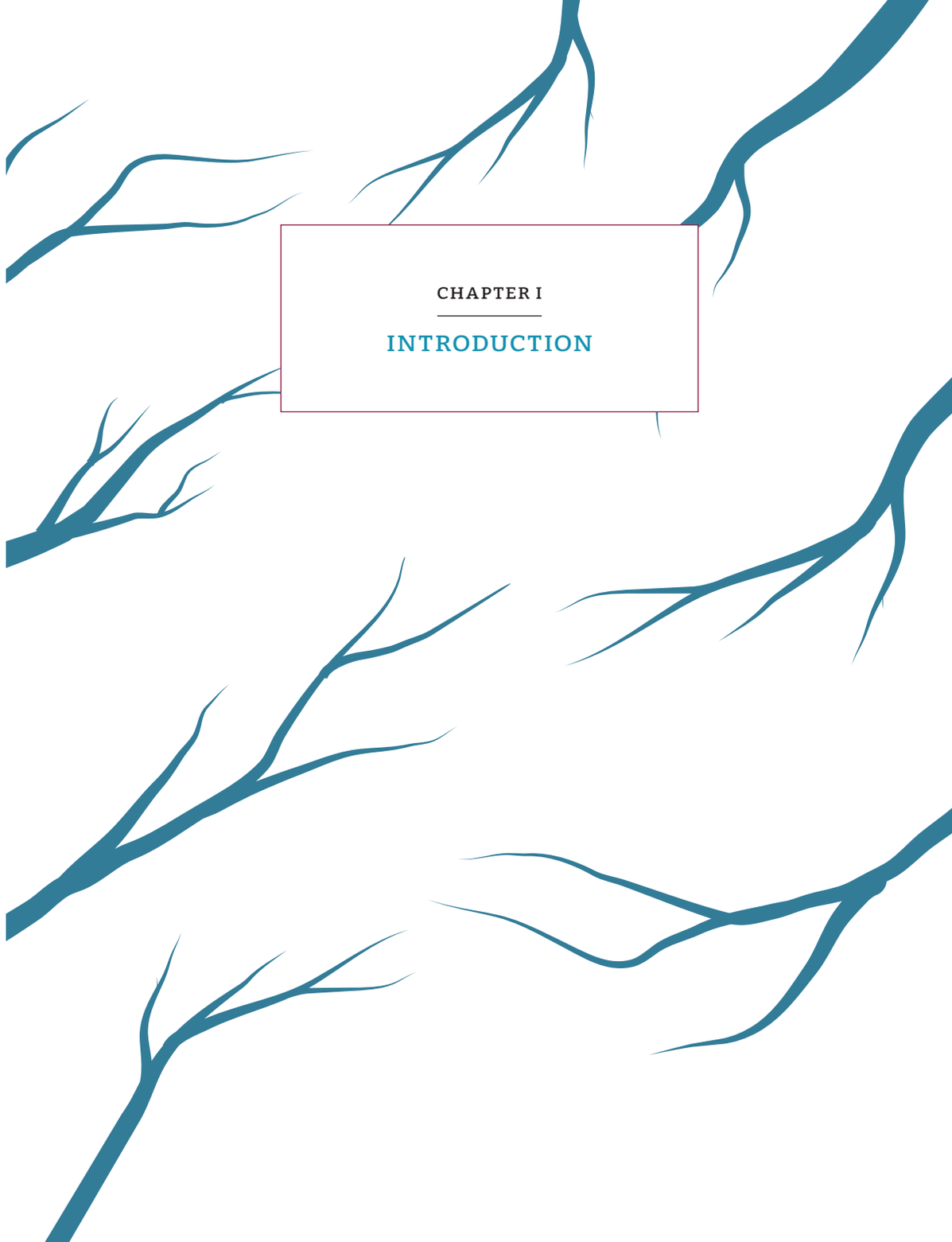
Lost in translation: the toll-like receptor 7 induced pharmacological challenge model of the skin

Assil, S.



CHAPTER I

INTRODUCTION

EARLY PHASE CLINICAL DRUG DEVELOPMENT

Drug development is an extensively well-established but resource-demanding, and lengthy process. The gap between drug discovery and market availability is generally estimated to span between 10 to 15 years.[1,2] Generally, the final stage in these programs is centred around clinical trials testing drug candidates. The conventional approach for clinical trials that is used by pharmaceutical industry, was established in the early 1960's as a guideline by FDA and divides clinical trials into four phases, i.e., phase I-III and a post- marketing phase (IV).[3] In this approach, pharmacokinetics, safety, and tolerability are generally prioritized as primary objectives in phase I/II trials.[4] However, with the current approach, insufficient or delayed early information is obtained on the pharmacodynamics and efficacy of the drug that is studied. This limitation can lead to high attrition rates reaching as high as 90% across all phases, with 55% attributed to lack of efficacy in phase 2/3, *Figure 1*.

As a consequence, drug candidates fail to progress to the market resulting in high losses of investment.[5-7] Implementing biomarkers into clinical trials, with the goals of patient selection, enhancing safety, and serving as a surrogate clinical endpoint, has resulted in a twofold increase in the probability of success (POS) compared to trials that do not utilize biomarkers (POS 10.3% versus 5.5%). While the use of biomarkers improves the POS in all phases, the effects were more pronounced in phase I and II which is also the case in the field of autoimmune and inflammation therapeutics.[7] Nonetheless, despite the numerous advantages of incorporating a biomarker into a clinical trial, it also comes with certain drawbacks. Making a go/no-go decision regarding a biomarker at the end of a phase I trial is only reasonable when the biomarker's predictive accuracy is established at 93.4%.[8] Hence, improvement is necessary, e.g., by augmenting the primary study objectives with research goals that specifically examine fundamental aspects of the drug's properties, such as its ability to reach the intended site of action and its target engagement, commonly known as proof-of-mechanism studies, which can be integrated into phase I/II trials.[9,10]

Proof-of-mechanism studies focus on the initial validation of the appropriateness of a target in a specific population (volunteers or patients), the most effective dosage regimen, and the duration of treatment allowing to minimize the resource wastage.[11] Selecting an appropriate patient population is vital for this type of studies. However, the absence of a disorder, e.g. an inflammatory condition, in healthy volunteers may impede the examination of these hallmarks. To overcome this problem, pharmacological challenge models or experimental models in humans can be established that temporarily mimic components of physiological and pathophysiological conditions. Such models are currently minimally available but can potentially be of great importance, e.g., in the field of immune-mediated inflammatory diseases (IMID).

