforces the hypothesis that epidermal keratinocytes are central to the initiation of cutaneous inflammation as well as the initiation of cutaneous response to injury, infection, and toxicity.

Thus, the current model for the cutaneous response to injury, regardless of the specific type or etiology of the injury, is initiated by epidermal keratinocyte recognition of PAMP or DAMPs via engagement of TLRs and/or NLRs, thus triggering pro-inflammatory signaling pathways an inflammatory cascade that leads to the generation of anti-microbial peptides such as β-defensins and cathelicidins, pro-inflammatory chemokines such as IL-8, CXCL1, CXCL9, CXCL10 and CXCL11, and cytokines such as IL-1β, TNF, IL-6 and IL-18. These keratinocyte-derived chemokines and cytokines further recruit and activate DCs and other leukocytes to elaborate additional cytokines and chemokines, such as IFNα from pDCs and IL-12 and IL-23 from dermal DCs which further recruit and activate T lymphocytes of both the Th1 and particularly the Th17/Th22 lineage to release pro-inflammatory cytokines such as IFNγ, IL-17 and IL-22, thus converting the initial innate immune response to an adaptive immune response, and providing cross-talk between the two arms of the immune system (Figure 2). This immune response is believed to
be central to the skin’s response to a wide range of injurious stimuli, regardless of the exact nature of the stimulus.

Figure 2. Skin-resident immune sentinels. Ultraviolet (UV) light, trauma, irritants or infection (essentially any type of barrier disruption) triggers a coordinated immune response to maintain skin homeostasis. Skin-resident immune cells are key sentinels for restoring homeostasis but can also be effector cells during cutaneous injury. Reprinted from Nestle F.O., et al. Skin immune sentinels in health and disease. Nat Immunol. 2009 9:679-91, with permission from Macmillan Publishers Limited.

Cutaneous toxicity that occurs as the result of immune-mediated mechanisms falls into the same categories as systemic immune-mediated diseases. Type I hypersensitivity is acute, and classically manifests cutaneously as urticaria mediated by IgE antibodies bound to the surface of mast cells and basophils. Type II hypersensitivity is cytotoxicity induced by IgG or IgM antibodies with complement activation. The various forms of pemphigus are an example of cutaneous type II hypersensitivity (Figure 3), with autoantibodies directed against keratinocyte antigens, including desmoglein 1 and desmoglein 3.

Figure 3. Skin from a human with pemphigus vulgaris illustrating subepidermal separation of epidermal keratinocytes from the underlying dermis (acantholysis) with the resultant formation of a subepidermal cleft containing rounded-up epidermal keratinocytes (acantholytic cells) and occasional red blood cells. Image courtesy of Richard Carr, MD., Department of Histology, Warwick Hospital, United Kingdom.
Type III hypersensitivity is mediated by IgG or IgM antigen-antibody immune complex deposition. Cutaneous toxicities due to type III hypersensitivity reactions are drug-induced cutaneous vasculitis and cutaneous lupus erythematosus. Approximately 20–30% of cutaneous vasculitides are drug-induced, generally arising 7–10 days following administration of the inducing drug substance. Vasculitis is characterized by inflammation of small cutaneous vessels with or without vascular necrosis and/or thrombosis. Cutaneous lupus can have a variety of histologic presentations, but is often characterized by an interface dermatitis consisting of a lichenoid inflammatory cellular infiltrate at the dermal-epidermal interface. Lichenoid drug eruptions have a very similar histopathologic appearance.

In contrast to type I, II and III hypersensitivities, which are systemic, type IV hypersensitivity is often a local reaction, and in the skin is referred to as allergic contact dermatitis. Different toxic agents can induce more than one immune-mediate mechanism. As an example, penicillin, which is often cited as the classic example of a drug acting as a hapten, can cause both an IgE-mediated type I hypersensitivity reaction manifesting as urticaria, as well as non-IgE mediated reactions that manifest as variable degrees of epidermal keratinocyte necrosis, ranging from erythema multiforme to toxic epidermal necrolysis and Stevens-Johnson Syndrome, severe conditions with widespread full-thickness epidermal necrosis and detachment from the underlying dermis (Figure 4).

Figure 4. Skin from a human with toxic epidermal necrolysis (TEN) illustrating widespread necrosis of the entire basal layer of the epidermis (arrows) with scattered necrosis of suprabasal keratinocytes (arrowheads) and epidermal-dermal separation and cleft formation (asterisks). The cleft is filled with a small amount of cellular debris and amorphous material. There is little or no associated inflammatory reaction in either the epidermis or dermis. Image courtesy of Dr. Phillip McKee.
The pathogenesis of immune-mediated cutaneous toxicity is not completely understood, although current understanding suggests involvement of both the adaptive and innate immune system. It had long been surmised that drugs and other xenobiotics acted as haptens, as described for penicillin, and when conjugated to proteins became presented to the immune system and elicited an immune response. More recently, the involvement of the innate immune system has come to the forefront.

