Translational dermatology in drug discovery: perspectives for integrating humanized xenograft models and experimental clinical studies

Thomas K. Petersen and Poul Sørensen

Biological Research, Discovery, LEO Pharma, 55 Industriparken, DK-2750 Ballerup, Denmark

Application of humanized xenotransplantation disease models and experimental clinical studies in the context of translational research in drug discovery in dermatology is an opportunity to reduce failure due to lack of efficacy in clinical development stage.

Introduction

In the search for ways of increasing productivity by shifting failure earlier in drug discovery, there has, in recent years, been an increased focus on improving experimental disease models to become more predictive of clinical outcome [1]. The present review provides a future perspective on the application of translational research in drug discovery for skin diseases by exploring new opportunities for integrating humanized xenotransplantation disease models with experimental clinical studies. Moreover, as many of the underlying principles of skin diseases are shared with other autoimmune and inflammatory disorders, evolving general pathogenic concepts could be evaluated in these models, as not only visible disease characteristics but also more detailed histological, cellular and genomic features can be readily assessed for investigational analysis.

Many relevant *in vivo* pharmacological models exist for determining proof of principle in drug discovery for skin diseases in laboratory animals. Unfortunately, none of the current models is characterized to the same extent at the cellular and molecular level as human skin diseases; for example, it is not clear whether the same repertoire of leukocyte subsets is represented in skin. Additionally, the molecular expression patterns of adhesion molecules in the skin of mice are somewhat different from those of normal human skin [2,3], and this becomes an issue since lymphocyte homing and the recirculation process play a crucial role in chronic inflammatory skin diseases [4,5]. On the contrary, xenotransplantation of normal or affected skin from patients has the advantage that human tissue is used, containing relevant human leukocyte cell subsets and adhesion molecules. It is, therefore, not surprising that the application of humanized xenotransplantation skin models has increased considerably over the past decade and has been exploited, in particular, in investigation of new therapeutic concepts targeting lymphocyte migration [6].

The majority of skin diseases are classified as inflammatory and immune mediated, with or without the involvement of bacteria, and include psoriasis, atopic dermatitis (AD), acne and contact eczema. The present review places special emphasis on psoriasis and AD as model diseases, as these two indications represent very different immunopathological aspects of the immune system and cover a wide range of immune-mediated mechanisms present in inflammatory skin diseases.

Atopic dermatitis

Atopic dermatitis is a chronic inflammatory disease associated with skin hyperreactivity to environmental triggers that are innocuous to normal non-atopic individuals. Approximately 80% of the patients are associated with IgE-mediated sensitization. Sparse perivascular T cells and an increased number of Th2 cytokine-expressing cells are present in unaffected AD skin as compared with healthy skin. In the acute lesions, there is a marked infiltration of CD4+ activated memory T cells and Th2 polarized T cells as well as IgE-expressing Langerhans cells, macrophages and mast cells. In the chronic lesions, the number of antigen-presenting cells (APCs) is increased, the mononuclear dermal infiltrate is dominated by macrophages and the cytokine response is more polarized towards Th1. Eosinophils are present to a lesser extent than T cells as compared with the acute lesions, and greater number of IL-5, GM-CSF, IL-12 and IFN-γ expressing cells are present. Local tissue expression of a number of cytokines is prominent in resident dermal cells including keratinocytes, mast cells and dendritic cells, and they initiate and promote the recruitment and extravasations of leukocytes [7].

Corresponding author: Petersen, T.K. (thomas.petersen@leo-pharma.com)

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The hyperreactivity in AD is linked to an impaired skin barrier function, where a number of crucial components are described as being dysregulated, including decreased content of skin lipids [8], increased protease activity [9] and loss-of-function mutations in filaggrin [10]. Furthermore, a recent study has shown that the Th2 cytokines IL-4 and IL-13 downregulate filaggrin expression during the differentiation process [11]. A more detailed description and discussion of the pathogenesis of AD is available elsewhere [7].

