Rubor, calor, tumor, dolor: objective assessments of inflammation

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CHAPTER I

INTRODUCTION

Early-stage drug development in the field of dermatology has its challenges. The human skin, functioning as the body's largest organ, orchestrates a complex interplay of cellular interactions to regulate crucial processes such as inflammation, immune responses, wound healing, and angiogenesis.[1] This subtle interplay is disturbed in many inflammatory and autoimmune skin diseases and the development of novel dermatological agents focusses mainly on compounds modulating this disturbance.[2] In the early exploratory stage of clinical drug development, trials are conducted in the absence of clinical information about the drug. Uncertainty about its active dose, regimen and pharmacological activity contribute to a relatively low probability of success, amounting to 13.8% from phase I to market registration across all therapeutic areas. For drugs targeting immunosuppressive and anti-inflammatory processes, the likelihood of success is even lower at 6.3%.[3]

In an effort to increase the probability of success from early phases to market registration, an alternative, more rational approach was proposed, wherein pharmacodynamic properties are evaluated at the earliest clinical stage of drug development.[4] The European Medicines Agency (EMA) underscores the importance of assessing these pharmacodynamic properties, i.e., all possible effects of a drug in the body, including therapeutic effects, adverse effects, and side effects of drugs in the early exploratory stage of clinical drug development.[5] How does this paradigm shift impact early-stage drug development in clinical pharmaco-dermatology? From our perspective, exploring pharmacodynamic properties at such an early stage of drug development raises a few challenges: how to evaluate pharmacodynamic endpoints in early-stage dermatology trials and which specific pharmacodynamic endpoints should be considered for evaluation.

HOW TO EVALUATE PHARMACODYNAMIC ENDPOINTS IN EARLY-STAGE DRUG DEVELOPMENT

Using pharmacodynamic endpoints in early-stage clinical drug development is not new. With the increasing complexity of drugs and poor success rates, even more focus is put on gaining comprehensive insight into the mechanism of action at an early stage. This necessitates the understanding of downstream signalling events in proteins and cells. In the field of immuno-oncology, a notable 74% of phase I studies have integrated pharmacodynamic markers, with 94% of these being blood-based.[6] This trend is also observed in clinical trials focusing on immunological indications, driven by the availability of blood-based assays, a pathway-oriented development, and the wealth of experience gained in the oncology domain.

At the Centre for Human Drug Research (CHDR), a non-profit clinical research institute at the interface between academia and the pharmaceutical industry, objective pharmacodynamic endpoints have extensively been used over the past decade, either in studies in patients or by using pharmacological challenge models. Both pharmacokinetic and quantitative pharmacodynamic endpoints have supported critical decisions in drug development, also in the field of dermatological drug development. For example, antimicrobial peptides have been shown not to improve clinical symptoms of atopic dermatitis as quantified using objective pharmacodynamics.[7]

SKIN CHALLENGE MODELS

One essential issue when evaluating the pharmacodynamic properties of (novel) dermatological agents in an early-stage dermatology trial in healthy volunteers is the absence of a disorder, which can impede the exploration of key aspects of drug development. Inflammation, for example, is a significant factor in various dermatological conditions. Skin inflammation represents an immune response to invading pathogens, skin injuries, and exposure to xenobiotics, microbes, and parasites. Inflammation manifests itself clinically through features such as erythema, pain, heat, and swelling.[8] A range of immune cells from the innate and adaptive systems collaborate to eliminate the invading pathogens. Imbalance among these immune cells can contribute to the development of chronic skin conditions like psoriasis vulgaris, atopic dermatitis, and acne vulgaris.[9–11] Evaluating potential anti-inflammatory agents in healthy volunteers is complicated and necessitates a challenge model capable of mimicking the physiological and pathophysiological processes associated with an inflammatory skin disease. In that case, pharmacological challenge tests are needed to provide unequivocal proof of pharmacological activity.