As described earlier, keratinocytes sense danger associated molecular patterns (DAMPS) through their toll-like receptors (TLRs), which triggers the release of antimicrobial peptides such as S100A8/S100A9 complexes, cytokines and chemokines that in turn recruit and activate leukocytes. Completing the loop, leukocytes that are recruited to the epidermis then release cytotoxic factors, such as perforin and granulysin from CD8+ cytotoxic cells and NK cells, as well as TWEAK, TRAIL, Fas ligand and other TNF family members from macrophages and DCs that in turn induce the keratinocyte death that underlies drug-induced blistering syndromes such as erythema multiforme and toxic epidermal necrolysis.

References:

**S-5**

**An In Vivo Delayed-type Hypersensitivity (DTH) Model in Non-human Primates and Applications in Nonclinical Studies Supporting Biotherapeutic Drug Development**  
*Christina M. Satterwhite, PhD, Charles River Laboratories Preclinical Services, Reno, NV*

The DTH response is a Type IV hypersensitivity antigen-specific reaction involving cell-mediated immunity initiated by CD4 and CD8 T cells; the classic DTH response is a feature of *Mycobacterium tuberculosis* infection. With many immunomodulatory drugs in development, there is an increased need for assays that can evaluate the effects of biotherapeutics and their ability to augment or suppress antigen specific T-lymphocyte responses. A series of studies were conducted to develop a model that comprised characteristics of the classic DTH response, utilizing a combination of clinical signs (induration and erythema), histologic findings, and immunohistochemistry, and to compare these measurements in the absence or presence of a known immunosuppressant compound in an effort to have a reproducible and robust method. For each component of assay development/characterization, six male rhesus monkeys (3/Group) previously sensitized with BCG (*Mycobacterium bovis*-Bacillus Calmette-Guérin) were challenged with Old Tuberculin (OT); to evaluate effects of immunosuppression, the DTH response was compared in animals that had received Dexamethasone or saline prior to OT challenge. In assay development experiments, all animals demonstrated measurable inductions greater than 3 mm in diameter at either 24, 48 or 72 hours post challenge; the DTH reaction was characterized by inflammatory cell infiltrates (predominantly CD4+ and CD8+ lymphocytes and CD68+ macrophages) surrounding most small blood vessels in the superficial and deep dermis and in the underlying subcutaneous tissue, most notable at 48 hours after challenge. The concurrent administration of Dexamethasone resulted in a reduction in size of inductions and an overall decrease in perivascular cellular infiltrates (decrease in CD4+ and CD8+ lymphocytes and a decrease/absence of CD68+ macrophages). These studies confirmed that BCG administration followed by tuberculin challenge provided a consistent assay methodology for evaluation of the DTH response. In addition, case studies will be presented to demonstrate applications in nonclinical studies that support biotherapeutic drug
development programs in cynomolgus monkeys utilizing immunization with BCG and challenge with Tuberculin.

S-6

Assessment of Cutaneous Phototoxicity for Regulatory Submission: Does Microscopic Evaluation Enhance Visual Evaluations?
Douglas B. Learn, PhD, Charles River Laboratories Preclinical Services, Horsham, PA

Evaluation of xenobiotic-induced cutaneous phototoxicity for risk assessment has traditionally been based on clinical observations, e.g., erythema, edema, and flaking. The need for histopathological examination of the skin arises from time to time, as this is a classic method for determining a toxic effect in a target organ. The time course of responses in the pigmented and non-pigmented skin in the Long-Evans rat was evaluated after oral administration of the phototoxins sparflaxacin and 8-methoxypsoralen (8-MOP) and a single exposure to ultraviolet B and A (UVR). UVR exposure alone did not result in visual changes, and minimal microscopic changes were noted. Sparflaxacin demonstrated skin responses of erythema and edema, while 8-MOP demonstrated skin responses of primarily erythema over the evaluation period. Primary microscopic findings in the sparflaxacin group was epidermal hyperplasia (Days 2-4) and in the 8-MOP group was single cell necrosis of keratinocytes (Day 2) and ulceration (Days 3-4). Inflammation, hemorrhage and edema was seen throughout with increased severity (Days 3-4) with 8-MOP. Findings were similar in the pigmented and non-pigmented skin. While the histopathologic changes differ, the findings collaborate but do not change the interpretation of positive clinical observations of both phototoxins.