INFLAMMATION AND PHARMACOLOGICAL CHALLENGE MODELS

Inflammation is the first response of our body to pathogens and ancient physicians practicing Ayurvedic medicine in the Indian peninsula, as early as 1500 BC, possessed prior knowledge of this phenomenon.[12,13] Aulus Celsus described the four characteristics of inflammation around 30 BC namely rubor (erythema), calor (increased heat), dolor (pain) and tumor (swelling), while Galen subsequently, in the third century, introduced the fifth sign, functio laesa (loss/disturbance of function) in the affected tissue/organ. Altogether, the four characteristics served as fundamental hallmarks referring to an acute inflammatory response while loss of function is an universal sign that accompanies all inflammatory processes.[14-16] Currently, inflammation is defined as the physiological reaction of the body to injury or infection, wherein the release of chemical mediators initiates an immune response aimed at contesting infections or enabling restoration of the impaired tissue.[17,18]

The prolonged continuation of inflammation over an extended period frequently contributes to the development of diverse chronic inflammatory conditions including auto-inflammatory diseases and IMID, which very often have cutaneous manifestations. The incidence of these kind of (skin) diseases in Europe is rising and the prevalence remains high with poor quality of life and major impact on socioeconomic burden.[19-21] The prevalence for diseases such as atopic dermatitis (5.5%), alopecia (5.8%), psoriasis (2.9%), chronic urticaria (1.4%) and cutaneous lupus erythematosus (0.065 - 0.85%) is quite high, whilst therapeutic options are still limited.[22-25] This highlights that there is a high medical need for novel therapeutics. Currently, a growing array of therapeutic agents is being explored in this field, with e.g., over 70 novel compounds specifically for atopic dermatitis in the development phase.[26] Despite this increase, being a direct result of improved understanding regarding the fundamental mechanism of the disease, drug development still is a lengthy process often spanning up to a decade and beyond from bench to bedside.[1,2] Therefore, innovative strategies are required to optimize and accelerate the process of drug development for bringing clinically effective therapies to the patients in need. Hence, the development of a robust challenge model for skin inflammation can be an important step in the optimization and acceleration process.

One of the pharmacological skin challenge models that has demonstrated high potential in preclinical mice studies is a model with imiquimod (IMQ) application. This model shows clinical features similar to psoriasiform lesions, but also activates immunological pathways that are of importance for various other auto-inflammatory/immunity diseases, such as type I interferonopathies (e.g., cutaneous lupus erythematosus).[27-31] A fully characterized translation of this model to humans has not yet been accomplished. Therefore, the objective of this thesis is I) to set up an experimental imiquimod challenge model in healthy volunteers, based on murine models, II) to suppress the IMQ-induced response with the anti-inflammatory prednisolone and III) to test applicability of the model with a novel investigational drug.

IMIDAZO[4,5-C]QUINOLINES

IMIQUIMOD Imiquimod or 1-(2-methylpropyl)-1H-IMIDazo[4,5-c]quinolin-4-amine (*Figure 2A*) is a synthetic, small molecule with a molecular weight of 240.3 g/mol, logP (2.65 – 2.83) that functions as an immunomodulating agent.[32] This small sized, highly hydrophobic nucleoside analogue is the active ingredient in Aldara® 5%, a topical cream marketed by 3M Pharmaceuticals in 1995 for the treatment of external genital warts and perianal warts and in 1997 for actinic keratosis, and superficial basal cell carcinoma.[33-37] IMQ is available on the market as a 5% cream, however it is also available in lower concentrations, 2.5% and 3.75% (Zyclara). IMQ acts as a Toll like receptor (TLR)7 agonist (TLR7, EC50=10.7 µM) and primarily activates the innate immune pathways followed by adaptive upon topical application, leading to the production of cytokines.[38,39] Whilst imiquimod's potential antiviral, antitumor, and immunoregulatory effects make it a compelling choice for the treatment of a diverse range of dermatologic conditions, it has also been used as a challenge agent in mice and rats to induce inflammation and psoriasiform lesions.[28,31,40]

RESIQUIMOD Resiquimod or 4-amino-2-ethoxymethyl-α,α-dimethyl -1H-IMIDazo[4,5-c]quinolin-1-ethanol (*Figure 2B*) is a synthetic small molecule with molecular weight of 314.4 g/mol, logP (1.72-2.24) and was also developed by 3M Pharmaceuticals. While belonging to the same chemical family as imiquimod, resiquimod can activate both TLR7 (EC50=1.5 ± 0.3 µM) and TLR8 (EC50=4.5 ± 3.2 µM) and therefore also being a good candidate for the treatment of genital herpes and actinic keratosis.[39,41] However, unlike imiquimod, resiquimod has never reached market approval due to inconsistent results in clinical trials.[42]