Psoriasis

Like AD, psoriasis is a disease that develops as a result of both genetic and environmental factors. Monozygotic twins have a concordance of psoriasis between 35 and 50%, and the disease is associated with certain HLA haplotypes and certain susceptibility loci. Leukocytes present in the psoriatic lesions include T cell subsets polarized as Th1, CD4+ and T cytotoxic, CD8+, and probably also a population of Th17 cells; they are present in dermis and epidermis in association with mast cells, dendritic cells and macrophages [12]. The proliferation of keratinocytes in psoriatic lesions is increased in parallel with a dramatically decreased differentiation time and, to date, there is a broad consensus that inflammation, including T cell activation, precedes and leads to disturbed keratinocyte homeostasis and epidermal hyperproliferation [13]. A more comprehensive description and discussion of the pathogenesis of psoriasis is available elsewhere [12].

Xenotransplantation models for AD and psoriasis

To date, the development and description of humanized AD models has been relatively limited, probably because of the difficulty in obtaining human biomaterial for study compared with other skin diseases. Primarily, these models are based on humanized allergic skin inflammation that involves transfer of PBMCs from patients with AD [14,15]. The donors have to be defined as allergic to some specified antigen (typically the house dust mite). Initially, the PBMCs are administered intraperitoneally to SCID mice, together with an allergen and staphylococcal enterotoxin B (SEB). After a week, the mice receive an additional allergen challenge followed in another week by intradermal injection of PBMCs from the same donor followed by 5 days of topical application of allergen and SEB to the skin. Intradermal injection of PBMCs must be included in order to bring the human cells as close as possible to the site of reaction and to achieve a pronounced inflammatory response in the skin. The human T cells have previously been shown to interact weakly with the murine vascular endothelial cells [16] and this is likely to explain this observation. The marked morphological changes in the skin reflect some of the features of AD lesions, and elevated hu-IgE levels are associated with the disease. The model allows the study of *in vivo* human allergic responses and may represent a tool for the examination of proof of concept strategies for specific therapies, although none has yet been reported. The major limitation of these models is that they do not involve transplantation of AD skin, which excludes the possibility for a more complete study of the skin immune system and barrier function *in vivo* in relation to AD.

In contrast to AD, xenotransplantation models have been used more widely for the investigation of psoriatic disease or for the discovery of new molecules for this indication. In the human psoriasis xenograft SCID mouse model, keratome-biopsies (split-skin) or full-thickness skin biopsies of chronic plaque-stage psoriasis are transplanted onto SCID mice, where the graft survives and maintains the psoriatic phenotype for six to eight weeks [17]. During this time it is possible to test molecules, either by systemic or topical administration [18,19]. Histology of harvested grafts serves as a robust readout for anti-psoriatic effect. Modifications of the model exist where uninvolved psoriatic skin is transplanted to SCID mice in combination with stimulated PBMCs from the same donor [20]. In this model a psoriatic plaque starts to evolve in the transplanted skin within a few weeks and is very useful for the investigation of the disease process in the early stages of psoriasis. The model is estimated to have a fairly good predictive value [6,18], as established therapies are efficient in the different modifications of model (Table 1). Recently, the model has been further validated for topical treatment where calcipotriol (Daivonex® ointment) and betamethasone dipropionate showed efficacy comparable to that observed in clinical studies [19] (Figure 1). The psoriasis xenograft models have not only been valuable for testing

TABLE 1

Correlation between efficacy of (A) established and (B) innovative (not yet established) anti-psoriatic drugs in the psoriasis SCID mouse model and clinical experience (modified from reference [18])

Drug	Treatment protocol (SCID)	Outcome in SCID model	Clinical experience
Calcipotriol (Daivonex®) [19]	Topically	+	+ (A)
1α,25-Dihydroxycholecalciferol [18]	i.c.	+	+ (A)
Dexamethasone [18]	p.o.	+	+ (A, rarely used)
Clobetasol propionate [18]	Topically	+	+ (A)
Betamethasone dipropionate [19]	Topically	+	+ (A)
Cyclosporine A [18]	i.p.	+	+ (A)
Efalizumab (Raptiva®) [18]	i.p.	+	+ (A)
Infliximab (Remicade®) [64]	i.p.	+	+ (A)
Troglitazone [18]	p.o.	+	+ (B, five patients)
Efomycine [18]	s.c.	+	+ (B, pilot study)
PS519 (proteasome inh.) [18]	i.p.	+	Not reported (B)
LEO15520 [56]	p.o.	+	Not reported (B)