A range of pharmacological challenge models and tests have been validated in the past years.[12] Except for the UV exposure model, most skin challenge models in dermatology trials share a commonality in the administration of a pharmacological agent to provoke an immune response. An example of intervention using a pharmacological agent is used within the KLH challenge model where researchers have been able to detect dose-dependent responses of a novel monoclonal antibody against OX40 ligand, developed for atopic dermatitis by using a KLH challenge model.[13] However, physical interventions on the skin could also provoke an immunologic and inflammatory response and mimic a dermatological disease, which is illustrated by the UVB exposure model in pain studies and more recently in cutaneous lupus erythematosus.[14] Another

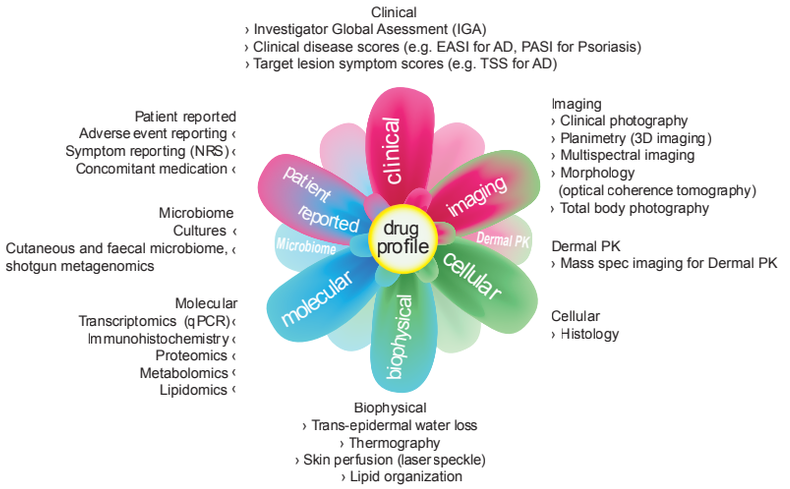
trauma-based challenge model is the wound healing model, which finds application in both preclinical and clinical research.[15,16] Various wound healing models are utilized in humans, including techniques based on tape stripping, induction of blisters or abrasion, laser-induced wounds, split-thickness, and biopsies.[16] In general, techniques in wound healing models can be categorized into two classes: partial thickness (e.g., tape stripping, blister, abrasion, laser-induced wounds, split thickness) and full thickness (e.g., full thickness biopsy techniques). The choice of method depends on the specific research question. Full thickness biopsies necessitate comprehensive dermal, epithelization, and subcutaneous procedures for healing, whereas studies employing partial thickness or blister wounds predominantly focuses on epidermal healing and processes influenced within the epidermis or dermis.

The demonstrated success of skin challenge models in early drug development trials in general is evident. However, CHDR has limited experience with wound healing models, including both partial thickness and full thickness models. Additive to the development of pharmacological skin challenge models, new trauma-based derma-immunological challenges, such as the partial and full thickness wound models, hold substantial promise not only for advancing our understanding of human (patho)physiology, but also increasing our knowledge about pharmacodynamics early in the drug development process.

A BLUEPRINT FOR CONDUCTING EARLY-STAGE DERMATOLOGY TRIALS

There was only limited guidance on how to execute early-stage clinical trials involving innovative topical or systemic drugs situated at the intersection of dermatology and clinical pharmacology. In 2020, a blueprint was proposed outlining the methodology for conducting early-stage clinical pharmacology studies within the field of clinical pharmaco-dermatology.[17] In short, this blueprint highlights five cornerstones capturing the essential facets of an early-stage clinical pharmacology study (Figure 1). These cornerstones entail the exploration of pharmacokinetic and pharmacodynamic properties of a new drug, the inclusion of sensitive and objective endpoints, a multidisciplinary setup, and integrating data from different domains. Rissmann underscores the significance of adopting a multidisciplinary framework, necessitating collaborations with fellow researchers on a global scale. Adhering to the principles outlined in these five cornerstones may enhance the likelihood of early detection of undesirable drug features, whether related to safety, pharmacokinetics, or pharmacodynamics, thereby mitigating the risk of drug failure during pivotal trials.

Figure 1 Blueprint for conducting early-stage clinical pharmacology studies in the field of clinical pharmaco-dermatology.