MECHANISM OF ACTION TLR7/8 AGONISTS Currently, there has been significant progress made in understanding the mechanism of action of imiquimod (Aldara®) and resiquimod. Recent evidence suggests that the main pathway is TLR dependent for both IMQ and resiquimod. The

other two inferior pathways following IMQ application are related to inflammasome activation and inhibition of the adenosine receptor.[27,41,43-45] The main pathway which is TLR-dependent leads to IMQ or resiquimod engaging with the pathogen recognition receptors (PRR) such as TLR7 and 8 respectively, that are predominantly expressed among other cells in humans on plasmacytoid dendritic cells (PDCS) and myeloid dendritic cells (MDCS), thereby activating the signal transduction cascade downstream of these receptors.[46,47] These receptors belong to the family of transmembrane glycoproteins containing an ectodomain of leucine- rich motifs allowing involvement in recognition of certain components of microbes. Furthermore, TLRs also have a transmembrane domain and a cytoplasmic tail domain that is primarily responsible for the initiation of intracellular cascades.[48-54] Upon binding of IMQ or resiquimod to TLR7 and TLR8, the TLR dimerizes and undergoes conformational changes resulting in recruitment of the adapter protein called myeloid differentiation primary response 88 (MYD88) which in turn interacts with members of interleukin (IL) 1 receptor associated kinases (IRAK) protein kinase family via death domain. This leads to phosphorylation of IRAK4 which activates IRAK1.[55] Upon phosphorylation of these two kinases, dissociation from MYD88 occurs leading to interaction with tumour necrosis factor (TNF) receptor associated factor (TRAF)6 with E3 ubiquitin ligase activity.[56] This formed scaffold phosphorylates the inhibitor of κB (IκB) – protein with a primarily function of keeping nuclear factor kappa B (NF- κB) in the cytoplasm - and therewith degrades IκB promoting translocation of NF- κB to the nucleus.[57] Activation of this downstream pathway is important in an early immune response such as secretion of pro-inflammatory cytokines including, TNF, IL-1, IL-1RA, IL-6, and IL-8[58-61] , *Figure 3.* Furthermore, the formed scaffold activates the AP-1 family transcription factors via phosphorylation of the mitogen- activated protein kinase (MAPK) pathway leading to production of interferon (IFN)β and TNF. Additionally, IRAK1 via TRAF3 interacts with interferon regulatory factor (IRF) 7 which is highly expressed in specific cell subsets among which PDCs. Phosphorylation of IRF7 shifts into the nucleus and triggers the production of type 1 IFNs, such as IFN-α and IFN-β.[62-64]

The second pathway is TLR-independent and presumably Aldara indirectly activates the inflammasome via NLRP3, which also triggers activation of caspase 1 and leads to pyroptosis accompanied by the secretion of IL-1β and IL-18.[43,65] An alternative pathway of Aldara® is also TLR- independent and involves the activation of adenosine receptors. Imiquimod selectively binds to the A_1 and A_2A subtypes of adenosine receptors and potentially exerts an antagonistic effect on these receptors.[44,45,66] This results in the inhibition of adenyl cyclase activity, which, in turn, prevents the conversion of adenosine monophosphate to cyclic adenosine monophosphate, and allows for the unimpeded transcription of proinflammatory cytokines, such as TNF, IL-4 and IFN-γ. This cascade potentially contributes to augmentation of inflammation synergistically of the TLR-mediated proinflammatory activity.

IMIQUIMOD IN (PRE)CLINICAL STUDIES

Initially imiquimod was proposed as a challenge agent to drive psoriasis-like inflammation in mice, in addition to its marketed applications in the treatment of basal cell carcinoma and actinic keratosis, by *van der Fits et al.* In this research, IMQ was applied to the shaved back and the right ear for 5 to 6 consecutive days resulting in a daily dose of 62.5 mg. Within 2-3 days of application, the first clinical inflammation symptoms such as erythema, scaling and skin thickening became apparent. The epidermal thickening was also confirmed with Hematoxylin and Eosin (H&E) stained sections, which also showed increased numbers of dermal DCs, PDCs, neutrophils, and T cells. On a molecular level, elevated levels of IL-17/IL-23 measured by quantitative polymerase chain reaction (QPCR) were observed. Furthermore, a significant 2-fold spleen enlargement was observed after mice have been subjected to 5 to 6 days of IMQ application with increased percentages of macrophages, DCs and PDCs.[31] These findings indicate that topical IMQ application in mice leads to systemic effects.

Subsequently the mouse model of psoriasis-like skin inflammation gained popularity and has been utilized in more than 150 preclinical published studies.[29,67] This model offers several benefits, including its

ease of implementation, cost-effectiveness, and ability to induce acute skin inflammation.[68-70] However, it is not without drawbacks. Some of the disadvantages include unintended systemic effects from topical treatment, its excessive usage with limited validation studies, histological misinterpretation, and its limited representation of various aspects of human psoriasis.[29,70-75] Another challenge is the difficulty in translatability to humans given the presence of interspecies differences. In general, human and murine skin share similar cellular compositions in the dermis and epidermis, but they primarily differ in thickness. Murine skin is characterized by thin (less than 25 µM) and loose structure while human skin is firm and thick (100 µM) and is firmly adhering to the underlying tissues with 5-10 layers of the epidermis, compared to murine which has only 2-3 layers.[76-80] Beyond the morphological distinctions, there are also significant immunological differences between human and murine skin. For instance, human skin contains the cytokine IL-8, which is absent in murine skin.[81] This particular cytokine plays a crucial role in neutrophil attraction in humans and is secreted upon activation of the TLR7 receptor and involvement of the NF-κB pathway. However, since mice lack this cytokine, its absence, as well as the absence of potentially other cytokines, may lead to discrepancies between the expected mode of action of IMQ and the observed response in mice. This highlights the necessity for achieving translatability of the IMQ model, ensuring that the findings from mouse studies can be effectively translated and applied to humans.