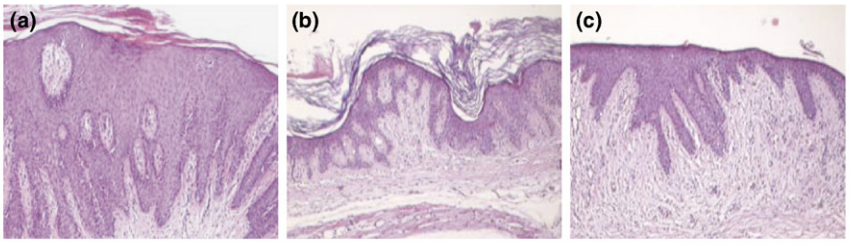

FIGURE 1

Histology of biopsies from the psoriasis xenograft SCID-mouse model: Psoriatic keratome biopsies (1.5 cm × 1.5 cm) were transplanted onto SCID mice and 10 days after transplantation, the lesions were treated topically twice daily for four weeks with the test formulations. As compared to placebo (Daivonex® ointment base) **(a)**, there is a clear antipsoriatic effect at the histological level following topical treatment with Betamethasone dipropionate in Daivonex® ointment base **(b)** and Daivonex® ointment **(c)**.

novel therapeutic concepts but have also provided experimental evidence that bacterial superantigens trigger psoriasis [21], that T lymphocytes play a central role in the onset of disease [22] and that TNF-α is a key regulator of local T cell proliferation and disease development [23]. The advantage of the psoriasis xenograft models is that an almost complete psoriatic skin immune system is present, including the process of leukocyte homing and recirculation. It is important to be aware that components of the residual murine skin immune system including NK cells, DCs, macrophages and neutrophils are present in the murine tissue surrounding the human graft. These immune cells may influence the inflammatory response in the psoriatic graft.

A number of other humanized xenograft models that only involve healthy tissue from donors are relevant to consider when evaluating new therapeutic concepts for both AD and psoriasis. The first model, the human skin allograft injury model, allows the study of the human immune reaction *in vivo* [24,25] (Table 2). In brief, PBMCs are injected into SCID mice engrafted with healthy human skin from a different donor. In this model, a graft-versus-host response is elicited in the skin graft, which shows a reproducible pattern of progressive human T cell infiltration and human microvascular injury that resembles human first-set skin graft rejection. This model provided the first evidence of *in vivo* function of human-specific immune modulators of LFA-3. As mice and rats do not express LFA-3, it was not previously possible to evaluate its role in animals. This therapeutic concept has later been proven effective in psoriasis patients treated with alefacept—a human LFA-3/IgG(1) fusion protein [26].

The second model, the 'trans *in vivo* delayed hypersensitivity (DTH) model' is a relatively simple model for human DTH testing [27,28] (Table 2). It comprises an antigen-specific immune response that is relevant for both AD and psoriasis. In brief, human PBMCs plus antigen (e.g. tetanus toxoid) are injected into the pinnae or footpads of naive mice, and within 24 h, swelling of pinnae or footpad develops. The model has provided *in vivo* proof of principle of novel small molecule antagonists directed against LFA-1, which consists of CD11a and CD18 subunits, and thereby targets T cell trafficking [28]. No works on efficacy of these small

TABLE 2

Correlation between targets evaluated in patients and in human xenotransplantation models: The human skin allograft injury model, trans in vivo delayed hypersensitivity (DTH) model with or without skin transplantation