S-7

UV-induced Melanin Chemiexcitation: A New Mode of Melanoma Pathogenesis
Douglas E. Brash, PhD, Yale University, New Haven, CT

Mutations in sunlight-induced melanoma arise from cyclobutane pyrimidine dimers (CPDs), DNA photoproducts usually created picoseconds after an ultraviolet (UV) photon is absorbed at thymine or cytosine. Surprisingly we found that, in melanocytes, CPDs were generated for hours after UVA or UVB exposure. These “dark CPDs” constituted the majority of CPDs in cultured human and murine melanocytes and in mouse skin, and they were most prominent in skin containing pheomelanin, the melanin responsible for blonde and red hair.

The mechanism was also a surprise. Dark CPDs arise when UV-induced superoxide and nitric oxide combine to form peroxynitrile, one of the few biological molecules capable of exciting an electron ("chemiexcitation"). Excitation occurred in fragments of melanin, creating a quantum triplet state that had the energy of a UV photon but induced CPDs by radiationless energy transfer to DNA. UVA and peroxynitrile also solubilized melanin and permeabilized the nuclear membrane, allowing melanin to enter. Melanin is evidently carcinogenic as well as protective.
These findings may underlie the dependence of UV-induced and spontaneous skin cancers on melanin type. The results also validate the long-standing suggestion that chemical generation of excited electronic states – the source of bioluminescence in lower organisms – is important in mammalian biology. Chemiexcitation might also occur in internal tissues, because the same chemistry should arise wherever superoxide and nitric oxide arise near cells that contain melanin.

S-8

In Vitro Skin Models and Their Predictability in Defining Normal and Disease Biology, Pharmacology, and Toxicity
Dmitry M. Danilenko, DVM, PhD, DACVP, Genentech, Inc., South San Francisco, CA

Three-dimensional in vitro human skin model systems have been increasingly used in recent years for both toxicologic applications as well as for basic pathophysiologic research. These models have also recently gained increased acceptance for the toxicologic evaluation of topical irritation and corrosion.

These in vitro models have also been increasing used for the assessment of cutaneous toxicity and biology from systemically administered agents. The most commonly utilized 3-dimensional human skin models are EpiDerm®, EpiSkin®, and SkinEthic®. All three are reconstituted human epidermal models that are composed of normal human epidermal keratinocytes that form a three-dimensional reconstitution of normal human epidermis containing all viable and nonviable cell layers (stratum basale, stratum spinosum, stratum granulosum, and stratum corneum). All models are metabolically and mitotically active, have a lipid and ceramide profile very similar to normal human epidermis, and express normal epidermal differentiation markers such as keratin 1/10, pro-filaggrin and involucrin. They are all grown in serum-free media system and are highly reproducible lot to lot.

These reconstituted human epidermis (RHE) models have been extensively evaluated for their ability to correctly predict topical skin irritation/corrosion, and have all demonstrated very good accuracy and reproducibility in their ability to accurately predict skin corrosion (90+%). Their ability to predict skin permeability/transport and phototoxicity has been less extensively evaluated, but they have also gained acceptance for those uses as well.

In addition to the evaluation of topically applied substances, RHE models have also been used for the evaluation of skin toxicity from systemically administered substances, the biologic/physiologic effects of a variety of cytokines and growth factors, as well investigations into the pathophysiology of the cutaneous diseases such as psoriasis. For example, the epidermal growth factor receptor (EGFR) inhibitors, cetuximab and erlotinib, have a clinical dose-limiting toxicity of rash. In the EpiDerm® RHE model, both EGFR inhibitors induced a decrease in epidermal thickness with the presence of occasional necrotic keratinocytes (Figure 1A), and also induced a marked decrease in the expression of phosphoS6, a Ser/Thr protein kinase that is activated/phosphorylated in response to EGFR activation (Figure 1B).
As an example of the use of the RHE model in pathophysiologic investigation, the IL-10 cytokine family members IL-19, IL-20, IL-22, and IL-24 were all found to induce morphologic, biochemical, and molecular changes consistent with some of the features found in psoriatic epidermis. IL-22, in particular, elicited many of the same features in the EpiDerm® RHE model that are found in psoriatic epidermis (Figure 2). Some of the morphologic and differentiation features that IL-22 induces in RHE, such as hypogranulosis (Figure 2A) and up-regulation in the expression of the hyperproliferative cytokeratin 6 (Figure 2B), the antimicrobial peptide S100A7 (Figure 2C), and activation/phosphorylation of the nuclear transcription factor Stat3 (Figure 2D) are features shared by psoriatic epidermis, in contrast to those elicited by EGF, a recognized growth factor with effects on numerous epithelia, including epidermis.
The ability of cytokines such as IL-22 and oncostatin M to induce morphologic and differentiation features in keratinocytes that mimic those found in psoriatic epidermis, as well as activate keratinocytes to upregulate many of the same genes known to be upregulated in psoriatic skin in the absence of blood vessels and leukocytes is remarkable, and reinforces the hypothesis that epidermal keratinocytes are central to the initiation of cutaneous inflammation as well as the initiation of cutaneous response to injury, infection and toxicity.