IMQ has not earlier been tested as a challenge compound in humans, however the safety and efficacy were tested extensively in healthy volunteers and in patients. Furthermore, it has proven to be effective for the treatment of certain viral infections such as perianal and genital warts, superficial basal cell carcinoma, superficial squamous cell carcinoma, actinic keratosis, and certain superficial malignant melanomas.[82] Application of IMQ depends on the skin disease, however in general, patients are exposed to 5% IMQ at least three to four times per week for a minimal period of four weeks.[83] While topically applied IMQ has shown to be locally effective, neglectable amounts (<0.9% of the dose) of the compound after single dose reaching systemic circulation were reported.[84] Following topical application of IMQ local skin reactions were observed including local pruritus, burning sensation, erythema, excoriation, and oedema. In general, the drug appears to be well-tolerated.[85-90]

OBJECTIVE CHARACTERIZATION OF (SKIN) INFLAMMATION

The use of validated biomarkers in the context of clinical drug development has the potential to yield significant time and cost saving by obtaining early validation of pharmacological effects or "proof- of-pharmacology". We define a biomarker as objectively measurable characteristics that serve as indicators of normal biological processes, pathological processes, or pharmacological response to an intervention.[91]

In the fields of immunology and dermatology, many biomarkers and endpoints that are used in clinical settings provide only one-dimensional information. For instance, in the context of skin inflammation, biopsies are considered the conventional method for obtaining information about the cellular and molecular process within the skin.[92,93] Moreover, the impact on the skin's surface is frequently visualized using imaging techniques to gain valuable insights into erythema.[94] Whilst these assessments are commonly used in clinical settings they offer only limited, one-dimensional information on skin inflammation, despite its multifaceted nature as a complex biological process. Hence, there is need for a multimodal characterization of skin inflammation. To address this need, we introduce a novel and comprehensive approach covering different domains, i.e. imaging, biophysical, molecular and cellular and physicians score, *Figure 4*. The goal of this approach is to have a more complete profile regarding skin inflammation that can be used to characterize drug specific effects in early phase clinical trials.

AIMS AND OUTLINE OF THIS THESIS

The primary aim of this thesis is developing and characterizing a mechanistic model to investigate skin inflammation on a mechanistic basis in healthy volunteers by applying imiquimod, for utilization in drug development programs. Extensive characterization of the inflammatory response to imiquimod is performed using an array of assessments focusing on imaging based, biophysical, cellular, and molecular changes.

In **chapter 2**, we broaden the scope and present a comprehensive overview of other challenge agents for mechanistical models in healthy volunteers studying inflammation, pruritus and models that target the adaptive immune response.

Chapter 3 describes the development of a temporary skin inflammation model in healthy volunteers. Cutaneous inflammation in this model is induced by the topical TLR7 agonist IMQ (Aldara®).

In **chapter 4** of this thesis, a clinical study is performed to investigate whether the immune response driven by IMQ could be suppressed. This study involves the administration of a registered anti-inflammatory drug, i.e. oral prednisolone compared with a placebo. In addition, a technique called suction blistering is implemented in this study to further characterize the molecular and cellular aspects of the immune response.

The objective of **chapter 5** is to conduct a translational study that further studies the IMQ-induced skin inflammation in healthy volunteers by comparing short and long IMQ exposure. The study aims, amongst other objectives, to determine whether the complement system played a role in the underlying mechanisms associated with the induced skin inflammation as earlier described in preclinical studies and if prolonged IMQ exposure enhances the cellular effect. Also, this chapter attempts understanding the role of neutrophil in this pharmacological model. Furthermore, we aim to gain a deeper comprehension of the potential translational gap that exists in this model.

Chapter 6 predominantly centres on the application of the IMQ model, in combination with a novel drug candidate called omiganan, belonging to the group of cathelicidins, to examine if application of both compounds results in synergy of the IMQ-induced inflammatory response, as evidenced from in vitro studies.[95]

Lastly, **chapter 7** provides a synthesis of the main findings of this thesis, accompanied by a general discussion and recommendations regarding suitability of the model for future drug development programs.

Figure 1 Process of conventional drug discovery and the failure rate at each step. *Created with BioRender.com*

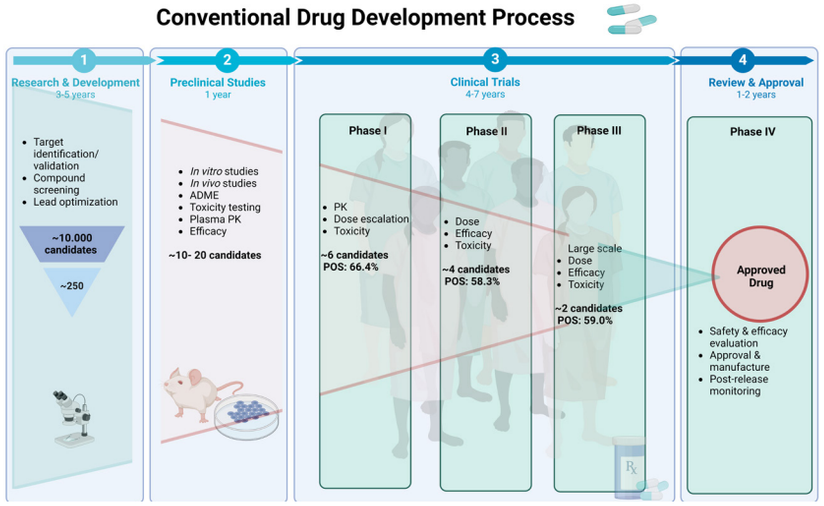
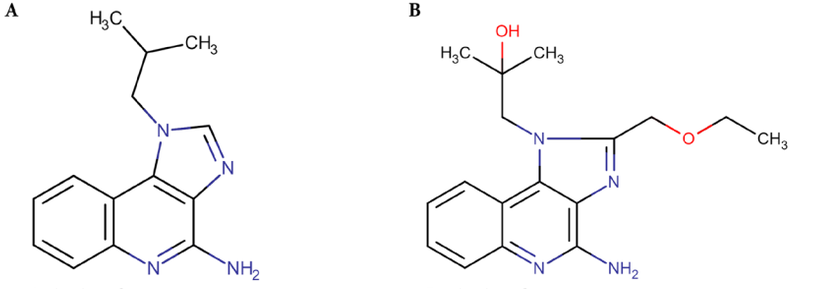