WHICH PHARMACODYNAMIC ENDPOINTS TO CONSIDER

Although the number of novel techniques to assess the pharmacodynamic properties of a (novel) dermatological agent has expanded in the field of dermatology, pivotal dermatology trials are dominated by physician-evaluated scores. Clinical efficacy scales, such as the Eczema Area and Severity Index (EASI) for atopic dermatitis (AD), Psoriasis Area and Severity Index for psoriasis vulgaris (PV) and inflammatory lesion count for acne vulgaris (AV) or investigator global assessments, play a vital role in the assessment of drug efficacy in early-stage dermatology trials. Irrespective of the specific dermatological disease, clinical efficacy scales are based on the number of lesions, the area affected, and the severity of the disease, often classified based on color. Most of these scores use discrete numbers from 0-3 (e.g., EASI). Despite efforts towards objectivity, most clinical scores necessitate a clinical judgment from the physician. The subjective nature of clinical scores, influenced by factors such as clinical training, color perception, and even ambient lighting conditions, can impact the physician's judgment. Their limited objectivity, potential inter-rater variability and lack of sensitivity might compromise unbiased conclusions, while assessments to evaluate the pharmacodynamic properties of a (novel) compound ought to be as valid, consistent, accurate, reproducible, and error-free as possible.

Typically, clinical efficacy scales find application in pivotal phase 3 trials involving large patient cohorts. These outcomes are often associated with the prevention or decrease in symptoms, such as erythema, scaling, or itch. Detecting significant effects on such endpoints necessitates trials with large sample sizes and prolonged follow-up periods. Incorporating clinical scores into phase I research might lead to trials with inadequate sample size and short follow-up periods, precluding robust statistical analyses. Consequently, phase I studies are often underpowered to decisively approve or reject a compound for further development.

To gain more rigorous insight into the pharmacodynamic effects of a novel dermatological agent, objective outcome measures are needed. For example, monitoring microcirculation can provide insights into the severity, progression, and response to treatment of various skin diseases.[18] Microcirculation is just one aspect of skin health; the comprehensive evaluation of skin responses to dermatological therapeutics may involve multiple biomarkers. Lack of insight into the mechanisms and processes of wound healing, for example, has hindered the development of new interventions and therapies so far.[19,20]

A biomarker refers to a broad subcategory of medical signs of clinically significant patient outcomes that can be accurately and reproducibly measured.[21] Ideally, a parametric and accurate biomarker of the pharmacological influence of a critical pathophysiological process is available to reflect the pharmacodynamic effect of a therapeutic agent. Given the intricate interplay of physiological processes in human skin, including inflammation, immune responses, wound healing, and angiogenesis, these processes offer valuable insights into assessing the pharmacodynamic impact of therapeutic treatments. Physiological processes, exemplified by erythema, wound healing, barrier function, and skin surface biomarkers, present opportunities to identify readily applicable biomarkers in early-stage dermatology trials. However, the challenge remains in identifying biomarkers accurate enough to reflect the pharmacological influence of critical pathophysiological processes and determining the tools capable of precise measurement.

OBJECTIVE MEASURES OF PHARMACODYNAMIC ENDPOINTS IN DERMATOLOGICAL DRUG TRIALS

The selection of an appropriate pharmacodynamic endpoint is of paramount significance in the context of early-stage drug trials. Recent advancements in the development and application of digital tools, imaging techniques and combinations of serum biomarkers have substantially advanced the realm of objective measures as pharmacodynamic endpoints in dermatology trials.

A rapidly growing list of tools is currently available for the comprehensive characterization of drug effects in the individual patient. The earlier-introduced blueprint on how to perform early-stage dermatological drug trials also offers guidance on how to include different sensitive and objective endpoints and integrate data from different domains, assessed by multiple techniques. The so-called 'DermaToolbox' encompasses innovative methodologies applicable in early-stage drug development trials, introducing a multidimensional approach spanning clinical, imaging, biophysical, molecular, cellular, microbiome, and physician- and patient-reported biomarkers. Digital indices, for instance, demonstrated by the digital Psoriasis Area and Severity Index (PASI) widely used in psoriasis trials, and the digital Eczema Area and Severity Index (EASIdig) in atopic dermatitis trials, have succeeded their non-digital counterparts.[22]

Transepidermal water loss (TEWL) quantifies the amount of evaporated water moving through a fixed area of the stratum corneum to the skin surface within a given time frame. TEWL has become the most widely used objective measurement for evaluating the barrier function of skin in healthy individuals and in patients with skin diseases linked to skin barrier dysfunction, such as atopic dermatitis.[23]