Model	Protocol	Drug/target	Outcome in model	Correlation with clinical studies[a]
Human skin allograft injury model [24,25]	Transplantation of healthy skin to SCID mice and PBMCs (i.p.) allogeneic to the skin [24,25]	Murine Mab to human LFA-3 or human LFA-3-IgG1 fusion protein [24]	Inhibition of immune reaction [24]	Human LFA-3-IgG1 fusion protein (Alefacept®) has effect in psoriasis [26]
		Cyclosporine A and rapamycin in combination [25]	Inhibition of immune reaction [25]	Cyclosporin A has an effect in psoriasis
Trans *in vivo* DTH model without skin transplantation [28]	Co-injection of tetanus toxoid and human PBMCs from tetanus-sensitized donors into footpads of naive mice [28]	LFA-1 antagonists [28]	Inhibition of immune reaction [28]	No reports for LFA-1 antagonists, but Efalizumab (Raptiva®) has an effect in psoriasis [29]
Trans *in vivo* DTH model with skin transplantation [16]	Transplantation of healthy skin to SCID mice and PBMCs (i.p.) from tetanus-sensitized donors [16]	Anti-human CD4 Mab [16]	Inhibition of proliferation of T cells [16]	Moderate effect of anti-CD4 antibody (HuMax-CD4®) in a study in psoriasis [31]

[a] It is necessarily not the same molecules that have been used both in the models and the patients.

molecules in clinical studies have been reported so far, but efalizumab, a humanized anti-CD11a monoclonal antibody, has shown efficacy in psoriasis [29]. To obtain a more complete immune system for the study of the human DTH-response, human skin grafts must be transplanted in combination with PBMCs from tetanus toxoid-sensitized donors [16,30] (Table 2). After healing, mice are injected intraperitoneally with PBMCs from the same donor. Tuberculin and diluent are injected intradermally, and 72 h later, inflammation including perivascular T cell infiltration can be observed in the graft. Perivascular T cell accumulation is only present when the antigen is injected into the human skin, confirming that these cells specifically recognize human skin as homing sites. Proliferation can be blocked with monoclonal antibodies to human major histocompatibility complex antigens and with anti-human CD4 monoclonal antibodies. In psoriasis patients a moderate effect of treatment with a humanized monoclonal anti-CD4 antibody (HuMax-CD4®) has been reported [31] indicating that the model may be used in relation to certain studies of T cell targeted therapies. The model lends itself to studies of endothelium T cell interactions, T cell activation within skin and chronic inflammatory skin diseases [16,30]. The limitation of the models utilizing healthy skin is that they do not reflect the skin immune response of a specific disease, but their advantage is that they are easier to work with because healthy skin is easier to obtain, that is, from removal in relation to cosmetic surgery, compared with skin from patients.

Experimental clinical studies in AD and psoriasis

Experimental clinical studies preceding traditional clinical studies have been used for obtaining early proof of concept in AD [32] and psoriasis [33], patients.

For experimental clinical studies in AD, the allergic patch test is the most commonly used, as pharmacologic studies in spontaneous AD can be difficult to standardize because patients with AD differ in the stage of their skin disease (i.e. acute, subacute, chronic). Typically, the test site is treated one to three weeks before induction of the atopy patch test reaction, followed by induction by topical application of allergen. Topical anti-inflammatory treatments, including corticosteroids [34,35], tar [34] and certain emolients [36] have shown efficacy at the clinical and histological level, whereas tacrolimus [35] and pimecrolimus [37], which both are approved for AD, have been reported not to have a significant efficacy in the atopy patch test. Systemic treatment with anti-IL-5 (Mepolizumab) also does not show any efficacy in the atopy patch test [38]. Very recently, an RxR antagonist (BAL10092) has been reported to show an effect in a proof of concept study in the AD patch test. In this study only three days of treatment was used [32]. The major limitation of the AD patch is that it is an allergic reaction that only involves the very acute phase of AD. This may also explain the non-significant effect of the calcineurin inhibitors. Pretreatment has only been described in this model except for one study [32], although therapeutic treatment of established subacute or chronic lesions is preferable in order to obtain a complete and more relevant evaluation of novel therapeutic concepts.

For experimental clinical studies in psoriasis, the psoriasis plaque test, derived from the KJ Dumas and JR Scholtz method, is one of the most widely applied assays [39,40]. In addition to screening for anti-psoriatic effects, the psoriasis plaque test can help answer other questions, including frequency of use, dose relationship and comparison of formulations. The psoriasis plaque test is relatively simple, not time-consuming and allows simultaneous testing of multiple substances. Compounds can be applied topically, either occlusively using Finn Chambers or non-occlusively, where only the test sites will be covered with a non-occlusive gauze. The occluded approach favours the penetration of the compound into the skin, whereas the non-occluded approach resembles the clinical situation more closely. A prerequisite for optimal performance is knowledge about the dermal permeation of the compound in an adequate formulation. Intradermal drug administration may also be applied if a suitable formulation does not exist for late discovery stage molecules.