Figure 2 Chemical properties of imiquimod and resiquimod. *Created with BioRender.com*



Chemical properties:
- Molecular weight: 240.3 g/mol
- Target: TLR7 (EC50 = 10.7 µM)
- logP: 2.65 – 2.83

Chemical properties:
- Molecular weight: 314.4 g/mol
- Target: TLR7 (EC50=1.5 ± 0.3 µM) and TLR8 (EC50=4.5 ± 3.2 µM)
- logP: 1.72 - 2.24

Figure 3 Potential mechanism of TLR7 agonists: IMQ and resiquimod. (I) MY-D88 dependent pathway. (II) Via NLRP3. (III) MY-D88 independent pathway via adenosine receptor. *Created with BioRender.com.*

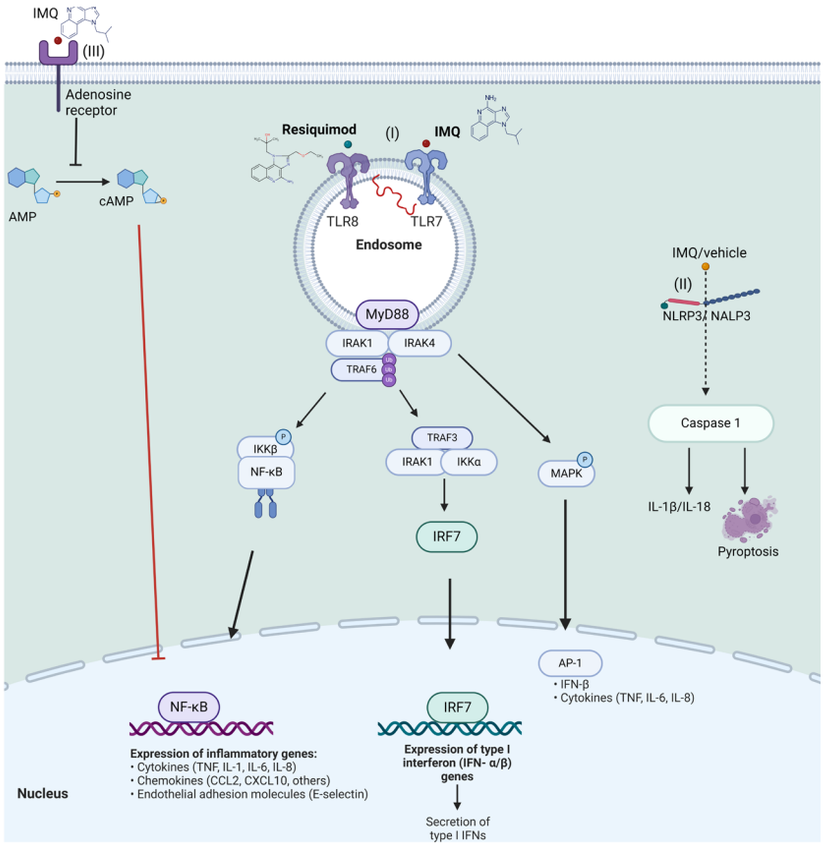
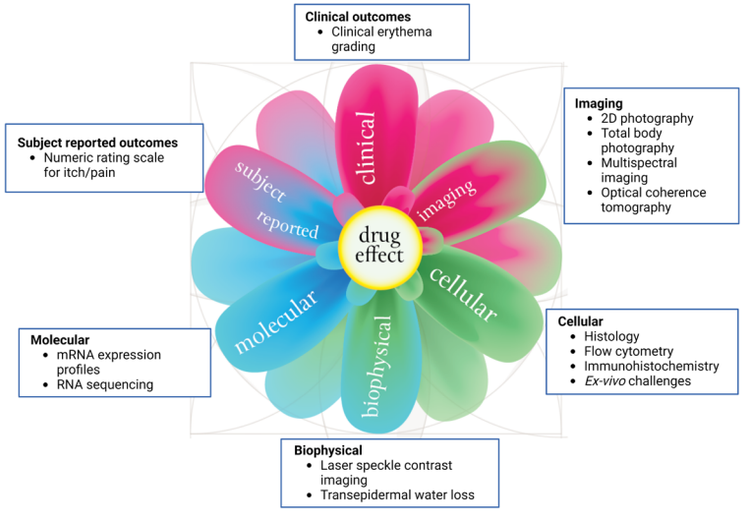


Figure 4 Multimodal approach to characterise drug effect. Derma flower created by F. van Meurs, adapted for this chapter with BioRender.com



REFERENCES

1 Hinkson, I. V., Madej, B. & Stahlberg, E. A. Accelerating Therapeutics for Opportunities in Medicine: A Paradigm Shift in Drug Discovery. Front. Pharmacol. 11, 1–7 (2020).

2 Sun, D., Gao, W., Hu, H. & Zhou, S. Why 90% of clinical drug development fails and how to improve it? Acta Pharm. Sin. B 12, 3049–3062 (2022).

3 Stuart J. Pocock. Clinical trials. Cancer vol. 33 (1989).

4 Umscheid, C. A., Margolis, D. J. & Grossman, C. E. Key concepts of clinical trials: A narrative review. Postgrad. Med. 123, 194–204 (2011).