References:
Spontaneous Animal Models for the Study of Epidermal Diseases

Elizabeth Mauldin, DVM, DACVD, DACVP, University of Pennsylvania, Philadelphia, PA

Introduction:
The stratum corneum (SC), the outermost layer of the epidermis, was once regarded as a mere waste product of epidermal turnover. Over the past two decades, this unassuming layer has come to forefront of dermatology research. The SC is serves as the direct interface between the body and the ambient...
environment. One of the most important functions of the SC is to restrict water movement into and out of the skin (i.e., the SC keeps the body hydrated). The SC is well known to protect against pathogens and xenobiotics and offers some limited protection from ultraviolet light. More recently, impairment of SC barrier has been shown to allow cutaneous sensitization.

Formation of the SC barrier involves three major processes: 1) formation of compacted intracellular keratin, 2) intercellular lipid production from secreted lamellar body contents, and 3) desquamation via cleavage of corneodesmosomes by serine proteases. Mendelian defects in the formation of the SC may involve any of these processes and are manifest as scaling disorders. The severity of the clinical phenotype represents the attempt of the epidermis to repair the flawed barrier.

Patients with ichthyosis are at risk for developing secondary bacterial and fungal infections as well as atopic skin disease. Even more subtle defects (e.g., heterozygous FILAGGRIN mutations) in clinically normal individuals have been shown to permit epicutaneous allergen exposure and sensitization. Furthermore, these alterations may, in part, help to explain the dramatic rise of atopic dermatitis and allergic asthma in industrialized nations. In veterinary medicine, years of “allergy” dogma are being challenged as barrier function has moved to the forefront of research and treatment of canine atopic dermatitis.

**Why study epidermal diseases in dogs?**

Hundreds of genetic diseases have been described in the dog, many of which are analogous to human genetic diseases and have been invaluable for the understanding of both disease processes and a variety of therapies. In the United States, there are greater than 70 million dogs that live in 40 million households. 10% of those dogs suffer from atopic dermatitis. While mouse models have been invaluable to the study the skin barrier and atopic dermatitis, the inadequate clinical similarity and lack of disease complexity limits the translational impact. Dogs share the same environment (i.e., the same microbiome) as the human caregivers, and canine atopic skin disease has been shown to closely mimic the natural disease state in humans. The immune system of dogs, the gastrointestinal and respiratory tracts, and cutaneous anatomy are more analogous to humans than rodents. Canine atopic dermatitis has similar lesion distribution (e.g., flexural folds), life stage of onset, and IgE-specificity as human AD. Furthermore, dogs, like humans, are predisposed to developing secondary and recurrent Staphylococcal infections. There is reasonable evidence that dogs with AD have an altered skin barrier and this may play a role in allergen sensitization: deranged lipid profiles in the SC (e.g., decreased free ceramides), ultrastructural change in SC of atopic dogs, and increased transepidermal water loss (TEWL) in both normal and abnormal skin.

**SC and Mendelian disorders of Cornification in dogs**

Tremendous advances have been made in the clinical and genetic characterization of ichthyosis; however, there have been only modest advances in therapeutics. Dogs, like humans, develop a spectrum of ichthyosiform disorders. In humans, the disorders are classified by the clinical lesions (e.g., character of scale, distribution of lesions, erythroderma), syndromic or nonsyndromic, age of onset/pattern of inheritance, and histopathologic and ultrastructural features. Dog models are not yet
as detailed and most basic characterization is the light microscopy delineation as epidermolytic or nonepidermolytic. Homologues for the ichthyosiform disease in humans occur in several dog breeds: epidermolytic ichthyosis in Norfolk terriers, and nonepidermolytic ichthyosis in the Golden retriever, American bulldog, and Jack Russell terrier.

The umbrella term “autosomal recessive congenital ichthyosis” (ARCI) manifests as three skin phenotypes: harlequin ichthyosis, lamellar ichthyosis, and congenital ichthyosiform erythroderma. The phenotypes of LI and CIE are often overlapping, and some patients may switch phenotypes with age and treatment. Harlequin ichthyosis is a more severe disorder of cornification that is often fatal in infancy. Patients with LI have marked scaling with brown plate-like scale in absence of erythema; CIE patients have fine white scale with prominent erythema. To date, eight genes have been associated with ARCI: transglutaminase 1 (TGM1), ABCA12, two lipoxygenases (ALOXE3 and ALOX12B), a NIPA-like domain containing 4 (NIPAL4 or ICTHYIN), LIPN, CYP4F22, and PNPLA-2. In general, the phenotypes do not predict the mutational status. Harlequin ichthyosis, the most severe and often fatal phenotype, has only been documented in large animals. Dogs with ARCI may have lesions of LI or CIE.