Corticosteroids and vitamin D analogues are often included as reference compounds as they are efficient in the treatment of chronic plaque psoriasis [40,41]. The calcineurin inhibitors tacrolimus [42] and pimecrolimus [43,44] that are approved for use in AD have been shown to have an effect under occlusion in the psoriasis plaque assay. Contradictory results have been reported for tacrolimus in chronic plaque psoriasis, where some groups have been unable demonstrate an effect [45] yet others have been able to show an effect [46]. Lack of effect is probably because of the very poor diffusion of the active substances through the hyperkeratotic lesion, and other studies are currently underway with improved formulations of tacrolimus [47] and pimecrolimus [41]. The advantage of the psoriasis plaque test is that it is possible to use multiple test sites on the same patient and thereby different treatments can be compared intra-individually. Some sort of occlusion is needed, in order to secure that the treatment is not transferred to other test sites, and this occlusion may lead to different results from those obtained using open application in traditional clinical studies.

Test of novel concepts in the psoriasis plaque test has, so far, been rarely reported. An example of the early testing of a novel concept was recently reported for the target P2Y2 in 26 psoriasis patients [33]. Preclinical experimental data suggested P2Y2 to be a good target for psoriasis, as P2Y2 receptors play a major role in epidermal homeostasis [48]. A compound showing good effects on keratinocytes and neutrophils *in vitro* was identified. Only 75 g of GMP material was used for preclinical safety assessment and for the study. The limited toxicology programme was agreed upon with Medicines and Healthcare Products Regulatory Agency, UK (MHRA), and ethics and regulatory approval were obtained before the study in patients. The conclusion of the study was clear: There was no effect of the new compound on the total symptom score, whereas the reference compounds, corticosteroid and calcipotriol, showed a clear and significant anti-psoriatic effect. These human stop/go data were generated three to five years earlier than would have been possible using a more traditional drug development process.

Regulatory aspects of experimental clinical studies in drug discovery in dermatology

The authorities allow experimental clinical studies preceding that of a traditional development programme without an extensive preclinical package in AD [32] and psoriasis [33] as described above. Additionally, new regulatory guidelines for microdosing have opened up opportunities for testing drugs in experimental clinical studies in patients without the need for a traditional