5 Avorn, J. The $2.6 Billion Pill — Methodologic and Policy Considerations. N. Engl. J. Med. 372, 1877–1879 (2015).

6 Hwang, T. J. et al. Failure of investigational drugs in late-stage clinical development and publication of trial results. JAMA Intern. Med. 176, 1826–1833 (2016).

7 Wong, C. H., Siah, K. W. & Lo, A. W. Estimation of clinical trial success rates and related parameters. Biostatistics 20, 273–286 (2019).

8 Hurko, O. & Jones, G. K. Valuation of biomarkers. Nat. Rev. Drug Discov. 10, 253–254 (2011).

9 Cohen, A. F., Burggraaf, J., van Gerven, J. M. A., Moerland, M. & Groeneveld, G. J. The Use of Biomarkers in Human Pharmacology (Phase I) Studies. Annu. Rev. Pharmacol. Toxicol. 55, 55–74 (2015).

10 Hijma, H. J. & Groeneveld, G. J. Analgesic drug development: proof-of-mechanism and proof-of-concept in early phase clinical studies. Med. Drug Discov. 10, 100083 (2021).

11 Campbell, C. M., Gilron, I., Doshi, T. & Raja, S. Designing and conducting proof-of-concept chronic pain analgesic clinical trials. Pain Reports 4, (2019).

12 Pole, S. Ayurvedic Medicine. Ayurvedic Med. 86, 75–89 (2006).

13 Garodia, P., Ichikawa, H., Malani, N., Sethi, G. & Aggarwal, B. B. From ancient medicine to modern medicine: Ayurvedic concepts of health and their role in inflammation and cancer. J. Soc. Integr. Oncol. 5, 25–37 (2007).

14 Granger, D. N. & Senchenkova, E. Inflammation and the Microcirculation. (2010).

15 Silva, M. R. e. A brief Survey of the History of Linguistics. Course Gen. Linguist. 8, 86–90 (1978).

16 Chen, L. et al. Oncotarget 7204 www.impactjournals.com/oncotarget Inflammatory responses and inflammation-associated diseases in organs. Oncotarget 9, 7204–7218 (2018).

17 Scott, A., Khan, K. M., Cook, J. L. & Duronio, V. What is 'inflammation'? Are we ready to move beyond Celsus? Br. J. Sports Med. 38, 248–249 (2004).

18 Bennett, J. M., Reeves, G., Billman, G. E. & Sturmberg, J. P. Inflammation-nature's way to efficiently respond to all types of challenges: Implications for understanding and managing 'the epidemic' of chronic diseases. Front. Med. 5, 1–30 (2018).

19 Pärna, E., Aluoja, A. & Kingo, K. Quality of life and emotional state in chronic skin disease. Acta Derm. Venereol. 95, 312–316 (2015).

20 Lundberg, L., Johannesson, M., Silverdahl, M., Hermansson, C. & Lindberg, M. Health-related quality of life in patients with psoriasis and atopic dermatitis measured with SF-36, DLQI and a subjective measure of disease activity. Acta Derm. Venereol. 80, 430–434 (2000).

21 Hong, J., Koo, B. & Koo, J. The psychosocial and occupational impact of chronic skin disease. Dermatol. Ther. 21, 54–59 (2008).

22 Flohr, C. & Hay, R. Putting the burden of skin diseases on the global map. Br. J. Dermatol. 184, 189–190 (2021).

23 Richard, M. A. et al. Prevalence of most common skin diseases in Europe: a population-based study. J. Eur. Acad. Dermatology Venereol. 36, 1088–1096 (2022).

24 Bieber, T. Mechanisms of disease: Atopic dermatitis. N. Engl. J. Med. 358, 1483–1494 (2008).

25 Jarukitsopa, S. et al. Epidemiology of systemic lupus erythematosus and cutaneous lupus erythematosus in a predominantly white population in the United States. Arthritis Care Res. (Hoboken). 67, 817–828 (2015).

26 Bieber, T. Atopic dermatitis: an expanding therapeutic pipeline for a complex disease. Nat. Rev. Drug Discov. 21, 21–40 (2022).