**Epidermolytic ichthyosis** (a.k.a. epidermolytic hyperkeratosis) is a very rare disease in dogs. The light microscopic features (suprabasal keratinocyte vacuolation and lysis, hypergranulosis, and marked hyperkeratosis) are uniquely linked to mutations in epidermal keratins. This disorder has been identified in a few dog breeds (Rhodesian ridgeback, Labrador cross), but only well characterized in Norfolk terriers. The disorder in the terriers is autosomal recessive and caused by a splice site mutation in the KRT10 gene. The affected dogs have regions of mild pigmented scale with alopecia and roughening of the skin. Epidermolytic ichthyosis due to KRT10 mutations is similarly rare in people but usually autosomal dominant. Affected individuals have generalized erythema and blistering at birth followed by hyperkeratosis later in life.

**Nonepidermolytic ichthyosis** in the golden retriever (GR) is relatively common, mild, and unique in its variable presentation and age of onset. The phenotype is compatible with lamellar ichthyosis. GR ichthyosis is caused by a mutation in PNPLA1 (patatin-like phospholipase domain-containing protein) gene. Research on the dog mutation led to the identification of recessive PNPLA1 mutations in two human families with ARCI which previously had no genetic cause. The gene is thought to play a role in lipid organization and metabolism within the outer epidermis. In dogs, the gross lesions consist of large, soft, and white to grey adherent scale that is prominent on the trunk and may be associated with ventral hyperpigmentation. A definitive diagnosis can be achieved by skin biopsy, but the disease is so clinically unique that veterinary dermatologists often forego the biopsy procedure. The disease must have confounding factors because adult dogs that are homozygous for PNPLA1 mutation may not necessarily develop clinical lesions.

In **American bulldogs**, nonepidermolytic ichthyosis is easily recognized at 1-2 weeks of age but may be misinterpreted as failure to groom the puppies by the dam. The glabrous skin and flexural folds are erythematous and wrinkled with “saran-wrap”-like adherent scale. The remainder of the pelage has widely distributed large white scale. There is tremendous Malassezia overgrowth, which is contrary to
GR ichthyosis. This phenotype combines features of lamellar ichthyosis and congenital ichthyosiform erythroderma. The disorder has been linked a defect in the ICHTHYIN gene (currently referred to as NIPAL-4) but the precise mutation remains unknown. ICHTHYIN mutations are seen in about 16% of humans with ARCI. Unlike golden retrievers, the skin lesions in bulldogs do not wax or wane and are generally more severe. Ichthyin, like PNPLA-1 is involved in lipid metabolism and formation of the hydrophobic lipid barrier.

Nonepidermolytic ichthyosis in Jack Russell terriers has a more severe phenotype than the Golden retrievers and American bulldogs. The affected dogs have large, thick, adherent parchment paper–like scales. The dogs have defective formation of the cornified envelopes due to a mutation in the gene encoding transglutaminase-1 with a resulting deficiency of this enzyme. TGM1 is the major causative gene for ARCI in humans.

Summary:
Given the popularity of dogs and co-habitation with humans, much can be learned from natural spontaneous diseases to the benefit of both humans and dogs. Newer clinical trials divisions in veterinary schools are proving that even companion dogs can be used humanely for advancement in therapeutics to ease suffering in both humans and dogs.

S-10

The Hair Follicle: A Fascinating Mini-organ
Monika Welle, Prof DrMedVet, DECVP, University of Bern, Bern, Switzerland

The base of each hair shaft resides in a multicellular mini-organ called hair follicle (HF). Hair growth is needed to renew the protective covering of the body and it enables the seasonal change in the coat. Therefore, after the initial follicular morphogenesis, the HF is maintained by cycling through periodic stages which include a growth phase (anagen), a regression phase (catagen), and a quiescent phase (telogen). To sustain cyclic regeneration, each HF is dependent on its epithelial stem cells and on complex signaling events. Hair loss is a common complaint in mammals and possible underlying causes are numerous. Alopecia can be attributed, at large, to a decreased formation, a decreased regeneration, or an increased destruction of hair follicles and the consequence is that either the quality or the total density of hair shafts is altered. Whereas in mice knowledge about HF biology has rapidly progressed, the understanding of HF biology in other species is still very limited. However, newly available techniques may help in the future to understand alopecic disorders.