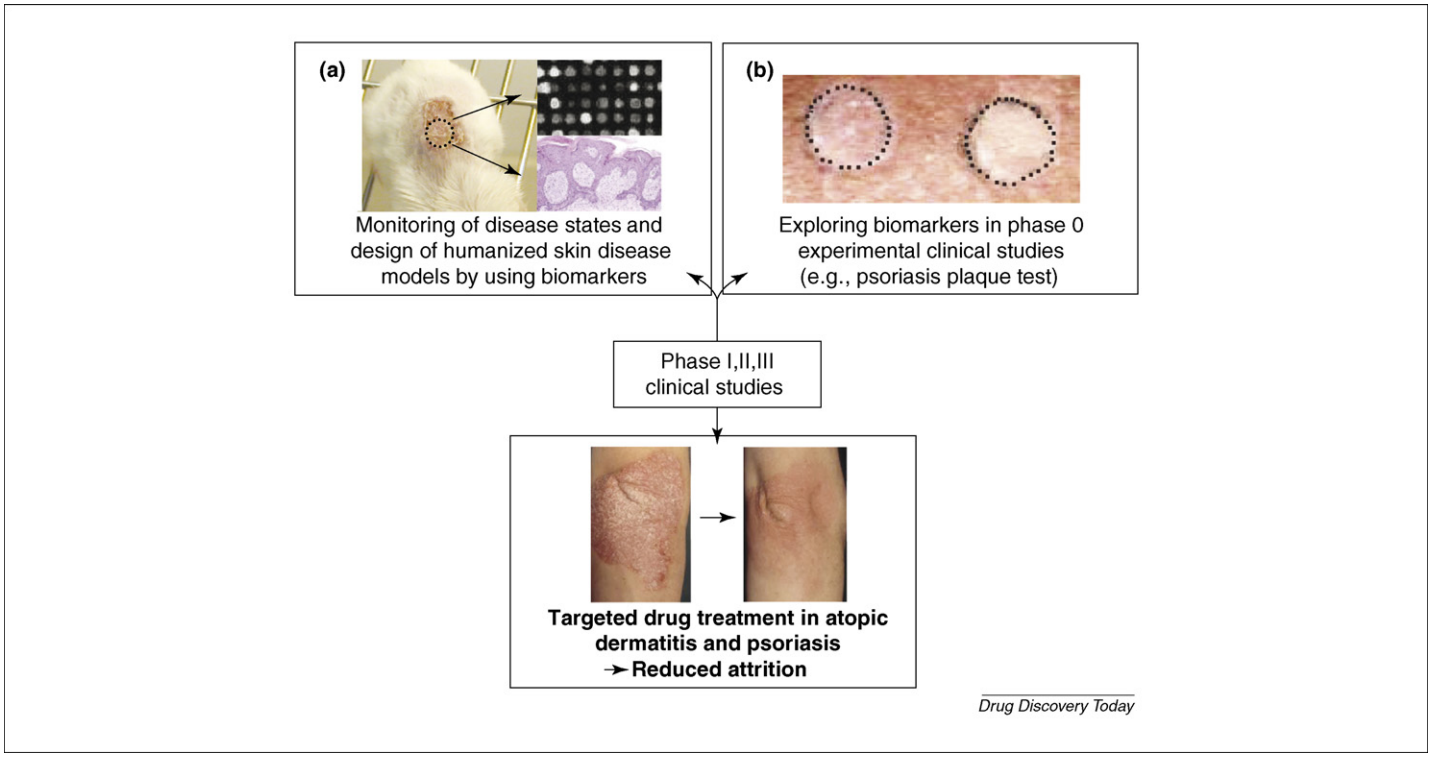

FIGURE 2

Application of humanized xenotransplantation disease models and experimental clinical studies in the context of translational research in drug discovery is an opportunity to reduce failure due to lack of efficacy in clinical development stage in dermatology. **(a)** Design, validation and selection of disease models are based on biomarker data from patients (e.g. the psoriasis SCID mouse model). **(b)** The psoriasis plaque assay: In experimental clinical studies biomarkers can be used to translate preclinical pharmacology to effects observed in experimental clinical studies.

preclinical safety package (FDA guidance on exploratory, investigational new drug application [49] and the position paper 'Non-clinical Safety Studies to support Clinical Trials with a Single Microdose' published by the European Medicines Agency [50]). The primary purpose of these guidelines is to determine pharmacokinetic properties of new drugs, using systemic administration, but at levels so low that one would not expect to see either pharmacological or toxic effects. The principles outlined in the guidelines may be extended to experimental clinical studies with new drugs in dermatology: By administrating the drugs locally to skin, to a limited area (e.g. as in the psoriasis plaque test), it is possible to achieve therapeutically relevant doses and thereby achieve a pharmacological effect and subsequent proof of concept. This can be considered as 'microdosing' using the defined criteria by the authorities, as the systemic exposure following such limited and local administration to the skin is minimal provided that the skin penetration is low. This can be estimated by skin penetration studies *in vitro* [51]. Although to some extent it has been possible to perform such experimental studies for many years, the new guidelines highlight these possibilities, indicating that the authorities endorse these types of studies and view them as important. Thus, such experimental studies would provide an important tool in the drug discovery process for achieving earlier proof of concept than a traditional development programme.