27 Flutter, B. & Nestle, F. O. TLRs to cytokines: Mechanistic insights from the imiquimod mouse model of psoriasis. Eur. J. Immunol. 43, 3138–3146 (2013).
28 Gudjonsson, J. E., Johnston, A., Dyson, M., Valdimarsson, H. & Elder, J. T. Mouse models of psoriasis. J. Invest. Dermatol. 127, 1292–1308 (2007).
29 Hawkes, J. E., Gudjonsson, J. E. & Ward, N. L. The Snowballing Literature on Imiquimod-Induced Skin Inflammation in Mice: A Critical Appraisal. J. Invest. Dermatol. 137, 546–549 (2017).
30 Schon, M. P. Animal models of psoriasis: a critical appraisal. Exp. Dermatol. 17, 703–712 (2008).
31 van der Fits, L. et al. Imiquimod-Induced Psoriasis-Like Skin Inflammation in Mice Is Mediated via the IL-23/IL-17 Axis. J. Immunol. 182, 5836–5845 (2009).
32 Chollet, J. L. et al. Development of a topically active imiquimod formulation. Pharm. Dev. Technol. 4, 35–43 (1999).
33 Flutter, B. & Nestle, F. O. TLRs to cytokines: Mechanistic insights from the imiquimod mouse model of psoriasis. Eur. J. Immunol. 43, 3138–3146 (2013).
34 Caperton, C. & Berman, B. Safety, efficacy, and patient acceptability of imiquimod for topical treatment of actinic keratoses. Clin. Cosmet. Investig. Dermatol. 35–40 (2011).
35 Edwards, L. et al. Self-administered topical 5% imiquimod cream for external anogenital warts. Arch. Dermatol. 134, 25–30 (1998).
36 Lebwohl, M. et al. Imiquimod 5% cream for the treatment of actinic keratosis: results from two phase iii, randomized, double-blind, parallel group, vehicle-controlled trials. J. Am. Acad. Dermatol. 50, 714–721 (2004).
37 Tambunlertchai, S., Geary, S. M. & Salem, A. K. Topically Applied Resiquimod versus Imiquimod as a Potential Adjuvant in Melanoma Treatment. Pharmaceutics 14, (2022).
38 Bhagchandani, S., Johnson, J. A. & Irvine, D. J. Evolution of Toll-like receptor 7/8 agonist therapeutics and their delivery approaches: From antiviral formulations to vaccine adjuvants. Adv. Drug Deliv. Rev. 175, (2021).
39 Hemmi, H. et al. Small anti-viral compounds activate immune cells via the TLR7 MyD88-dependent signaling pathway. Nat. Immunol. 3, 196–200 (2002).
40 Gangwar, R. S., Gudjonsson, J. E. & Ward, N. L. Mouse Models of Psoriasis: A Comprehensive Review. J. Invest. Dermatol. 142, 884–897 (2022).
41 Jurk, M. et al. Human TLR7 or TLR8 independently confer responsiveness to the antiviral compound R-848. Nat. Immunol. 3, 499 (2002).
42 Fife, K. H., Meng, T.-c., Ferris, D. G. & Liu, P. Effect of resiquimod 0.01% gel on lesion healing and viral shedding when applied to genital herpes lesions. Antimicrob. Agents Chemother. 52, 477–482 (2008).
43 Köllisch, G. et al. Various members of the Toll-like receptor family contribute to the innate immune response of human epidermal keratinocytes. Immunology 114, 531–541 (2005).
44 Schön, M. P., Schön, M. & Klotz, K. N. The small antitumoral immune response modifier imiquimod interacts with adenosine receptor signaling in a TLR7- and TLR8-independent fashion. J. Invest. Dermatol. 126, 1338–1347 (2006).
45 Schön, M. P. & Schön, M. Immune modulation and apoptosis induction: two sides of the antitumoral activity of imiquimod. Apoptosis 9, 291–298 (2004).
46 Petes, C., Odoardi, N. & Gee, K. The toll for trafficking: toll-like receptor 7 delivery to the endosome. Front. Immunol. 8, 1075 (2017).
47 Doxsee, C. L. et al. The immune response modifier and Toll-like receptor 7 agonist S-27609 selectively induces IL-12 and TNF-α production in CD11c+ CD11b+ CD8− dendritic cells. J. Immunol. 171, 1156–1163 (2003).
48 Takeuchi, O. & Akira, S. Pattern recognition receptors and inflammation. Cell 140, 805–820 (2010).
49 Kawai, T. & Akira, S. TLR signaling. Cell Death Differ. 13, 816–825 (2006).
50 Kawagoe, T. et al. Essential role of IRAK-4 protein and its kinase activity in Toll-like receptor–mediated immune responses but not in TCR signaling. J. Exp. Med. 204, 1013–1024 (2007).
51 Leulier, F. & Lemaitre, B. Toll-like receptors—taking an evolutionary approach. Nat. Rev. Genet. 9, 165–178 (2008).
52 Kaushik, D., Kaur, A., Petrovsky, N. & Salunke, D. B. Structural evolution of toll-like receptor 7/8 agonists from IMIDazoquinolines to IMIDazoles. RSC Med. Chem. 12, 1065–1120 (2021).
53 Janssens, S. & Beyaert, R. A universal role for MyD88 in TLR/IL-1R-mediated signaling. Trends Biochem. Sci. 27, 474–482 (2002).
54 Akira, S., Takeda, K. & Kaisho, T. Toll-like receptors: critical proteins linking innate and acquired immunity. Nat. Immunol. 2, 675–680 (2001).
55 Patinote, C. et al. Agonist and antagonist ligands of toll-like receptors 7 and 8: Ingenious tools for therapeutic purposes. Eur. J. Med. Chem. 193, (2020).
56 Adhikari, A., Xu, M. & Chen, Z. J. Ubiquitin-mediated activation of TAK1 and IKK. Oncogene 26, 3214–3226 (2007).
57 Kawai, T. & Akira, S. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. Nat. Immunol. 11, 373–384 (2010).
58 Karin, M. Nuclear factor-κB in cancer development and progression. Nature 441, 431–436 (2006).
59 Reiter, M. J., Tester man, T. L., Miller, R. L., Weeks, C. E. & Tomai, M. A. Cytokine induction in mice by the immunomodulator imiquimod. J. Leucoc. Biol. 55, 234–240 (1994).
60 Gibson, S. J. et al. Cellular requirements for cytokine production in response to the immunomodulators imiquimod and S-27609. J. Interf. cytokine Res. Off. J. Int. Soc. Interf. Cytokine Res. 15, 537–545 (1995).
61 Megyeri, K. et al. Stimulation of interferon and cytokine gene expression by imiquimod and stimulation by Sendai virus utilize similar signal transduction pathways. Mol. Cell. Biol. 15, 2207–2218 (1995).
62 Bell, E. Intracellular trafficking, IRF7 and type-I-IFN responses. Nat. Rev. Immunol. 5, 361 (2005).
63 Urosevic, M. et al. Disease-independent skin recruitment and activation of plasmacytoid predendritic cells following imiquimod treatment. J. Natl. Cancer Inst. 97, 1143–1153 (2005).
64 Sauder, D. N. Imiquimod: modes of action. Br. J. Dermatol. 149, 5–8 (2003).
65 Kanneganti, T.-D. et al. Bacterial RNA and small antiviral compounds activate caspase-1 through cryopyrin/Nalp3. Nature 440, 233–236 (2006).
66 Schön, M. et al. Tumor-selective induction of apoptosis and the small-molecule immune response modifier imiquimod. J. Natl. Cancer Inst. 95, 1138–1149 (2003).
67 Schön, M. P., Manzke, V. & Erpenbeck, L. Animal models of psoriasis—highlights and drawbacks. J. Allergy Clin. Immunol. 147, 439–455 (2021).
68 Grine, L. et al. Topical imiquimod yields systemic effects due to unintended oral uptake. Sci. Rep. 6, 20134 (2016).
69 Grine, L., Dejager, L., Libert, C. & Vandenbroucke, R. E. Dual inhibition of TNFR1 and IFNAR1 in imiquimod-induced psoriasiform skin inflammation in mice. J. Immunol. 194, 5094–5102 (2015).
70 Walter, A. et al. Aldara activates TLR7-independent immune defence. Nat. Commun. 4, 1560 (2013).
71 Luo, D.-Q., Wu, H.-H., Zhao, Y.-K., Liu, J.-H. & Wang, F. Different imiquimod creams resulting in differential effects for imiquimod-induced psoriatic mouse models. Exp. Biol. Med. 241, 1733–1738 (2016).
72 Lebre, M. C. et al. Human keratinocytes express functional Toll-like receptor 3, 4, 5, and 9. J. Invest. Dermatol. 127, 331–341 (2007).
73 Amberg, N., Holcmann, M., Stulnig, G., Glitzner, E. & Sibilia, M. Effects of depilation methods on Imiquimod-induced skin inflammation in mice. J Invest Dermatol 137, 528–531 (2017).
74 Matos, T. R. et al. Clinically resolved psoriatic lesions contain psoriasis-specific IL-17–producing αβ T cell clones. J. Clin. Invest. 127, 4031–4041 (2017).
75 Swindell, W. R. et al. Genome-wide expression profiling of five mouse models identifies similarities and differences with human psoriasis. PLoS One 6, e18266 (2011).