S-11

Scleroderma as a Model for the Dilemma of Fibrosing Diseases: Where Do We Go When Molecular Biology and Animal Surrogates Inadequately Inform?
Robert Dunstan, DVM, MS, DACVP, Biogen Idec, Cambridge, MA
Fibrosis is a feature of most chronic inflammatory and many neoplastic disease processes. Although it represents a homeostatic mechanism to repair tissues, often the deposition of collagen and other extracellular matrix components can interfere with organ function. When progressive, this can lead to end-organ failure and death. The impact fibrosis has on morbidity and mortality is largely unappreciated but when one considers the frequency of hepatic, pulmonary, renal, and cardiac fibrosis, one can understand why 45% of all human deaths in Western developed countries have been attributed to fibrosing diseases and the mortality in underdeveloped countries is likely to be higher. However, in spite of a good understanding of the molecular and cellular basis of fibrosis, there are few effective therapies. Herein lies the dilemma of the fibrosing diseases—where do researchers go next to effectively and efficiently develop drugs to control the excessive and inopportune deposition of the extracellular matrix?

Arguably the best example of this dilemma lies in scleroderma (SSc), a disease that is not on the differential of veterinary pathologists even specializing in skin diseases because it is so rare or because it does not occur in domestic animals. In humans, scleroderma is defined as a chronic, systemic disease characterized by thickening and hardening of the skin with internal organ involvement. There are two forms of the disease: 1) limited scleroderma (ISSc) that involves the skin below the knees and elbows and 2) diffuse scleroderma (dSSc) that has the same distribution as ISSc but also involves the skin above and below the knees and elbows as well as the rest of the body.

Patients with both ISSc and dSSc develop systemic disease. In ISSc, this is most commonly represented by pulmonary arterial hypertension, a condition that may take decades to develop after the initial diagnosis of scleroderma. DSSc tends to be much more aggressive with patients exhibiting pulmonary (most common), renal, or cardiac involvement. A feature common to both forms of the disease is Raynaud’s phenomenon, where there is a vasoconstrictive reduction of blood flow to the fingers and toes that is associated with digital palor and occasionally, avascular necrosis. The diagnosis of scleroderma is largely based on clinical features such as Raynaud’s phenomenon and the progression of the disease is assessed by manually pinching the skin in 17 standardized locations and grading skin thickness/pliability on a 0-3 score (the modified Rodnan skin score, mRSS). The 10 year survival for limited and diffuse forms of the disease is 70% and 50%, respectively. As is typical of the majority of fibrosing diseases, there is no approved disease modifying therapy.

Although scleroderma has been recognized as a clinical entity since the 1700s and there has been an increasing interest in the disease by rheumatology centers and pharmaceutical companies, the cause of scleroderma has proven elusive. In 1996, LeRoy defined scleroderma as a disease “with distinct abnormalities of three systems, immune, vascular, and microvascular, and mesenchymal extracellular matrix that lead to exuberant fibrosis.” Today, a variation of this statement begins almost every manuscript on this topic. What is not known is what is the initiating factor in scleroderma and whether to effectively treat the disease one needs to manipulate one or multiple systems described by LeRoy.
Molecular analyses of scleroderma from genomic and transcriptomic perspectives have been informative but have not resulted in innovative therapies and are no more predictive than the mRSS as a biomarker of disease progression. In addition, molecular classification of skin from SSc subjects has identified diffuse 1, diffuse 2, inflammatory, limited, and normal-like signatures but these have yet to be confirmed by histologic evaluation. To further add to confusion, these signatures do not appear to change as the disease progresses (although dSSc can change dramatically over time) and clinically unaffected sites are molecularly indistinguishable from affected sites.5-8

Because so little is understood about SSc, murine surrogates have been used to inform the basic processes defined by LeRoy. A summary of emerging/most widely used murine animal models is below. None of the current models mimic the human disease where they can be considered homologues and most are poor analogues. The first difference is physical. Human skin is far thicker than mouse skin because more structural support is needed for a species that can easily outweigh its rodent counterpart by a factor of 3,000. This means collagen-cross linking is more abundant and fibers much thicker. The second difference is anatomic/histologic. Mouse skin is heavily haired, the superficial vascular plexus is less well developed and there is a panniculus muscle. The third difference in the models themselves: even if murine skin replicated the skin of humans more closely, the models themselves do not recapitulate SSc.9-14