Translational dermatology and beyond

At present there is a situation where the authorities highlight the possibilities for performing experimental clinical studies, and this has only been applied to a very limited extent in drug discovery over the years. Attractive opportunities with regard to translational research within drug discovery for AD and psoriasis can be utilized by integrating the selection and design of animal models, experimental clinical studies together with biomarkers at the molecular and genomic level (Figure 2). The accessibility of the skin as an organ has enabled studies of molecular, cellular and genomic features in tremendous detail in AD [52,53] and psoriasis [52–55] that, in turn, facilitates the identification of disease-related and target-related biomarkers and the discovery of novel therapeutic targets. The disease-related biomarkers, which describe the disease biology, are used to determine if the drug has the desirable effect on the disease. An example of this is the measurement of number of Ki67-positive keratinocytes that is increased in psoriasis. These are reduced in number and normalized following treatment with a p38 MAP kinase inhibitor in the psoriasis SCID mouse model [56]. The target-related biomarkers are used to determine if the drug has the desirable effect on target. An example of this is the measurement of p38 MAP kinase downstream targets that are upregulated in lesional psoriasis [57] and are normalized following treatment with p 38 MAP kinase inhibitors *in vivo* (Kathrine Abell, unpublished data).

Application of disease-related and target-related biomarkers in preclinical disease models will enable us to determine how valid the models are in relation to the specific disease and target, which subsequently will enable the selection of those models that most resemble the human disease. This will improve the predictivity of the animal models in relation to outcome in clinical trials. Furthermore, the molecular and genetic understanding of the human skin diseases makes it possible to produce tailor-made genetically

engineered mice or to establish new improved humanized xenotransplantation models that are even more relevant and predictive for the human skin disease.

In dermatology, clinical endpoints and histopathology are straightforward and relatively cheap to perform and, in general, efficacy of a topical treatment must be observed within two to three weeks in order to have any relevance in clinical practice. Biomarkers at the molecular or genomic level are, therefore, not needed for simple short-term assessment of efficacy unless the experimental studies do not allow for either clinical or histological endpoints to establish efficacy. However, validated biomarkers at the molecular or genomic level are needed in order to conclude on-target pharmacology. If the expected efficacy on biomarkers for disease activity and drug target is absent in early experimental studies, then development can be terminated. Instead, discovery efforts should be directed towards finding alternative molecules with improved chances for having the predicted and required pharmacology in humans. The biomarkers may also reveal improved understanding of mechanism of action of novel or established therapies or be able to identify additional pharmacological effects beyond those expected, leading to the identification of new targets.

The models and studies described herein also provide opportunities for drug discovery outside the indication area of dermatology. Application of humanized xenotransplantation models and experimental clinical studies within psoriasis provide interesting opportunities beyond that of skin diseases in the context of autoimmunity.

Psoriasis is an inflammatory disease that shares immunological and pathological principles with a number of other inflammatory diseases including rheumatoid arthritis, Crohn's disease, systemic lupus erythematosus, multiple sclerosis and juvenile-onset diabetes [54,55,58]. This overlap is observed at the level of biochemical pathways and in relation to the locations of loci mapped by linkage analysis [52,59]. Genes that have been shown to be shared nearly always encode products that regulate immune system activation including PTPN22, SUMO4, TNF-α, IL12B/IL23R, and interleukin cluster on chromosome 1q31–q32 [55]. Overlap is also observed at the therapeutic level, where anti-TNF-α therapies are efficacious in psoriasis [12], rheumatoid arthritis [60] and Crohn's disease [60,61]. This makes psoriasis an attractive indication for exploring new therapeutic concepts for other autoimmune diseases targeting human-specific immune molecules; psoriasis may even be used as a model disease for the development of new treatments for these related indications. Likewise, AD may be considered as a model for diseases with atopic phenotypes including asthma and allergic rhinitis, as regulatory T cells share a similar phenotype characterized by diminished IL-10 production [62] in these diseases together with a significant involvement of eosinophils and mast cells. In addition to this, recent genomic mapping patterns in AD have revealed an overlap with psoriasis disease loci and those mapped for other inflammatory or autoimmune diseases, including Graves' disease, systemic lupus erythematosus and rheumatoid arthritis [63].

Conclusion

In future drug discovery and development, we believe that, the application of biomarkers in humanized xenotransplantation models and experimental clinical studies in the context of translational research will improve experimental disease models. As a result, the models will be more predictive to the clinical outcome and thereby reduce cost for more comprehensive preclinical safety testing and efficacy trials and reduce the failure rate due to lack of efficacy for the benefit of patients and society. Experimental clinical studies must be integrated in the overall drug discovery strategy in the context of translational research at a much earlier stage than the clinical drug candidate, as this will otherwise delay the overall development of a candidate drug for the particular target.

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