76 Pasparakis, M., Haase, I. & Nestle, F. O. Mechanisms regulating skin immunity and inflammation. Nat. Rev. Immunol. 14, 289–301 (2014).
77 Mestas, J. & Hughes, C. C. W. Of mice and not men: differences between mouse and human immunology. J. Immunol. 172, 2731–2738 (2004).
78 Eming, S. A., Martin, P. & Tomic-canic, M. Wound repair and regeneration: mechanisms, signaling, and translation. Sci. Transl. Med. 6, 265sr6-265sr6 (2014).
79 Wong, V. W., Sorkin, M., Glotzbach, J. P., Longaker, M. T. & Gurtner, G. C. Surgical approaches to create murine models of human wound healing. Biomed Res. Int. 2011, (2011).
80 Abdullahi, A., Amini-Nik, S. & Jeschke, M. G. Animal models in burn research. Cell. Mol. life Sci. 71, 3241–3255 (2014).
81 Hu, Y. et al. The role of interleukin-1 in wound biology. Part II: In vivo and human translational studies. Anesth. Analg. 111, 1534–1542 (2010).
82 Wagstaff, A. J. & Perry, C. M. Topical imiquimod: A review of its use in the management of anogenital warts, actinic keratoses, basal cell carcinoma and other skin lesions. Drugs 67, 2187–2210 (2007).
83 EMA. Bijlage I samenvatting van de productkenmerken 1 Apidra. 1–24 (2017).
84 Monograph, P. Aldara ® P. 1–39 (2013).
85 Beutner, K. R. et al. Treatment of genital warts with an immune-response modifier (imiquimod). J. Am. Acad. Dermatol. 38, 230–239 (1998).
86 Korman, N. et al. Dosing with 5% imiquimod cream 3 times per week for the treatment of actinic keratosis: results of two phase 3, randomized, double-blind, parallel-group, vehicle-controlled trials. Arch. Dermatol. 141, 467–473 (2005).
87 Schulze, H. J. et al. Imiquimod 5% cream for the treatment of superficial basal cell carcinoma: results from a randomized vehicle-controlled phase iii study in Europe. Br. J. Dermatol. 152, 939–947 (2005).
88 Geisse, J. et al. Imiquimod 5% cream for the treatment of superficial basal cell carcinoma: results from two phase iii, randomized, vehicle-controlled studies. J. Am. Acad. Dermatol. 50, 722–733 (2004).
89 Geisse, J. K. et al. Imiquimod 5% cream for the treatment of superficial basal cell carcinoma: a double-blind, randomized, vehicle-controlled study. J. Am. Acad. Dermatol. 47, 390–398 (2002).
90 Szeimies, R. M. et al. Imiquimod 5% cream for the treatment of actinic keratosis: results from a phase iii, randomized, double-blind, vehicle-controlled, clinical trial with histology. J Am Acad Dermatol 51, 547–555 (2004).
91 Aronson, J. K. Biomarkers and surrogate endpoints. Br. J. Clin. Pharmacol. 59, 491 (2005).
92 Harvey, N. T., Chan, J. & Wood, B. A. Skin biopsy in the diagnosis of inflammatory skin disease. Aust. Fam. Physician 46, 283–288 (2017).
93 Berekméri, A. et al. Non-invasive approaches for the diagnosis of autoimmune/autoinflammatory skin diseases—a focus on psoriasis and lupus erythematosus. Front. Immunol. 10, 1931 (2019).
94 Ruccia, F., Zoccali, G., Cooper, L., Rosten, C. & Nduka, C. A three-dimensional scar assessment tool for keloid scars: Volume, erythema and melanin quantified. Ski. Res. Technol. 27, 1007–1016 (2021).
95 Grievink, H. W. et al. Antimicrobial Peptide Omiganan Enhances Interferon Responses to Endosomal Toll-Like Receptor Ligands in Human Peripheral Blood Mononuclear Cells. Clin. Transl. Sci. 13, 891–895 (2020).