What has been lacking in SSc research is a cross-disciplinary “interactome” approach. Currently, there are two definitions of SSc: a molecular definition based on differential expression of molecules and a structural definition based on histologic features. The information provided by these evaluations needs to be merged as alone, neither gives a complete picture of the disease. Only 40% of the variation in protein concentration can be explained by knowing mRNA concentration. This correlation is probably much lower for a disease primarily of the extracellular matrix. At the same time, the morphology of SSc is surprisingly ill-defined for a disease whose pathology was first described in detail over 60 years ago. The reason for this is that the emphasis in anatomic pathology has been largely based on establishing a diagnosis rather than quantifying the morphologic changes associated with SSc (“morphomics”). We are just beginning to apply quantitative analysis to SSc; however, even in these early stages, human SSc can be shown to be a structurally heterogeneous disease and sections from some SSc patients have large areas of dermal collagen that are morphologically indistinguishable from normal skin, suggesting the need for in situ analysis to capture disease-specific molecular features of SSc. Applying “morphomics” to animal models provides a more reproducible method to define structural homology (or lack thereof) with the disease in humans it is meant to inform.

In conclusion, SSc represents not only a prototypical example of a fibrosing disease but serves as an example of how the future of anatomic pathology will transition from descriptive to quantitative and how “morphomics” will be used in combination with molecular methods to give a complete picture of the pathophysiology of a disease.

*Out of completeness, there is a third major form of the scleroderma, localized scleroderma (also called morphea) that is disfiguring but benign and will not be further discussed.
References:

Selected Murine Models of Scleroderma

<table>
<thead>
<tr>
<th>Model</th>
<th>Vasculopathy</th>
<th>Inflammation</th>
<th>Autoimmunity</th>
<th>Fibrosis</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inducible</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Bleomycin-induced fibrosis9</td>
<td>Y</td>
<td>3+</td>
<td>2+</td>
<td>3+</td>
<td>Fibrosis starts in panniculus and extends upwards at injection site; will cause pulmonary inflammation/fibrosis with skin injection</td>
</tr>
<tr>
<td>Hypochlorous acid-induced fibrosis9</td>
<td>N</td>
<td>3+</td>
<td>2+</td>
<td>3+</td>
<td>Similar to bleomycin with abundant dystrophic mineralization, often: will cause pulmonary inflammation/fibrosis with skin injection</td>
</tr>
<tr>
<td>Lesion Description</td>
<td>Grade 1</td>
<td>Grade 2</td>
<td>Grade 3</td>
<td>Grade 4</td>
<td></td>
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<tr>
<td>-----------------------------------------------------------------------------------</td>
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<td>---------</td>
<td>---------</td>
<td>---------</td>
<td></td>
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<tr>
<td>Graft versus host disease, sclerodermic variant</td>
<td>N</td>
<td>3+</td>
<td>2+</td>
<td>3+</td>
<td></td>
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<tr>
<td>Lesions arise randomly and vary in severity; inflammation can be extensive and</td>
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<tr>
<td>appears as directed against epithelial elements as much as against the dermis</td>
<td></td>
<td></td>
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<tr>
<td>Angiotensin II</td>
<td>1+</td>
<td>1+</td>
<td>N</td>
<td>1+</td>
<td></td>
</tr>
<tr>
<td>Associated with cardiovascular remodeling; very little fibrosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>Genetic</strong></td>
<td></td>
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</tr>
<tr>
<td>Tight skin-1$^9$ (Fibrillin-1 mutation?)</td>
<td>N</td>
<td>N</td>
<td>1+</td>
<td>3+</td>
<td></td>
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<tr>
<td>Normal dermis, tight skin is due to tethering of subcutaneous fat to underlying</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>musculature, emphysematous lungs, abnormal bone formation</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Tight skin-2$^{11}$ (Gain of function mutation in Col3a1 gene)</td>
<td>N</td>
<td>N</td>
<td>1+</td>
<td>1+</td>
<td></td>
</tr>
<tr>
<td>Tight skin, increased elastin, non-inflamed, collagen morphology normal until</td>
<td></td>
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<tr>
<td>animals &gt;12 weeks old</td>
<td></td>
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</tr>
<tr>
<td>Stiff skin syndrome (Fibrillin-1 mutation)$^{12}$</td>
<td>N</td>
<td>2+</td>
<td>3+</td>
<td>3+</td>
<td></td>
</tr>
<tr>
<td>Supposedly a model for a condition in humans called the same; difference is that</td>
<td></td>
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<tr>
<td>this has been described as an inflammatory response and the human condition is</td>
<td></td>
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<tr>
<td>described as non-inflamed</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Fos-related antigen-2 transgenic$^{13}$</td>
<td>3+</td>
<td>3+</td>
<td>N</td>
<td>2+</td>
<td></td>
</tr>
<tr>
<td>Believed to have a microangiopathy</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