In addition, the spectrum of imaging techniques has expanded considerably over the years.[24] Examples are stereophotogrammetry, Optical Coherence Tomography (OCT) and Laser Speckle Contrast Imaging (LSCI). Stereophotogrammetry, also known as 3D photography, involves the simultaneous acquisition of two images of the lesion from slightly varied angles, allowing creation of a three-dimensional reconstruction. From this, a range of measurements can be obtained, including lesion dimensions, properties, and surface features.[25] OCT is applied for high-resolution, *in vivo* imaging of skin structure and vasculature to quantify and monitor inflammation in conditions like psoriasis and atopic dermatitis,[26] while measurements performed via LSCI are based on the dynamic change in backscattered light because of interaction with red blood cells; LSCI is used to objectively quantify perfusion in inflammatory skin lesions.[27] It is evident that non-contact and reliable imaging modalities hold promise in detecting slight changes that may often go unnoticed by the naked human eye.

PROBLEM STATEMENT

In summary, evaluating the pharmacodynamic properties of a dermatological agent seems indisputable for well-informed decision-making in the early stage of drug development. Despite the multitude of early-stage drug trials incorporating pharmacodynamical endpoints, there seems to be a lack of understanding how to integrate pharmacodynamic outcomes into early-stage dermatological

drug trials. Success stories from investigations employing objective pharmacodynamic endpoints in both disease and challenge models underscore the feasibility of integrating such endpoints during the initial clinical stages of drug development. The predominant focus of studies in the earlier stages of drug development remains on healthy volunteers, with challenge models being unequivocally endorsed in most cases. The introduction of novel trauma-based derma-immunological skin challenges, including partial and full-thickness wound models, holds promise for enhancing our comprehension of dermatological physiological processes and advancing early-stage pharmacodynamic knowledge.

Considering the presumed limitations in the objectivity of clinical efficacy scores, we advocate a more objective approach that incorporates imaging techniques and precise biomarkers as pharmacodynamic endpoints in early-stage dermatology trials. While outcomes reported by physicians and patients offer clinically relevant information and can be integrated into routine dermatologic practice and pivotal phase 3 dermatology trials, their applicability in early-stage trials is limited. There is a great need for more reliable and objective outcome measures, given the increasing development of compounds for dermatological-immunological disorders.

AIMS AND OUTLINE OF THIS THESIS

This thesis describes how pharmacological and trauma-based derma-immunological challenges are developed and how objective pharmacodynamic endpoints are evaluated in early-stage trials in the field of clinical pharmaco-dermatology. **Section I** of this thesis discusses the development of trauma-based derma-immunological challenges and how these models support the characterization of different immunological pharmacodynamic processes. **Chapter 2** covers the characterization of a cutaneous wound model to objectively test novel wound healing treatments. In this chapter, three- and four-mm full thickness punch biopsies were taken on the lower back of healthy volunteers and left to heal without intervention. In **chapter 3**, a suction blister model is developed to characterize epidermal wound healing. By using negative pressure, skin blisters were induced over approximately two hours. After removal of the epidermal sheet blisters were left untreated in the observational study and treated with a novel topical cream. The characterization of pharmacodynamic endpoints after the development of pharmacological challenge models is discussed in **section II**. **Chapter 4** describes the study of an intradermal challenge with substance P in healthy participants to understand and distinguish between wheal and flare responses following various substance P doses. In **chapter 5**, the pharmacodynamic activity of IRAK4 inhibitors is evaluated and characterized after inducing an inflammatory response via the topical application of imiquimod and systemic application of lipopolysaccharide. **Section III** illustrates how pharmacodynamic endpoints can be incorporated in a classical phase I feasibility and dosing study about microneedles. In **chapter 6**, we pre-explored the nature and extent of impact of repeated methotrexate injections via microneedles in children. In **chapter 7**, we investigated the feasibility of intradermal administration of adalimumab via hollow microneedles and conventional needles and evaluated the effects on percutaneous perfusion in a classical phase I feasibility and dosing trial. **Chapter 8** summarizes the results of all chapters and highlights future avenues for early-stage drug development in dermatology.

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