**S-12**

**Pragmatic Issues in Dermal Toxicity Studies: Expected and Unexpected**

*Christine L. Merrill, DVM, PhD, DACVP, GlaxoSmithKline, Research Triangle Park, NC; and Sundeep Chandra, BVSc, PhD, DACVP, GlaxoSmithKline, Research Triangle Park, NC*

It has been more than seven decades since the publication of the Draize scoring system, but the field of dermal toxicity continues to evolve in order to accurately predict dermal (and systemic) responses in humans to topically applied pharmaceuticals. Although the testing methods have undergone extensive refinements, idiosyncrasies and unexpected issues during the conduct of these studies are not unusual. Dermal administration presents multiple challenges. Procedure-related issues unique to dermal studies such as clipping the skin or tape-stripping the epidermis to remove the stratum corneum; wrapping the torso to prevent ingestion of the test substance, and occluding the application site can impact interpretation. For the study pathologist, it is imperative to have knowledge concerning irritation potential of excipients used for dermal formulations, histological differences in skin morphology between different laboratory animals, study protocol-related microscopic changes, and spontaneous background lesions. Other factors to consider in evaluations of the skin include use of different scoring systems and discrepancies between in vivo observations, necropsy observations and microscopic pathology findings.
Pigs in Toxicology: Differences in Metabolism and Background Findings
Kristi L. Helke, DVM, PhD, DACVP, Medical University of South Carolina, Charleston, SC

In toxicological testing, both a rodent and non-rat rodent species are required. Historically, dogs and non-human primates have been the species of choice of the non-rat rodent portion of testing. Swine, especially the miniature swine or minipigs, are increasingly being used in preclinical safety as an alternate non-rat rodent species. The pig is an appropriate option for these tests based on metabolic pathways utilized in xenobiotic biotransformation. Phase I and phase II biotransformation pathways in humans and pigs highlighting similarities and differences will be discussed. Numerous breeds of pigs are discussed along with specific breed and sex differences in these enzymes that are known. Although swine have been used extensively in biomedical research, there is also a paucity of information in the current literature detailing the incidence of background lesions and differences in incidence between commonly used breeds. Background lesions with consideration of breed, sex, and geographical location will also be covered.

Selected References:

Cutaneous Toxicity in Non-human Primates
Annette Romeike, DMV, DACVP, Covance Laboratories SAS, Porcheville, France

Cutaneous drug eruptions are among the most common types of adverse reaction to drug therapy. Preclinical safety studies are typically employed to identify and characterize hazards arising from new medicinal products under development, but concordance of the toxicity observed in humans with that observed in experimental animals is very poor, particularly when it comes to cutaneous reactions. This is not surprising considering that cutaneous toxicity is caused by very diverse mechanisms and that physiology and anatomy of the skin differ largely between generally used experimental animals and man.

Monkeys, specifically cynomolgus macaques, are increasingly used for preclinical safety studies, because of their high similarity to humans. This also applies to their skin. However, most researchers conducting preclinical safety studies do not pay a lot of attention to the skin. Analysis is typically limited to
superficial external observations and to microscopical examination of a tiny piece of skin around the mammary gland.

This presentation aims at increasing the understanding of cutaneous physiology and pathology with particular emphasis on the cynomolgus monkey. We propose a systematic approach towards examining and recording skin lesions in a macaque safety study, and we provide examples, where a detailed examination of features of the skin has identified and characterized specific safety signals that are important for assessing the risk for patients.

S-15

A Case of Drug-induced Cutaneous Toxicity Observed in Cynomolgus Monkeys

Rie Kikkawa, DVM, PhD, DABT, DJCVP, DJSOT, DACVP, Novartis Institutes for BioMedical Research, East Hanover, NJ

The purpose of this presentation is to demonstrate a case of drug-induced cutaneous toxicity observed in cynomolgus monkeys. The test-article was a small molecule with a ubiquitously distributed target, especially in rapidly dividing cells, and which modulated cell cycle regulation. After seven consecutive days of oral dosing, animals developed multifocal skin lesions. The lesions were characterized clinically by scab and vesicle formation and were distributed mainly in thin-skinned areas of the body including cheek, chest, abdomen and inner limbs. Microscopically, the lesions were confirmed as epidermal ulceration and vesicle formation. Immunohistochemical staining revealed that the levels within the epidermis where separation (vesicle formation) occurred were not consistent. Our efforts to elucidate the mechanism of toxicity using in-house database searches, immunohistochemistry, and differential diagnoses for vesicular skin lesions, will be presented.

To the best of our knowledge, similar cutaneous toxicity has not been reported previously, although there are many reports of other types of cutaneous toxicities. Understanding the mechanism of the toxicity and human relevance are very important when developing drugs. Our investigative efforts can be utilized when unusual skin toxicity is observed in the future.